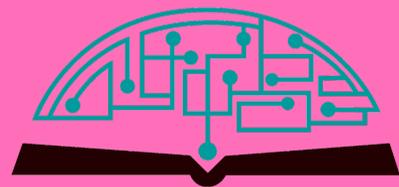


# IJHSR

International  
Journal of  
High School  
Research



**March 2026 | Volume 8 | Issue 5**

*[ijhsr.terrajournals.org](http://ijhsr.terrajournals.org)*

ISSN (Print) 2642-1046

ISSN (Online) 2642-1054



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# Spatial Transcriptomics and Adaptive Multi-Modal Ensemble Encoding: Early Metastasis Profiling and Targeting

Nidhi Yadalam

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**ABSTRACT:** Lung cancer remains the leading cause of cancer-related mortality, primarily due to its high metastatic potential and the difficulty of early metastasis detection. Current clinical approaches rely on low-sensitivity assessments and can also cause complications. To address these challenges, this project introduces a computational framework that predicts metastatic potential directly from the primary tumor site, thereby eliminating the need for secondary site biopsies and enabling earlier intervention. This framework leverages spatial transcriptomics, a cutting-edge technology that maps tumor tissue, capturing cellular interactions that are missed by traditional methods. By analyzing the spatial and molecular features of primary tumors, this system identifies high-risk tumor regions and key metastatic drivers, integrating neural networks, autoencoders, and unsupervised clustering. In-silico validation aligned the received outputs with known signatures from open datasets. Building on these findings, a deep learning-guided lipid nanoparticle optimization pipeline was developed to design drug carriers for siRNA, aiming to silence the inhibition of genetic pathways. Further supervised analysis and validation revealed the heightened properties of the enhanced carriers. This study bridges AI-driven metastasis prediction and treatment with precision nanomedicine for metastatic cancer at its earliest stages by shifting from late-stage intervention to proactive, gene-targeted suppression.

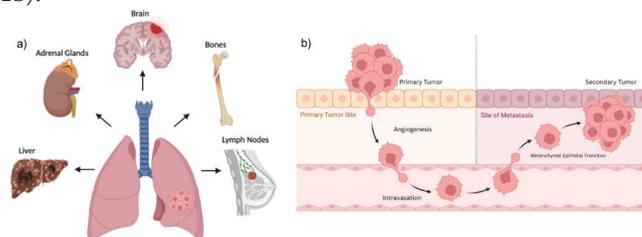
**KEYWORDS:** Computational Biology and Bioinformatics, Computational Pharmacology, Multimodal Ensemble Learning, Spatial Transcriptomics, Metastasis Prediction and Treatment.

## Introduction

Bronchogenic Carcinoma, or Lung Cancer (LC), is deemed to be the leading cause of death by cancer in the United States, with 234,580 new cases of LC and 125,070 deaths just in 2024. LC accounts for 20% of all cancer-related deaths, accounting for more than breast, colon, and prostate cancers combined.<sup>1</sup> LC is a disease caused by the uncontrollable division of mutated cells, which can cause abnormalities, including tumor masses.<sup>2</sup> A common cause involves repeated exposure to carcinogens, which can lead to an abnormal growth pattern of the epithelial cells lining the lung pathways, resulting in lung cancer if left untreated.<sup>3</sup> There are two main categories of LC: Small Cell Lung Cancer (SCLC) and Non-Small Cell Lung Cancer (NSCLC).<sup>4</sup> SCLC is a cancer that grows and spreads at an exceptionally high rate, with a centrally localized lung mass or bulky thoracic lymph node involvement; the main subtypes of NSCLC are adenocarcinoma, squamous cell carcinoma, and large cell carcinoma.<sup>5</sup>

Lung Cancer Metastasis (LCM) refers to the dynamic process by which the cancer cells escape the primary tumor, migrate, and colonize distant organs. The process involves several complex mechanisms and commonly affects specific organs, contributing to the lethality of most forms of lung cancer. The spread of lung cancer cells is influenced by various factors, including interactions within the tumor microenvironment (TME) and the process of epithelial-mesenchymal transition (EMT). EMT enables cancer cells to acquire migratory and invasive properties.<sup>6</sup> Lung cancer can metastasize to almost any part of the body, but the most common locations for metastasis

include the liver, bones, brain, and adrenal glands (Figure 1a).<sup>7</sup> The process begins with angiogenesis, where new blood vessels form to supply nutrients and oxygen to the tumor. This supports rapid tumor growth and provides a pathway for cancer cells to escape the primary site.<sup>8</sup> Through EMT, lung cancer cells lose their epithelial characteristics, such as tight cell-cell adhesion and polarity, and gain mesenchymal features such as increased motility and invasiveness. This transition enables cancer cells to invade the surrounding tissue, penetrate the extracellular matrix, and enter the bloodstream or lymphatic system, a process known as intravasation. Once in circulation, the cancer cells survive various stresses (such as the immune surveillance system) by developing resistance mechanisms.<sup>9</sup> To establish secondary tumors, the cells undergo a mesenchymal-epithelial transition (MET), reverting to an epithelial phenotype that supports proliferation and colonization (Figure 1b).<sup>10</sup>



**Figure 1:** Biological context for metastasis. a) Anatomical illustration of primary lung cancer and its most frequent metastatic destinations. b) Schematic representation of the general metastatic cascade: angiogenesis provides vascular access, followed by intravasation into the bloodstream, and eventual colonization of distant tissues through mesenchymal-epithelial transition.

The primary challenge in managing metastatic lung cancer is the difficulty in early detection and precise identification of metastatic spread. Unlike localized tumors that can be surgically removed or targeted with localized radiation, metastases often involve multiple distant sites, requiring systemic treatment approaches that are less effective in advanced stages.<sup>11</sup> Current diagnostic techniques rely on imaging scans (CT, PET, MRI) and biopsies from suspected secondary sites.<sup>12</sup> However, these methods have several invasive limitations – posing risks of complications, infections, and additional stress for patients.<sup>13</sup> Furthermore, metastatic tumors are often heterogeneous, meaning that biopsies from one part of the metastatic site may not fully capture the molecular characteristics of the entire metastatic population.<sup>14</sup> Many metastases also remain undetectable until they reach a clinically significant size, by which point treatment options become limited. Some micrometastases are too small to be detected through conventional imaging, leading to missed diagnoses and delays in targeted treatment.<sup>15</sup>

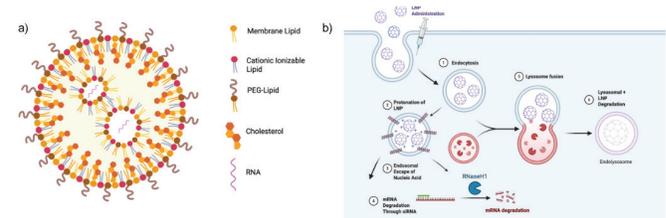
Recent technological advancements have revolutionized the ability to analyze gene expression while preserving spatial characteristics, providing valuable insights into the interactions within the TME, unlike traditional bulk RNA-sequencing methods, which average gene expression across an entire tissue. Spatially resolved gene expression, also known as spatial transcriptomics (ST), is a technique that enables scientists to study gene expression within the physical context of intact tissue.<sup>16</sup> This approach provides valuable insights into how cells interact with one another and their environment, which is critical for understanding cell functions and tissue dynamics.<sup>17</sup> By analyzing intact tissue, spatial transcriptomics retains critical spatial information, offering a more holistic view of cell biology.<sup>18</sup>

However, despite its potential, the vast, high-dimensional datasets generated by spatial transcriptomics remain difficult to analyze and interpret. Machine Learning (ML) has emerged as a transformative approach to addressing technical challenges and analyzing patterns.<sup>19</sup> Utilizing deep learning allows for an understanding of the complex ST organization of tumor cells in their microenvironment.

RNA-based therapeutics, particularly small interfering RNA (siRNA), offer a highly specific approach to cancer treatment by silencing genes that drive metastasis at the translational level.<sup>20</sup> Unlike traditional treatments, siRNA can target specific oncogenes or metastatic regulators, shutting down pathways responsible for tumor invasion, angiogenesis, and immune suppression.<sup>21</sup> This precision therapy has the potential to revolutionize cancer treatment by directly inhibiting metastatic progression rather than broadly targeting cell division. However, siRNA delivery presents a major challenge. Naked siRNA is highly unstable, rapidly degrading in the bloodstream and failing to reach tumor sites effectively.<sup>22</sup> Additionally, siRNA molecules must overcome multiple biological barriers, including immune clearance, poor cellular uptake, and endosomal degradation, before they can exert their therapeutic effects.<sup>23</sup> To address these challenges, Lipid Nanoparticles (LNPs) have emerged as a promising delivery platform for RNA-based

therapies, offering enhanced stability, targeted delivery, and efficient cellular uptake (Figure 15).<sup>24</sup>

LNPs are engineered drug delivery systems designed to encapsulate and protect siRNA, improving its stability and ensuring efficient transport to tumor cells (Figure 2a). LNPs are widely used in gene therapy, immunotherapy, and vaccine development, proving their effectiveness in delivering nucleic acids with high efficiency. The core of an LNP typically contains ionizable cationic lipids that bind to and condense the negatively charged RNA, surrounded by structural lipids such as cholesterol and phospholipids that enhance membrane stability. A polyethylene glycol (PEG)-lipid is incorporated on the outer surface to improve circulation time and reduce immune clearance.<sup>25</sup> Upon reaching the target tissue, the acidic environment of endosomes activates the ionizable lipids, promoting endosomal escape and releasing the siRNA into the cytoplasm, where it silences gene expression by degrading target mRNA (Figure 2b).<sup>26</sup> Compared to viral delivery systems, LNPs offer greater biocompatibility, lower immunogenicity, and scalable production—making them ideal for siRNA-based cancer treatment.



**Figure 2:** RNA-loaded lipid nanoparticles and delivery mechanism. a) Schematic of an LNP showing its major components: ionizable lipids, cholesterol, membrane lipids, PEG-lipids, and encapsulated RNA. b) Mechanism of siRNA delivery via LNPs: cellular uptake, endosomal escape triggered by pH-dependent activation of ionizable lipids, and gene silencing through RNA-induced silencing complex (RISC) activation. Modified from ref 36.

Despite their potential, optimizing LNPs for siRNA delivery remains a complex challenge. The ideal LNP formulation must strike a balance between stability, size, charge, and encapsulation efficiency, while ensuring precise targeting of metastatic cells. Current research focuses on improving LNP composition, surface modifications, and ligand-based targeting to maximize therapeutic efficacy.<sup>27</sup> By leveraging AI-driven optimization techniques, LNP formulations can be tailored for metastatic inhibition, marking a significant step toward next-generation cancer therapeutics.

Recent years have seen rapid progress in integrating spatial transcriptomics with advanced computational methods – particularly deep learning and multimodal learning – to dissect tumor and microenvironment architecture in cancer types. For example, a 2024 study by Zuani *et al.* demonstrated that combining single-cell and spatial transcriptomics in NSCLC enables a high-resolution “map” of tumor signaling modules that drive cellular reprogramming of macrophages.<sup>28</sup> Furthermore, Wang *et al.* utilized a combination of scRNA-seq and ST in human multiple primary lung cancer lesions to map a novel epithelial sub-population and reveal inter-lesion spatial heterogeneity.<sup>29</sup> On the computational front, deep-learning frameworks have begun to integrate histopathological images

with ST maps. For example, Zhao *et al.* introduced a model named GIST, which fuses histology and spatial transcriptome data to predict cell-type distributions and latent spatial signatures.<sup>30</sup> Another model, TransST, uses transfer-learning and spatial factor modelling to improve deconvolution of ST datasets and reveal biologically meaningful areas of interest in tumor samplings.<sup>31</sup>

In parallel, deep, generative learning is rapidly accelerating the optimization of LNPs and ionizable lipids for nucleic acid delivery, critical for siRNA and mRNA therapies. The AG-ILE platform, developed by Xu *et al.*, uses a combination of deep learning neural networks and combinatorial chemistry to determine the best possible ionizable lipids for specific cell types.<sup>32</sup> Similarly, Wang *et al.* applied AI-driven virtual screening with LightGBM to predict lipid pKa and delivery efficiency, yielding novel candidates outperforming traditional lipid candidates such as DLin-MC3-DMA.<sup>33</sup> Comprehensive reviews, such as Dorsey *et al.*, highlight challenges and opportunities in ML-guided formulation, including data sparsity, feature engineering, and multi-objective optimization of particle size, charge, and targeting.<sup>34</sup>

This study aims to utilize spatial transcriptomics and machine learning to analyze the molecular and spatial characteristics of primary lung tumors. This method will enable the early identification of tumors that are most likely to metastasize by analyzing the gene expression patterns associated with metastasis, the spatial organization of tumor cells, and the TME interactions. Using the encodings learned from the metastasis profiling, the pipeline also aims to provide an optimized nucleic acid-based therapy to inhibit metastasis. This will allow clinicians to intervene earlier and more precisely, tailoring treatments to suppress metastatic progression before it becomes widespread.

## ■ Methods

### *Data Preprocessing:*

The spatial transcriptomics datasets used in this study were obtained from the Visium Spatial Gene Expression platform, provided by 10x Genomics.<sup>35</sup> The Visium platform enables high-resolution spatial transcriptomic profiling, capturing gene expression data alongside spatial coordinates within tissue sections. The datasets include spatial coordinates for tissue spots, corresponding gene expression profiles, and cluster annotations derived from prior analyses.

To prepare the data for downstream analysis, spatial coordinates were normalized to maintain consistency across samples. High-variance genes were selected from the gene expression data, as they are more likely to capture biologically significant patterns. Modified from the SpatialPCA reduction methodology proposed by Shang and Zhou, dimensionality reduction was then applied using Principal Component Analysis (PCA).<sup>36</sup> This retains the top 50 principal components to reduce noise and computational complexity while preserving key features of the data. Finally, the spatial coordinates, transcriptomic data, and cluster information were merged into a unified dataset, creating a comprehensive foundation for subsequent analysis, including clustering and spatial modeling.

This preprocessing pipeline ensures that the high-dimensional data is optimized for accurate and robust exploration of spatial gene expression patterns.

### *Graph Neural Network for Feature Learning:*

The spatial and transcriptomic data are processed using a graph neural network (GNN), which is designed to effectively capture the relationships between spatially adjacent regions and their gene expression profiles. The GNN operates on a graph structure where each spatial spot or cell is treated as a node, and edges represent spatial proximity. The input to the GNN consists of a graph where nodes are characterized by the PCA-reduced gene expression data and their spatial coordinates. The graph edges are constructed based on a k-nearest neighbor (k-NN) algorithm applied to the spatial coordinates, ensuring that each node is connected to its most relevant neighbors.<sup>37</sup> Edge weights are computed using a Gaussian kernel on spatial distances, allowing closer neighbors to have a stronger influence while reducing noise from distant nodes.

The GNN is composed of multiple graph convolutional layers designed to propagate and aggregate information across the graph.<sup>38</sup> Each layer updates the features of a node by aggregating the features of its neighbors, weighted by the edge connections. This equation is expressed mathematically as:

$$h_i^{(l+1)} = \sigma \left( W^l \sum_{j \in N(i)} \frac{h_j^{(l)}}{|N(i)|} + b^l \right)$$

Where:

$h_i^{(l)}$  is the feature vector of the node  $i$  at layer  $l$ .

$W^l$  is the trainable weight matrix at layer  $l$ .

$N(i)$  represents the neighbors at the node  $i$ .

$b^l$  is the bias term at the layer  $l$ .

$\sigma$  is a non-linear activation function, such as the rectified linear unit (ReLU).

The aggregation function combines information from neighboring nodes, ensuring that each node's updated representation incorporates both its local features and its spatial context. After passing through the GNN, each node is represented by a high-dimensional embedding that captures both the spatial relationships of the tissue and the transcriptomic variation. These refined features are then passed into the autoencoder for dimensionality reduction and further representation learning in the latent space. By propagating and aggregating information across spatially connected nodes, the GNN enables the model to capture the subtle spatial dependencies and gene expression patterns crucial for tasks such as clustering and domain identification.

### *Latent Space Representation:*

The autoencoder plays a pivotal role in learning meaningful latent space representations by processing data through its input, bottleneck, and output layers.<sup>39</sup> The input to the autoencoder is derived from the encodings of the GNN, which combines PCA-reduced gene expression data and the corresponding spatial embeddings. The latent space, or bottleneck,

is the most critical component of the autoencoder architecture. This layer reduces the input to a compact 10-dimensional representation, forcing the network to focus on the most essential features of the data. The latent space effectively captures a distilled version of the spatial transcriptomic data, encoding both the spatial relationships and the most significant patterns in gene expression. This compact embedding serves as a versatile representation, enabling tasks like clustering to uncover meaningful spatial domains or regions with shared biological properties. The process of distillation in the latent space also ensures that noise and irrelevant features are filtered out, leaving only the core biological signals for downstream analysis. The output of the autoencoder seeks to reconstruct the input as closely as possible. Starting from the 10-dimensional latent space, the decoder progressively transforms the reduced representation back into the original input dimensionality. The accuracy of the reconstruction is measured using the mean squared error (MSE) loss function, which quantifies the difference between the original input and the reconstructed output. Minimizing this loss ensures that the autoencoder has constructively captured the most critical features in the latent space while preserving the integrity of the data during reconstruction. By training the autoencoder on this task, the latent space representation becomes highly expressive, capturing both the spatial and transcriptomic relationships present in the input.

### Clustering:

Clustering is a crucial step in analyzing spatial transcriptomic data, as it enables the identification of biologically meaningful patterns and spatial domains within the tissue. In this study, k-means clustering was applied to the learned 10-dimensional latent representations generated by the autoencoder to group data points into distinct clusters based on their feature similarity. The algorithm begins with the initialization step, where cluster centroids are randomly placed in the latent space, as shown in previous studies.<sup>40</sup> Each centroid represents the potential center of a cluster.<sup>41</sup> In the assignment step, each data point, represented as a vector in the 10-dimensional latent space, is assigned to the nearest cluster centroid based on the Euclidean distance metric:

$$d(x_i, c_k) = \sqrt{\sum_{j=1}^{10} (x_{ij} - c_{kj})^2}$$

Where:

$x_i = (x_{i1}, x_{i2}, \dots, x_{i10})$  represents a datapoint in the latent space.

$c_i = (c_{i1}, c_{i2}, \dots, c_{i10})$  represents a datapoint in the latent space.

$d(x_i, c_k)$  is the Euclidean distance between the datapoint  $x_i$  and the centroid  $c_k$ .

Each data point is assigned to the cluster  $k^*$  that minimizes the Euclidean distance:

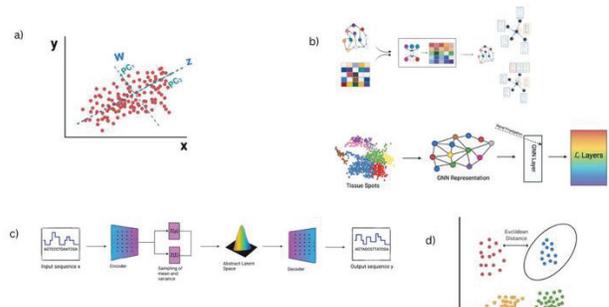
$$k^* = \underset{k}{\operatorname{argmin}} d(x_i, c_k)$$

This ensures that data points with similar gene expression and spatial characteristics, as encoded in the latent space, are

grouped. The recalculation of the centroid iteratively occurs until a solution is identified where the clusters are well-defined and separated.

By clustering in this reduced-dimensional space, k-means identifies distinct groups of spatially and transcriptionally similar regions, corresponding to biologically or pathologically distinct spatial domains. To facilitate interpretation of the clustering outcomes, spatial coordinates from the original data are used to create scatter plots where each data point is plotted according to its physical location within the tissue. The clusters assigned by k-means are visualized by coloring each point according to its cluster label. Once clusters are identified, they are visualized in two dimensions. Spatial coordinates are then color-coded by cluster labels, allowing the mapping of clusters back onto the tissue to reveal their spatial distribution and biological significance.

To validate the biological relevance of the identified clusters, Gene Set Enrichment Analysis (GSEA) is performed. This analysis determines whether specific gene sets, such as those associated with metastatic pathways, are enriched within each cluster. For instance, gene sets related to epithelial-mesenchymal transition (EMT), cellular invasion, migration, proliferation, or angiogenesis can reveal clusters likely representing metastatic regions. By linking clusters with well-documented metastatic pathways, GSEA provides a critical layer of validation, enabling researchers to hypothesize about the role of each cluster in metastatic progression.



**Figure 3:** Pictorial diagram of the ensemble multi-modal machine learning pipeline. a) Principal Component Analysis (PCA) reduces dimensionality of high-throughput transcriptomic data. b) Graph Neural Networks (GNNs) capture spatial and transcriptional relationships between tissue regions. c) A variational autoencoder learns abstract latent features from gene expression profiles. d) K-means clustering is applied in latent space to group biologically relevant tissue domains based on Euclidean distance.

### Metastasis Risk Score (Supervised Post-Analysis):

After identifying clusters potentially associated with metastatic behavior, a Metastasis Risk Score is calculated for each spatial location within the tissue. The computation of the Metastasis Risk Score considers three primary components: gene expression signatures, spatial context, and cluster membership.

The first component, gene expression signatures, involves evaluating the expression levels of known genes associated with metastasis. Regions with elevated expression of these genes are considered to have a higher likelihood of exhibiting metastatic behavior. The second component, spatial context, assesses the proximity of a spatial location to the primary tumor and evaluates the spatial patterns of gene expression. For instance, locations closer to the primary tumor or showing spatial char-

acteristics consistent with metastatic progression may receive higher scores. Lastly, cluster membership is incorporated by assigning higher weights to clusters identified as enriched for metastatic pathways through GSEA, linking the unsupervised clustering results to the risk-scoring process.

To refine the Metastasis Risk Score, supervised machine learning models such as Support Vector Machines (SVMs) and Random Forests (RFs) are employed. These models take input features derived from the latent space embeddings, expression levels of metastasis-associated genes, spatial proximity to high-risk clusters, and pathway enrichment scores. By training on labeled datasets where metastatic status is known, these models learn to predict the likelihood of metastasis at each spatial location. Incorporating known metastatic markers as features further enhances the accuracy of the risk predictions while maintaining biological interpretability.

### Unsupervised Therapy Design:

The Variational Autoencoder (VAE)-Bayesian inference method is implemented in this study because it allows for a continuous, molecule-based algorithm that can derive features from its own latent space, inspired by the methodology from Ochiai *et al.*<sup>42</sup> This method is modified from the chemical LNP optimization methodology proposed by Nidhi Yadalam in 2024 for cystic fibrosis therapeutics.<sup>43</sup> As the input is a valid Simplified Molecular-Input Line-Entry System (SMILES) entry of a combinatorially formulated LNP (composed of the four lipids as described previously), the VAE traverses through its encoder/decoder network, recognizing the principal components of the entry.

The primary chemical formulation system is manned by principles of iterative, combinatorial chemistry to initiate different syntheses of LNPs. After manually retrieving various cationic ionizable lipids, cholesterol, phospholipids, and PEG-lipids, the client class iterates through the database, identifying the SMILES input for each compound. The canonical smiles were manually inputted at the beginning for easy access. The code then generates combinatorial libraries of molecules by enumerating possible combinations of R-groups on the scaffold of cationic ionizable lipids. The composition finalizes with a large database of LNPs.

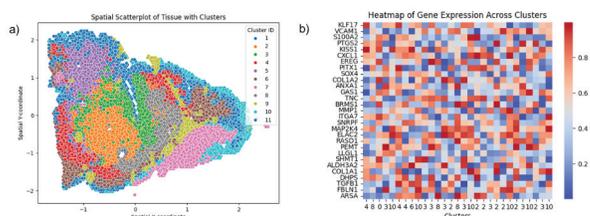
The encoder portion for the VAE is made based on Relational Graph Convolutional Networks (R-CGN). The main inputs for the encoder are the adjacency and feature matrices. After the relational convolutions, the dimensionality of the graph is then further reduced from 2D to 1D, allowing the molecule to be easily represented for random selection later. However, the 2D dimensionality is retained, allowing it to represent the latent space. In the latent chemical space, the features, or properties, of the compounds were reduced to lower dimensionality and then optimized using Gaussian properties and the loss function. The custom loss used in the VAE function consists of two terms: a reconstruction loss and a KL divergence loss. The reconstruction loss term measures how well the model reconstructs the input data, while the KL divergence term encourages the learned latent space to resemble a predefined prior distribution.

The decoder reconstructs the primary input SMILES from the latent space. After defining the latent space input, densely connected layers are applied inside the latent space to learn a nonlinear mapping from the latent space representation to the adjacency matrix and feature matrix. Therefore, the generated outputs capture meaningful graph structures and node features while mitigating the risk of overfitting. The decoder's dense layers are then mapped to a continuous adjacency tensor and reshaped to match the specified adjacency shape, generating a representation of the graph's adjacency matrix in the SMILES format.

## Results and Discussion

### Overview of Identified Clusters:

Unsupervised clustering methods, including k-means and Density-Based Spatial Clustering of Applications with Noise (DBSCAN), were applied to the latent space generated by the autoencoder. These methods identified distinct spatial clusters representing biologically meaningful groupings based on gene expression patterns. Scatterplots of spatial coordinates, colored by cluster labels, demonstrated clear separation between tissue regions, with clusters exhibiting high variability in gene expression predominantly located at the tumor's invasive front or distant metastatic sites. Figure 4a and Figure 4b show the groupings of spatial coordinates and clusters, as well as the transcriptome and heatmap of genes for the Human Lung Cancer FFPE tissue tumor block.

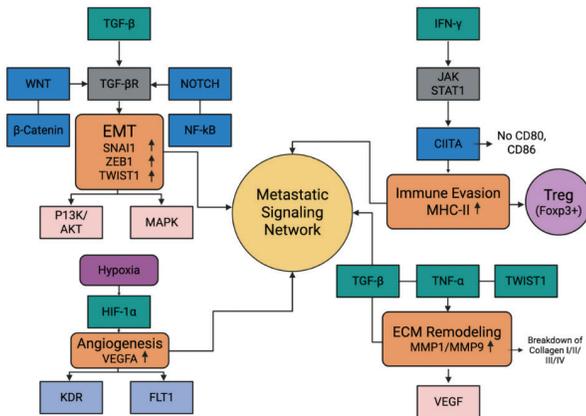


**Figure 4:** Spatial heterogeneity of gene expression. a) Spatial scatterplot showing transcriptionally distinct clusters across tissue regions, each color-coded to indicate unique spatial domains. b) Heatmap displaying gene expression variation across clusters, highlighting differential expression of metastasis-related genes.

### Metastatic Pathways Identified:

Pathway enrichment analysis of metastatic clusters revealed significant associations with key metastasis-related pathways for each specific dataset. Clusters demonstrated strong enrichment for EMT, as evidenced by the upregulation of genes such as SNAI1, ZEB1, and TWIST1, indicating enhanced cell mobility and invasiveness. This is demonstrated in publications that support the EMT signaling pathway, as mediated by the most famous EMT-related cytokine, transforming growth factor  $\beta$  (TGF- $\beta$ ).<sup>44</sup> Additionally, angiogenesis pathways exhibited elevated expression of genes such as VEGFA, which has been shown to bind with receptors such as FT1 and KDR — supporting vascular remodeling and tumor expansion.<sup>45</sup> Immune evasion pathways were also enriched, marked by upregulation of MHC class II genes in the absence of co-stimulatory signals, enabling the tumor to suppress immune responses through regulatory T cell induction.<sup>46,47</sup> ECM modeling was observed through the overexpression of matrix

metalloproteinases (e.g., MMP1, MMP9), which correlated with tissue invasion and metastasis.<sup>48</sup> All the different metastatic capabilities were discovered through the various tests of the individual datasets and compared with existing literature, with certain biochemical integrations shown (Figure 5). Dynamic comparisons with metastasis-related gene sets from TCGA and GEO datasets confirmed the robustness of these clustering results. A real-time correlation with the published cancer datasets was also run to ensure the practicality of the clustering results and the pathway likelihood (Figure 6a).



**Figure 5:** Integrated signaling pathways in lung cancer metastasis. Key pathways contributing to metastasis, including EMT, immune evasion, angiogenesis, and ECM remodeling, are shown converging on a central metastatic signaling network. Arrows indicate activation or upregulation of downstream effectors.

### Gene Set Enrichment Analysis:

Gene Set Enrichment Analysis (GSEA) further validated the enrichment of metastatic pathways within the identified clusters. EMT and invasion pathways displayed normalized enrichment scores (NES) greater than 3, with false discovery rates (FDR) of less than 0.05. A high NES indicates strong and statistically significant enrichment of these pathways within the metastatic clusters, suggesting that genes involved in EMT and invasion are highly activated compared to background gene expression levels. The low FDR ensures that these findings are not due to random chance, increasing confidence in the biological relevance of these pathways to metastasis. This provides robust evidence that EMT and invasion processes play a critical role in driving metastatic progression within the identified clusters, reinforcing the validity of the clustering and enrichment analysis. Additionally, immune suppression and angiogenesis pathways exhibited consistent enrichment across multiple metastatic clusters. Enrichment maps visualizing these findings revealed cluster-specific pathway activation, underscoring the heterogeneity in metastatic mechanisms across different tissue regions (Figure 6a and 6b).

### Cross-Validation with External Datasets:

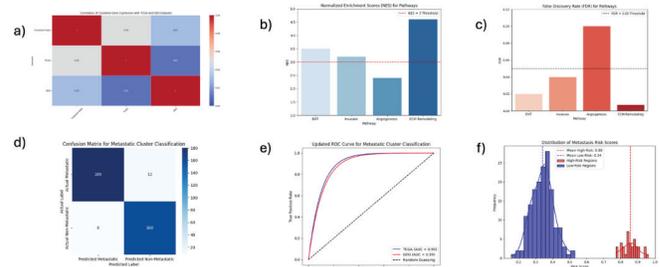
External validation was conducted using publicly available transcriptomic datasets from The Cancer Genome Atlas (TCGA) from the Genomic Data Commons (GDC) Portal<sup>49</sup> and Gene Expression Omnibus (GEO)<sup>50</sup> to assess the robustness of the identified metastatic clusters. To ensure consistency,

metastatic signatures were extracted from independent patient-derived tumor samples in these datasets and compared against the gene expression profiles from the identified clusters. The enriched pathways are known to be statistically and biologically significant, with the False Discovery Rate (FDR) values of less than 0.05 (Figure 6c).

Validation was performed by applying the trained clustering model to these external datasets and computing classification performance metrics. The metastatic clusters demonstrated high predictive accuracy, with an average precision of 94.2% (TCGA) and 91.5% (GEO), recall values of 92.8% (TCGA) and 90.2% (GEO), and F1 scores of 93.5% (TCGA) and 90.8% (GEO) (Figure 6d). Additionally, a receiver operating characteristic (ROC) analysis was performed, revealing high area under the curve (AUC) scores of 0.9 (TCGA) and 0.89 (GEO). The receiver operating characteristic (ROC) curve (Figure 6e) illustrates that metastatic classification shows clear separation between true positive and false positive rates.

### Metastasis Risk Scoring:

A Metastasis Risk Score was computed for each spatial region based on gene expression, spatial context, and cluster membership. High-risk regions, defined as those scoring in the top 20<sup>th</sup> percentile, exhibited a mean risk score of  $0.87 \pm 0.05$ , while low-risk regions scored significantly lower at  $0.34 \pm 0.07$  ( $p < 0.05$ ) (Figure 6f). The 20<sup>th</sup> percentile was chosen to capture the upper quantile of spatial regions exhibiting extreme metastatic gene-expression patterns while retaining sufficient statistical power for group comparisons. Cross-validation of the risk scores against clinical metastatic samples was performed using an independent dataset of histologically confirmed metastatic regions. The predictive accuracy of the risk scoring system was evaluated using sensitivity and specificity metrics, achieving a sensitivity of 92.8% and a specificity of 89.4%. A Spearman correlation analysis ( $\rho = 0.81$ ) further validated the strong association between predicted high-risk regions and actual metastatic outcomes, reinforcing the robustness of the risk assessment framework. These results highlight the effectiveness of the risk-scoring system in identifying regions with high metastatic potential.



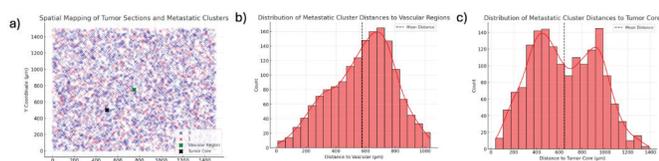
**Figure 6:** Validation of biological relevance and predictive strength of identified metastatic clusters. a) Clustered gene expression profiles show high correlation ( $r \geq 0.91$ ) with TCGA and GEO datasets. b) Enrichment analysis reveals strong activation of metastasis-related pathways, all with  $NES \geq 3$ . c) All enriched pathways show statistically significant FDR values ( $FDR < 0.05$ ). d) The confusion matrix shows accurate classification of metastatic vs. non-metastatic clusters with minimal error. e) ROC curves for TCGA and GEO confirm strong model performance ( $AUC = 0.90$  and  $0.89$ ). f) Metastasis risk scores show clear separation between high- and low-risk tissue regions.

### Spatial Analysis of Metastatic Clusters:

Mapping of identified clusters onto spatial tissue coordinates demonstrated that metastatic clusters were predominantly located at the tumor's invasive front or in distant regions. Quantitative spatial analysis revealed that for tumor sections ranging from 400 to 1200  $\mu\text{m}$ , metastatic clusters were positioned at an average distance of 600–950  $\mu\text{m}$  from the tumor core, whereas low-risk clusters were placed within 250–450  $\mu\text{m}$  of the tumor boundary. The spatial mapping (Figure 7b) illustrates that high-risk metastatic clusters (red) tend to be localized at the tumor periphery and distant regions. In contrast, low-risk clusters (blue) are concentrated near the tumor core. Most metastatic clusters exhibit a skewed distribution toward increased distances from the tumor core, reinforcing the notion that tumor cells preferentially disseminate away from the primary tumor mass. For the cluster distances to the vascular regions, the distances averaged at  $\sim 550$   $\mu\text{m}$  (Figure 7a), and for the cluster distances to the tumor core, the distances averaged at  $\sim 650$   $\mu\text{m}$  (Figure 7c).

Spatial autocorrelation analysis confirmed that metastatic clusters were non-randomly distributed (Moran's  $I = 0.67$ ,  $p < 0.03$ ), indicating strong spatial dependence of metastatic behavior. These findings suggest that metastatic clusters do not arise randomly within the tissue but instead follow predictable patterns of invasion towards vascularized and low-density extracellular matrix regions.

These findings were consistent with biological expectations of metastasis, particularly migration toward vascularized or low-density regions of the extracellular matrix. The statistical correlation between high-risk clusters and proximity to vasculature provided additional spatial validation supporting the identified clusters.



**Figure 7:** Spatial patterns associated with metastatic risk. a) Map of metastatic clusters overlaid on tissue coordinates, with high-risk (red) and low-risk (blue) areas, and annotated vascular regions and tumor core. b) Metastatic clusters are located closer to vascular regions on average ( $\sim 550$   $\mu\text{m}$ ), suggesting vascular-directed migration. c) Metastatic clusters show wider spatial spread from the tumor core ( $\sim 650$   $\mu\text{m}$  mean distance), indicating dispersal beyond the primary tumor mass.

### Unsupervised Therapy Analysis:

Following the development of the Variational Autoencoder (VAE) model and the exploration of its latent space to identify optimal molecular representations through decoding, an essential post-processing step ensures that only chemically valid SMILES strings are retained. Using RDKit modules, the model filters out invalid molecules by checking for structural feasibility, including bond integrity and atom valency. This step prevents the inclusion of synthetically impossible or unstable molecules. Beyond chemical validity, the model further evaluates the drug-likeness and pharmacological suitability of the generated lipid nanoparticles (LNPs) using two key metrics:

Quantitative Estimate of Drug-likeness (QED) and Lipinski's Rule of Five. The QED score quantitatively assesses how "drug-like" a molecule is based on a composite of physico-chemical properties. At the same time, Lipinski's rules provide guidelines (e.g., molecular weight  $< 500$  Da,  $\leq 5$  hydrogen bond donors,  $\leq 10$  hydrogen bond acceptors, and  $\log P \leq 5$ ) to gauge its oral bioavailability.

To further refine the selection, a supervised classification model is used to predict whether the newly generated LNP candidates are more optimized. This classifier integrates QED, Lipinski features, and additional molecular descriptors as inputs, enabling high-confidence predictions about the therapeutic viability of the generated molecules. The supervised classification model implemented a Random Forest Regressor, trained with built-in QED and Lipinski features for biomolecules and aggregates. The model received a 93% accuracy. This multilayered filtering process ensures that only chemically sound, biologically relevant, and pharmaceutically promising LNPs proceed to experimental validation.

### Significance and Limitations:

This study advances the field by integrating spatial transcriptomics, graph neural networks, and variational autoencoding to create a unified pipeline capable of predicting metastatic risk and generating optimized siRNA-loaded lipid nanoparticles. Previous spatial transcriptomics studies mainly characterized tumor microenvironments,<sup>16,18</sup> but few have connected spatial gene expression with actionable therapeutic design. By leveraging spatially-aware ML, this work identifies high-risk metastatic regions directly from the primary tumor—addressing a major clinical gap, as metastasis confirmation typically requires invasive secondary-site biopsies.<sup>13</sup> The model also demonstrates strong performance when validated against TCGA and GEO datasets, supporting its biological robustness. Furthermore, coupling metastasis profiling with AI-driven nanoparticle optimization introduces a new computational framework for targeted RNA therapeutics.

However, several limitations must be acknowledged. The first includes the reliance on Visium's spot-level resolution, which restricts single-cell interpretability. The absence of wet-lab validation prevents full confirmation of metastatic behavior or LNP performance. The Metastasis Risk Score uses an arbitrary percentile threshold that may not generalize across tumor morphologies, and QED/Lipinski filters do not fully capture biological delivery constraints. Finally, proteomic, metabolic, and other biophysical factors could enhance metastasis qualities as well as transcriptomic signatures. Future work integrating multi-omic datasets, higher-resolution spatial platforms, and experimental validation will strengthen the translational potential of this framework.

### Conclusion

Metastasis remains the greatest barrier to effective cancer treatment, responsible for over 90% of cancer-related deaths due to its late detection, lack of targeted therapies, and rapid resistance to conventional treatments. This study presents

a first-of-its-kind computational framework that integrates AI-driven metastasis prediction with RNA-based precision therapy, addressing critical gaps in early detection and targeted intervention.

By leveraging spatial transcriptomics and machine learning, this work introduces an AI-driven metastasis prediction model that can identify high-risk tumor regions before they spread, offering a non-invasive alternative to traditional biopsy-based diagnostics. Beyond detection, this study pioneers the development of an AI-optimized lipid nanoparticle framework, designed to stabilize and deliver siRNA therapeutics to metastatic cells with high precision. In silico validation confirms that the optimized therapies exhibit heightened potential for efficacious delivery at precisely targeted locations, as historically verified.

This research bridges the gap between computational oncology and precision nanomedicine, showing that AI can not only predict metastasis but also guide the design of next-generation therapeutics to stop it at the molecular level. By replacing invasive biopsies with AI-driven spatial analysis and broad-spectrum chemotherapy with gene-targeted siRNA delivery, this study presents a transformative approach to treating metastatic cancer. Future advancements will focus on *in vitro/vivo* validation, expanded clinical applications, and further refinement of AI, paving the way for the real-world implementation of non-invasive, patient-specific metastasis therapies.

This work attempts to establish a new standard in cancer therapeutics, demonstrating that AI and RNA-based medicine can collaborate to detect, target, and treat metastasis with unprecedented precision, thereby pushing the boundaries of what is possible in oncology.

## ■ Acknowledgments

The results here are in whole or part based upon data generated by the TCGA and GEO Research Networks: <https://www.cancer.gov/ccg/research/genome-sequencing/tcga> and <https://www.ncbi.nlm.nih.gov/geo/>. Figures made with Canva, Python, and BioRender.

Thank you to Dr. Lara Shamieh for her mentorship and guidance throughout this research project.

Continuation from prior work done with unsupervised therapy optimization for lipid nanoparticles.

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# ASMR and Its Effect on Adolescence

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**ABSTRACT:** Autonomous Sensory Meridian Response (ASMR) is an emerging internet phenomenon characterized by tingling sensations and relaxation in response to specific auditory or audiovisual stimuli. While adolescents widely use ASMR for relaxation, sleep, and stress reduction, most research has focused on adults, leaving a gap in understanding its impact on developing brains. This review synthesizes current findings on ASMR's effects on adolescent mental health, sleep, and anxiety, highlights the neurobiological mechanisms involved, and discusses limitations in existing studies. The paper advocates for more empirical research to clarify ASMR's potential as a non-pharmacological intervention for adolescent insomnia, anxiety, and depression.

**KEYWORDS:** Behavioral and Social Sciences, Neuroscience, ASMR, Adolescents, Mental Health.

## ■ Introduction

Despite its increasing popularity, Autonomous Sensory Meridian Response (ASMR) remains understudied, mainly hindering our understanding of its potential as a valuable tool for promoting mental health and well-being. A search conducted on January 29th, 2025, yielded only 472 published articles involving ASMR-related research. While ASMR is currently trending and widely discussed in popular culture, the common experience of ASMR, such as the pleasure experienced by sounds such as rain tapping on the window or the satisfying sound of typing, has always been present. Autonomous Sensory Meridian Response (ASMR) combines positive feelings, relaxation, and a distinct tingling sensation on the skin, typically triggered by auditory or audiovisual stimuli such as tapping, whispering, or role-playing.<sup>1,2</sup> The term came to view after an internet trend in 2010, after being coined by YouTube's Jennifer Allen.<sup>3</sup> Since its popularization in 2010, ASMR has become especially prevalent among younger audiences, with platforms like YouTube and Spotify serving as primary access points. Specifically, Spotify is popular with younger generations, with 62% of monthly users between the ages of 18 and 34. The largest age group is between 18–24, making up 31.5% of users.<sup>4</sup> YouTubers specializing in ASMR have gained millions of subscribers (for example, Joyceful Tingles), a testament to how popular this phenomenon has become. Along with its rise in popularity, recent research on ASMR suggests that it may be a novel tool to improve mental health, especially among the youth who are most likely to be exposed to ASMR and interact with it as it becomes increasingly popular on the internet. Despite its popularity, research on ASMR's effects in adolescents remains limited, with most studies focusing on adults. Adolescents, whose brains are still developing—particularly the prefrontal cortex—may be more sensitive to ASMR's effects, making it crucial to investigate its potential benefits and applications for this age group.

## *Goals of this Review Paper:*

The number of studies related to ASMR continues to rise, yet little is known about the relative impact of ASMR on adolescents. Deepening our understanding of the effects of ASMR on adolescents will allow for a clearer understanding of its effects on adolescents during development. Given the evidence of differences in youth's key sensory, emotional, and cognitive processes compared to adults, adolescents can be predicted to have a higher sensitivity to ASMR. The heightened reactivity of their sensory systems, as well as their development of the prefrontal cortex, potentially makes adolescents more sensitive to ASMR's stimulative effects. With its calming auditory and audiovisual stimuli, ASMR is an effective intervention to promote relaxation and enhance sleep quality, especially benefiting the youth. The possibility of using ASMR as a sleep aid for adolescents and using ASMR relaxation as an intervention for anxiety disorders has already been explored.<sup>5,6</sup> However, this research is still in its early stages.

This paper aims to advocate the essential need for more empirical research to determine the extent to which ASMR could be used to understand ways in which this phenomenon could be used as a temporary cure for insomnia, anxiety, and depression among adolescents. To do this, this paper will start by discussing how ASMR affects explicit areas of the brain and then discuss its effect on adolescents as a sleep aid that could combat insomnia and relaxation, which could combat anxiety.

## *Status of Adolescent Mental Health:*

Adolescent mental health is a growing concern, with the World Health Organization reporting that 14% of teenagers experience mental health problems.<sup>7</sup>

Rates of anxiety and depression have increased significantly in recent decades, and insomnia affects nearly a quarter of adolescents. Compared to 1990, the number of individuals with anxiety disorders increased by 18.42% by 2019.<sup>8</sup> Although not globally, a study conducted on 167,783 adolescents aged 12–17 in the United States of America had increased rates of depres-

sion, with 8.1% in 2009 compared to 15.8% in 2019.<sup>9</sup> Studies have shown that the number of adolescents with insomnia is as high 23.8% in some countries.<sup>10</sup>

### ***ASMR's Correlation with Mental Health:***

With ASMR's relaxation effects, how it could be used as a guide for some mental illnesses has sparked debate. A study conducted by Barratt and Davis in 2015 explores the extent to which ASMR can ease the symptoms of depression and chronic pain. This study gathered 475 participants, who were given a questionnaire to fill out and asked questions related to ASMR. When the participants were asked the reason why they engaged with ASMR, 70% agreed that it was "to deal with stress." 69% of the participants who scored moderate to severe on the BDI (Beck Depression Index) measuring the participants' mood (minimal: 0-13, mild: 14-19, moderate: 20-28, and severe: 29-63), with their reported scores improving with a mean of 38.75, in comparison to the participants who scored minimal or mild with an average improvement of 21.33, when listening to ASMR showing that ASMR eased their symptoms of depression.

Many efforts are effective when dealing with mental health, specifically, mental health medications. However, these methods are not always the preferred choice for everyone, as there are side effects to medication.<sup>11</sup> This includes as having drowsiness, restlessness, muscle spasms, tremor, dry mouth or blurring of vision for antipsychotics to treat psychosis or schizophrenia, constipation, blurred vision, weight gain for atypical antipsychotics also used to treat psychosis or schizophrenia, as well as impaired coordination, memory impairment, and drowsiness for benzodiazepines used to treat anxiety disorders, panic attacks, and phobias.<sup>12</sup> Traditional treatments, such as medication, often have undesirable side effects, highlighting the need for alternative interventions.

### ***The Adolescent Mind:***

Adolescence is a critical period of brain development marked by major changes in sensory processing and emotional regulation. It is often considered "a time of hypersensitivity to both appetitive and aversive emotional experiences."<sup>13</sup> During adolescence, the prefrontal cortex is still in development. This brain region controls our ability to regulate cognitive and emotional responses. In addition, other research has found differences in visual information processing in the striate and extrastriate cortices of youth compared to adults.<sup>14</sup> In a study that aimed to learn if adolescents would respond to high-stress or low-stress risk-taking, it was found that adolescent male decision-making under stress can lead to different behaviors compared to adults, which could be interpreted as them being more sensitive.<sup>15</sup>

One of the most important areas of development during this time is the prefrontal cortex (PFC), and within that is the medial prefrontal cortex (mPFC), which plays a crucial role in higher-order cognitive functions, including decision-making and emotional regulation. This brain region continues to mature well into the early 20s, meaning adolescents face difficulty in controlling their emotions and responses to sensory stimuli

compared to adults. This is due to the mPFC being more sensitive as it needs to absorb a larger amounts of information. Adolescence is also where there is increased sensitivity to emotional stimuli, often driven by heightened amygdala activity, which can lead to a more intense response to external triggers, including auditory and visual stimuli.<sup>17</sup>

## **■ Results and Discussion**

### ***Section 1: Neurobiological Effects of ASMR:***

As discussed before, ASMR (Autonomous Sensory Meridian Response) is a physiological phenomenon that is described as a "combination of positive feelings, relaxation, and a distinct, static-like tingling sensation on the skin."<sup>2</sup> The feeling of relaxation and pleasant tingling result from an ASMR intervention. Another study conducted in 2018 by Bryson C. Lochte does a comprehensive job of identifying what these pleasant tingling sensations and relaxation are and how they're created. The study had a simple design of 10 participants being put in an fMRI machine with 3 buttons each for a baseline, relaxing, or tingling sensation while watching 5 7-minute clips of ASMR they felt was the most stimulating for them. The study found that the feeling of "tingles" typically lasted about 5.9% of the whole ASMR experience and that they are the activation of the NAcc (nucleus accumbens), the mPFC (medial prefrontal cortex), and the insula.

ASMR triggers activation in brain regions associated with reward and relaxation, including the nucleus accumbens (NAcc), medial prefrontal cortex (mPFC), and insula.<sup>18</sup> During this experiment, the right and left Nacc showed significantly more positive action when participants felt tingle sensations than baseline or relaxing sensations. When participants experienced relaxing moments, activation appeared in the mPFC. Relaxation was found to be the most prevalent sensation during this study, showing that tingling sensations during ASMR are linked to increased activity in these areas, with relaxation being the most reported effect.<sup>18</sup>

Activation in the mPFC was small but significant during the relaxing moments compared to baseline moments. Relaxation occurs as oxytocin binds to receptors in the mPFC, meaning that with the activation of the mPFC during ASMR, there is a greater potential contribution of oxytocin to produce the relaxing sensation during ASMR.<sup>18</sup> In this study, the mPFC was mentioned several times; however, the ages of the participants are not specified, so we cannot know how the results would differ if it were conducted on different age cohorts individually. The mPFC, which continues to develop into early adulthood, is particularly relevant for adolescents, who may experience heightened sensitivity to ASMR due to ongoing brain maturation.<sup>16</sup>

### ***Section 2: ASMR as a Sleep Aid:***

Lack of sleep has become all too common among teenagers, and ASMR, as a tool, has great potential as a possible solution to this problem. Sleep deprivation is common among adolescents, often due to academic pressures and lifestyle factors.<sup>5</sup> Sleep deprivation can impact their cognitive functions, emo-

tional regulation, and overall well-being.<sup>19</sup> A large percentage of 23.8% of adolescents suffer from insomnia.<sup>10</sup>

ASMR has been shown to improve sleep quality, with studies indicating that regular ASMR sessions before bed can reduce sleep onset latency and increase effective sleep duration.<sup>20</sup> Many apps have been developed based on ASMR to improve sleep quality. These apps, such as Sleepmaker Rain1 and Sleepa, simulate sounds like rain against the window or wind to relax the user.<sup>21</sup> This is just one example of how ASMR has been used to improve sleep quality. ASMR has been proven to be a sleeping aid and even a temporary solution for sleeping problems such as insomnia.<sup>5</sup>

Much research has been conducted to show that ASMR can facilitate relaxation in adults.<sup>22</sup> Many teens use ASMR to relax before going to bed, smoothing the transition from wakefulness to sleep. The triggers, such as gentle whispering, tapping, or nature sounds, can create the feeling of “tingles,” which is a pleasant shivering feeling traveling down the spine.<sup>23</sup> Furthermore, as ASMR is a tool that can be controlled, adolescents can select when they would like to listen to ASMR, making it a voluntary activity and enhancing its effectiveness as a sleep aid. This personalization may make ASMR more appealing than traditional sleep aids,<sup>1</sup> as it allows users to curate their experience according to their preferences, ensuring that these adolescents feel safe, as they would have the power to control the duration, as well as the triggers of ASMR they are experiencing.

For example, a study involving high school students in China found that 20-minute ASMR interventions significantly improved sleep quality, suggesting that ASMR could serve as a non-pharmacological aid for adolescent insomnia. This was a 5-day study conducted by Z. Wu, thoroughly investigating the effect ASMR has on sleep quality. In this study, 60 participants, with an even number of female and male participants aged 15-19, were put into four groups, each experiencing a different duration of ASMR intervention sessions before sleep. Group A was the control group, Group B had an ASMR intervention session for 10 minutes, Group C for 20 minutes, and Group D for 30 minutes. In this study, after each group watched non-dramaturgic ASMR for the allocated time of each group, the participants slept. The participants' sleep quality was measured using the Pittsburgh Sleep Quality Index and a mobile phone app to gather data on sleep patterns; objective sleep duration, effective sleep time, and overall sleep quality.

The results indicated that the number of days of ASMR interventions influenced the timing of objective sleep onset; sleep onset is the time it takes to go to sleep, meaning that the longer the participants experienced the ASMR intervention, the earlier the sleep onset. However, it did not significantly impact the duration of sleep. The duration of ASMR intervention significantly impacted effective sleep duration, with Groups A and C resulting in significant increases in effective sleep duration compared to Group B, suggesting that 20 minutes of ASMR is beneficial to increase effective sleep duration. Although not confirmed in *post hoc* analysis, ANOVA analysis suggested that the duration of ASMR intervention influenced subjective sleep quality. These results show that there was a

“significant association between ASMR and improved sleep quality among high school students.” ASMR is a proven tool that could help adolescents sleep. Given the prevalence of insomnia in adolescents, this study gives hope that ASMR is a tool that should be considered to be used as a potential solution, as an alternate way to a pharmacological way of treating insomnia.

### ***Section 3: ASMR for Relaxation and Anxiety Reduction:***

ASMR's potential as a relaxation tool extends to anxiety reduction.<sup>6</sup> ASMR is suggested to be a relaxational tool that could be used as a potential mood lifter for depression.<sup>2</sup> Studies have demonstrated that ASMR can lower anxiety levels in teenagers, as measured by standardized scales such as the Hamilton Anxiety Rating Scale (HARS).<sup>6</sup> Synesthesia is the production of a sense impression relating to one sense or part of the body by stimulation of another sense or part of the body.<sup>24</sup> Similar to other phenomena, such as synesthesia that can be experienced through auditory stimuli, the tingling sensation felt is similar to the relaxation experienced when listening to music, for example, classical music.<sup>1,2,22</sup> The difference between ASMR and this other sensory phenomenon is that while synesthesia can't be controlled, ASMR can be turned off by pausing the audio/video. The ability to personalize ASMR experiences and control exposure may enhance its appeal and effectiveness compared to traditional relaxation techniques.

ASMR allows listeners to escape for a brief moment because of its ability to engage individuals. One can focus on what is happening around them, and they can use their senses at the same time. In the previous Lochte study, significant brain activation in the mPFC was observed during ASMR.

This region of the brain is responsible for self-awareness, social cognition, and social behaviors, including grooming, and when the chemical oxytocin binds to receptors in the mPFC, it mediates relaxation responses. The activation of the mPFC during ASMR suggests that more oxytocin is released, resulting in relaxing sensations.

As mentioned before, the PFC does not develop in humans until early adulthood, meaning adolescents are more sensitive to reward than adults.<sup>16</sup> Adding to the statistics mentioned before, it is wise to consider the possible use of ASMR as a tool of relaxation and at least a temporary solution to anxiety in adolescents. This idea of ASMR is further reinforced in a study conducted by A. Firmansya, which explores the effect of ASMR on anxiety in teenagers, showing how anxiety is not only used for relaxation but also as a remedy for mental health issues. This study explores this idea by collecting 17 adolescents (6 male, 11 female) participants from the city of Palembang, Indonesia, and testing anxiety levels before and after ASMR intervention, measuring their anxiety level based on HARS (The Hamilton Anxiety Rating Scale). Their results show that ASMR can allow teenagers who feel anxious to become calmer and more relaxed, reducing their anxiety levels.

#### **Section 4: Limitations:**

Although the studies discussed above help in highlighting and shedding light on this understudied area of research within ASMR, both papers have faults that should not be overlooked when referencing these papers.

The study by Wu *et al.* (2024) faces several limitations on the generalizability of the results. Due to the participants being from only 6 of the same schools in China, the results may differ if the participants in this study were from around the world, meaning that the study's results should not be applied globally. Furthermore, as the participants' sample size is only 60, the results have the possibility to significantly differ if the study were conducted on a larger scale, especially since the 60 people were broken into four groups, meaning there were only 15 people in each group.

To confirm the study's results and its generalizability, a recreation of this study on a larger scale with a larger diversity would be beneficial. However, this study also faces challenges with its ability to be recreated, as it misses some key information in its methods. First of all, although the study mentions that they used the Pittsburgh Sleep Quality Index to measure the participants' sleep quality, it does not mention the app they used to collect further data on sleep patterns, objective sleep duration, and effective sleep time. If this study were recreated, the same app would be used to get similar results, but this problem does not allow that. Furthermore, the study does not mention the participants' surroundings during the experiment. This study does not mention whether it was conducted within a lab or in the home of the participants. It doesn't mention the potential factors that could influence the time it takes for the participants to sleep, such as the participants' stress levels, the room temperature, or what specific time this experiment was conducted. We might get different results if the study were to be conducted in a more controlled environment of this study and yield more accurate results as to what would happen when interacting with ASMR before sleep.

The study done by Firmansyah *et al.* (2023) also has many limitations that affect its credibility. The first limitation of the study is its limited sample size, with only 17 participants. 6 of which are males, meaning there are about twice as many females in this study as males. The ratio is not very balanced, although it is important to mention that this fault is recognized in the discussion. A second issue is that this study does not have a diverse cast of participants, as all participants are from Palembang, Indonesia. Due to cultural differences, the stress of adolescents from Indonesia would differ from the stress of those who live in other parts of the world. These results cannot be applied to the global population and are only an analysis of what ASMR can do for the adolescents of Palembang, Indonesia, alone.

A recreation of this study with a larger sample size, wider diversity, and a more balanced ratio of females to males would further allow us to see the effect of ASMR on anxiety in all adolescents. The study's major limitation is its inability to be recreated as a study. It fails to mention the longevity of these ASMR interventions, failing to mention any controls or individual factors. This study only describes the individual factor

as an intervention and fails to mention the conditions of the participants, the participants' surroundings, and how long these participants were exposed to ASMR. We do not know how many times this study was repeated for the duration of the whole experiment. Even with these faults, this study helps as a guide and introduction to show the potential of ASMR in helping adolescents relax and even overcome anxiety. More studies should be conducted to truly understand the beneficial aspects of ASMR being used as a relaxational tool for teenagers.

#### **Section 5: Future Directions:**

ASMR has the potential to become a solution to a few mental health problems. Even though the benefits of ASMR are becoming more widely recognized, there is still a research gap that focuses exclusively on how it affects teenagers. Knowing how ASMR affects this group's emotional and cognitive health is essential because they are especially susceptible to mental health problems, as seen in the statistics. Future research should examine how ASMR affects adolescents' anxiety, sleep patterns, and general mental health over the long run.

The studies that this paper has reviewed provide beneficial insight into how adolescents could use ASMR to deal with some mental health issues. Despite this, the limitations of these studies leave much to be desired. In both studies, the sample is too small and is not diverse enough; there is a need for a large-scale study of the effect of ASMR on adolescents to see if the application of the results of the previous studies is only local or global.

Existing studies on ASMR and adolescents are limited by small, geographically homogenous samples and a lack of methodological detail, which restricts generalizability. Many studies do not account for individual differences such as personality traits, cultural background, or exposure to relaxation techniques, all of which may influence ASMR responsiveness. Larger, more diverse studies are needed to confirm current findings and explore the long-term effects of ASMR on adolescent mental health. Trait-based variables can influence the "need for cognition(NCS)" and "need to evaluate(NES)." The hypothesis is that one's NCS and NES may change how receptive an individual is to ASMR. The lower one's need to evaluate or need for cognition, the more likely they may be to not downregulate their physiological response to ASMR. Recognizing these factors would be a huge step in guiding the advancements in understanding ASMR.

#### **Potential Future Research:**

Future research should investigate the impact of ASMR on adolescents with different personality traits, such as introversion and extroversion, and explore the role of parasocial relationships with ASMR content creators. Adolescence is a critical period for brain development, particularly the pre-frontal cortex, and social relationships significantly influence this development. Introverted adolescents, who typically have fewer friendships, may be more inclined to form parasocial relationships with ASMRtists (content creators specializing in ASMR) than their extroverted counterparts, who often main-

tain a larger social circle. This study could provide valuable insights into how personality influences the perception and experience of ASMR in young people.

Another possibility to consider with this study is that extroverts who have few friends would be more inclined to form parasocial relations with ASMRtists instead of introverts, who may feel less need to feel these parasocial connections. Introverts tend to feel more energized when doing activities by themselves or in a smaller group, while extroverts feel energized when engaging with a large number of people. Introverts are more likely to be able to relax in a solitary environment, while extroverts are more likely to relax surrounded by many people. ASMR is an activity to relax. Extroverts with fewer friends, who may not meet their desire to socialize, may be more sensitive to building parasocial relationships with ASMRtists, and introverts with few friends who might not feel the desire to socialize may be less sensitive to building parasocial relationships with ASMRtists.

This study not only provides valuable insight into how ASMR can influence young people, but it also provides valuable insight into how parasocial relationships are built with one-sided intentions.

#### *Potential Future Mental Health Applications:*

Although ASMR is already a tool that can be accessed easily, these easily accessible ASMRs are not designed to treat mental health issues within adolescents specifically. There is also potential for developing ASMR-based interventions, such as personalized apps or school-based relaxation programs, to support adolescent mental health and well-being.

A way in which ASMR could be utilized is with an app that is designed to track sleep and health, whilst using ASMR to calm the user. Repetitive use of ASMR before bed has already been proven to be a factor that influences sleep onset; an app that allows the user to customize and personalize their own ASMR playlist to play before sleep onset would allow ASMR to be used as a sleep aid by adolescents, who struggle with sleep. Keeping track of sleep would allow them to see how their sleep quality improves as they use ASMR before bed. An app that acts as an intelligent assistant that schedules and customizes a time dedicated to relaxation would allow for better sleep and, therefore, better mental health.

Another way ASMR can be integrated into adolescents' daily lives is through the educational institution they attend. Educational institutions should consider implementing a 10-minute break during school hours to play ASMR to refresh and reduce cognitive workload, which can be measured with heart rate variability. High heart rate variability lowers mortality rates, while low heart rate variability increases mortality rates. An initiation to increase heart rate variability would positively influence adolescents and their health.

## ■ Conclusion

ASMR is a new topic in that it fosters a way for adolescents to deal with their mental health problems, making it an important area of research to study. ASMR is a promising tool for promoting relaxation, improving sleep, and reducing ad-

olescent anxiety. While preliminary studies suggest beneficial effects, more rigorous, large-scale research is needed to establish its efficacy and inform practical applications. ASMR's accessibility and low risk profile make it an attractive option for adolescents seeking alternatives to traditional mental health interventions. These studies give hope for the future of ASMR and its benefits, highlighting the need for further study of its potential positive impacts on the mental health of adolescents.

## ■ Acknowledgments

The author thanks Dr. Kimberly Clark for mentorship and guidance throughout the preparation of this review.

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# Identifying Mental Health Indicators from Social Media Activity Using AI

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**ABSTRACT:** Mental health challenges among youth have become a pressing issue, with rising rates of stress, anxiety, and depression highlighting the need for early detection and support systems. The purpose of this study is to explore the use of AI in identifying mental health signals among youth based on data collected from social media posts. Accordingly, a mental health dataset from Kaggle is used to train a Naïve Bayes classifier that categorizes social media posts into various mental health conditions. The results showed that the Naïve Bayes classifier was able to predict various mental health conditions with an accuracy of 64%, which is an encouraging baseline.

**KEYWORDS:** Computer Science, Artificial Intelligence, Naive Bayes, Machine Learning, Mental Health.

## ■ Introduction

Mental health is an ongoing crisis in our world. Every year, over 49,000 people die from suicide, equivalent to one death every 11 minutes.<sup>1</sup> Factors such as stress from school, family pressure, and peer expectations often contribute to these tragedies. In addition, issues like depression and anxiety are becoming increasingly severe, especially among young people. A study by the Centers for Disease Control and Prevention found that from 2009 to 2019, the percentage of high school students who reported persistent feelings of sadness or hopelessness rose from 26% to 37%,<sup>2</sup> showing a clear increase in stress signals among adolescents.

Consequently, it is important to understand the categories used to classify youth sentiment. In this study, a dataset with seven mental health categories (normal, depression, anxiety, suicide, self-harm, stress, and bipolar) was used. These categories reflect different emotional states that can be identified from text-based social media posts. Fortunately, many open-source datasets, such as those available on Kaggle, contain real-world examples labeled for these conditions. These labeled datasets allow researchers to train Artificial Intelligence (AI) models with the labeled data and help us uncover if there is any pattern between social media posts and mental conditions.

As part of this research, AI and Machine Learning (ML) models are used to classify data from the datasets and recognize emotional patterns. In particular, a key technique called Natural Language Processing (NLP), which allows machines to analyze text from forums, social media, or survey responses written by youth, is used. Natural Language Processing (NLP) is a field of computer science and AI concerned with the interaction between computers and human language.<sup>3</sup> Its primary goal is to endow machines with human-like abilities to understand, interpret, and produce natural language.

NLP was primarily driven by a rule-based system when it came to life in the 1950s, where experts wrote detailed grammar rules to teach computers how language works.<sup>3</sup> However,

rule-based systems have limitations (as highlighted in sections below), which paved the way for more sophisticated approaches. Over time, NLP evolved to use statistical methods and machine learning, allowing models to learn patterns from real-world data instead of relying only on fixed rules. Today, advanced NLP models can understand not just words, but also tone, context, and emotion, making them powerful tools for mental health analysis. As a result, it's critical to use technology like AI to detect sudden changes in sentiment, especially signs of self-harm, suicidal thoughts, or bullying, so that schools and mental health professionals can intervene effectively.

The motivation of this research comes from the fact that the author is part of and a witness to a competitive high school environment, where students often face pressure and are judged by academic performance, which impacts youth mental health. At the same time, the prevalence of AI/ML encourages the author to leverage this technology to improve the well-being of students in similar communities, while allowing building expertise in the area.

## ■ Methods

Given that this was the author's first foray into AI/ML, the primary focus was on learning the base concepts that are applicable in every AI/ML project before advancing to the more pointed research related to sentiment analysis. A key tip that also helped was implementing everything from scratch before using off-the-shelf libraries. Libraries are fast and easy to use, but building a model from scratch helps one understand the math and code that goes into making an ML model. Understanding the implementation under the hood is the best way to advance our learning in this field. This work leverages the NumPy library in Python,<sup>4</sup> for matrix calculations, and the Pandas library,<sup>5</sup> for handling datasets in the form of data frames.

**Understanding AI/ML Concepts:**

To start, the focus was on understanding the core idea behind ML: training a model to recognize patterns in data and make predictions. The two ideas that underpin most of the classical ML approaches are:

- Feature extraction: identifying measurable properties (like color/shape in fruit, or words/sentiment in text).
- Model training: using data to find a function  $f(x)$  that best separates the categories.

The above foundational exercise helped visualize how an AI model learns and generalizes from examples. This video also helped in the process of learning.<sup>6</sup>

**Probability and the Naïve Bayes Approach:**

Once the basics of classification were understood, a probabilistic algorithm, Naïve Bayes, which relies on conditional probability and the assumption that features are independent, was explored. This concept is important because the study would later dive into the use of Naïve Bayes to classify mental health posts. To use the Naïve Bayes approach, the following terms need to be computed:

- Prior probabilities represent how frequently each mental health category, such as “anxiety” or “depression,” occurs in the dataset.
- Likelihoods represent how often each word, such as “lonely” or “sad,” appears in a mental health category.
- Posterior probabilities (prediction probabilities) based on Bayes’ Theorem represent the final probability of how strongly a word falls under a certain mental health category after combining the likelihood and prior probability.

Understanding how to compute prior probabilities and likelihoods is the basis of the model, because the posterior probability (prior + likelihood) is the value that will be used to classify the sentence into one of the seven classes of the dataset.

This video cohesively explains how Naïve Bayes works.<sup>7</sup>

**Learning Text Preprocessing & NLP Techniques:**

Since the study involved analyzing text data, a concerted effort was made to dive into the details of Natural Language Processing (NLP). This included:

- Tokenization: breaking text into words.
- Stemming & Lemmatization: reducing words to their root forms.
- Count Vectorizer: converting words into numerical vectors for use in models.

This step was critical because computers cannot understand raw language—they need a numerical representation to process it. This process of creating a Bag-of-Words demonstrated how the conversion from the words in the dataset transformed into a numerical format, which is processable by a machine learning model.

**Exploring the Dataset:**

A labeled mental health dataset obtained from Kaggle was analyzed.<sup>9</sup> This dataset comprised seven emotional categories:

normal, depression, anxiety, suicide, self-harm, stress, and bipolar. The following dataset analysis was done:

- Class distribution (how many examples per class),
- Biases in the data, and
- Label definitions to understand the kind of language used in each mental state.

Understanding the dataset made it possible to determine the most effective preprocessing and modeling steps. Additionally, it is important to observe any biases in the data (less representation of one class over another) to explain why the model may not provide the most accurate representation of that specific sentiment, since the dataset is skewed (more details in the Discussion section). For more information on datasets: visit.<sup>10</sup>

**Building the Model – From Scratch:**

Then, a custom Naïve Bayes classifier was built from scratch, using NumPy to perform calculations. This hands-on implementation helped with understanding:

- How priors and likelihoods are stored and calculated,
- How predictions are made based on log probabilities,
- And how edge cases (like zero counts) are handled using techniques like Laplace smoothing.

This provided full control over every part of the model and facilitated a deeper understanding of how real algorithms operate under the hood. More information on Laplace smoothing.<sup>11</sup>

**Implementing the Library Model:**

To verify the correctness of the implementation, the scikit-learn library was used for comparison.<sup>12</sup> In addition, Scikit-Learn contains optimizations that provide a boost in the accuracy metrics. As is the good practice, the Scikit-Learn implementation of Naïve Bayes was leveraged for the project. Though the library version was faster, building a custom model helped develop a deeper understanding of the model-building workflow.

**Evaluating Performance:**

To measure how well the models performed, the following metrics were computed:

- Confusion matrices to see where the model was getting predictions right or wrong,
- Accuracy, precision, recall, and F1-score to evaluate performance from different angles.

These metrics gave insight into which emotional states were harder to detect and where the model needed improvement. Understanding the relationship between these metrics helped with understanding where the model was performing better and where it was performing worse.

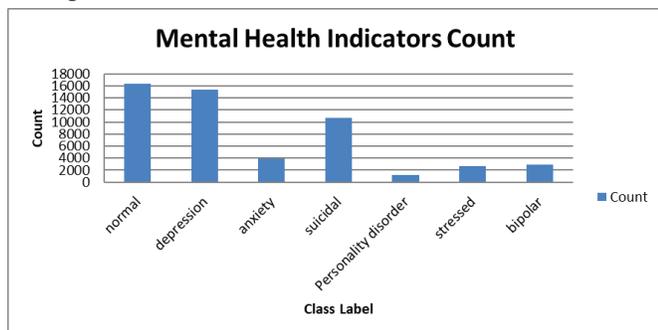
To get a better understanding of CMs (Confusion matrices) and metrics.<sup>13</sup>

**Dataset Details:****Source:**

The data set used in this study was obtained from Kaggle’s Sentiment Analysis for Mental Health.<sup>9</sup> It contains labeled social media posts aimed at detecting and classifying mental

health conditions. Its variety of mental health labels indicates a multi-class classification problem, making it suitable for Naive Bayes classification. The dataset is publicly available and licensed for research purposes.

#### Target Labels:



**Figure 1:** The bar chart shows the dataset is slightly imbalanced, with normal and depression being the most frequent classes.

#### Example Samples:

**Table 1:** Example mental health text samples.

Text Sample	Label
"Because this worries you."	Anxiety
"You're an addict that makes me doubt."	Normal

#### Preprocessing Steps:

All text was converted to lowercase, punctuation and special symbols were removed, and URLs were stripped out (Table 1). Tokenization was performed using Python's NLTK library, and stop words were removed to reduce noise. Lemmatization was applied to convert words to their root forms.

#### Custom Naive Bayes Implementation:

##### Priors/Likelihoods:

The custom Naive Bayes classifier was implemented from scratch to allow full control over preprocessing, feature extraction, probability computation, and learning purposes. The process began with preprocessing, which included lemmatization, stemming, stop word removal, punctuation removal, and conversion to lowercase. A Bag of Words representation was generated using a count vectorizer, transforming each text sample into a vector of word frequencies. These frequencies serve as the foundation for the Naive Bayes probability calculations. All this code can be found in the references section.<sup>14</sup>

Naive Bayes assumes independence of each word given the class label, allowing the likelihood of a text sample to be expressed as the product of the likelihoods (the probability of each word occurring given the class label) of all individual words. This likelihood is represented as  $P(w/C)$ . The class prior probability,  $P(C)$ , was computed by taking the ratio of the number of text samples in each class to the total number of text samples across all classes. This prior probability provides insight into how frequently each class occurs within the dataset. For example, the depression class occurs 29% of the time because 29% of the dataset is composed of depressive text samples.

The likelihood  $P(w/C)$  was calculated using Laplace smoothing to address zero-frequency terms. When computing the probability of a certain word occurring in a class, it is possible that the word does not appear in that class at all, resulting in a probability of zero. Since the overall likelihood is calculated by multiplying the probabilities of all words, a single zero would cause the entire product to become zero. This would discard valuable information from other words in the sample. To prevent this, a small constant (in this case, 1) is added to each count, ensuring that no probability is ever exactly zero.

The priors are calculated as follows:

$$P(C) = \frac{\text{Number of Text Samples in Class } C}{\text{Total Number of Text Samples}}$$

Laplace smoothing is included in the calculation of likelihoods as shown:

$$p(w | C) = \frac{\text{count}(w, C) + 1}{\sum_{j=1}^V \text{count}(w_j, C) + V}$$

where,

$w$  - a specific word we are calculating the probability for.

$w_j$  -  $j^{\text{th}}$  word in the entire vocabulary. Since vocabulary is a set, all the items are unique.

$C$  - class (category)

$\text{count}(w_j, C)$  - number of times the word ( $w_j$ ) appears in class  $C$ , and we sum this for every word in the vocabulary count given by  $V$ . Since Laplace smoothing adds 1 to every word's count, the total word count for the class increases by  $V$ .

##### Predictions:

Predictions with the custom Naive Bayes implementation rely on the combination of prior/likelihood probabilities to make up the posterior probability. We can loop through each of the classes and compute a likelihood by either using  $P(w/C)$  if the feature is present, or  $1 - P(w/C)$  if the feature is absent. By using the prior probability for that specific class, in combination with the likelihood, the posterior probability is appended to the array. Finally, by appending the highest probability from the posterior array into the predictions array, we can classify the text sample into one of the seven classes.

#### Scikit-learn Implementation:

##### Overview:

To benchmark the custom Naive Bayes model, scikit-learn's built-in Multinomial Naive Bayes was used. This model is designed for text data represented as word counts by using a count vectorizer. The model already includes built-in smoothing, making it a strong and fast baseline for sentiment classification. Although the author implemented a Custom Naive Bayes (for understanding), this work proceeded to leverage the Scikit-Learn library for the sake of robustness, as this is a well-tested and widely used implementation.

### Implementation Details:

The data was normalized by cleaning and preparing the text, including tokenization, converting to lowercase, stemming, stop word removal, lemmatization, and punctuation removal.

The train/test split function splits the dataset into training data and testing data based on user-provided splitting criteria. It's important that the model gets to see enough samples, so around 70% of the data was used for training. To evaluate the model's performance, the other 30% of the data that the model had never seen before (test data) was used. A random fixed seed was used so that every time the model is tested, it operates the same, mainly for reproducibility.

The vocabulary (unique set of all words in the dataset) was learned from the training set using a fitting function that maps each word to a unique index and transforms the text samples into matrices of word counts.

Test data transformation was completed, ensuring that no new words were added to the features. Tests are meant to be performed on new data, but still the same dataset and vocabulary that the model was trained with.

Training was done by using the training data and the training labels to fit the model. Lastly, Laplace smoothing (default  $\alpha=1.0$ ) was incorporated to handle zero-frequency words.

The trained model predicted labels for the test data, producing outputs for each of the seven classes in the dataset.

### Evaluation Metrics:

Accuracy, precision, recall, F1-score, and confusion matrix were chosen as the evaluation metrics for the proposed approach. By using `sklearn.metrics` to import `classification_report`, the full rundown of these metrics was obtained.

**Accuracy.** The share of correctly classified objects in the total number of objects (citation). In other words, it shows how often the model is right overall. In this mental health study, accuracy is calculated as the proportion of times the predicted labels ( $y_{pred}$ ) match the true labels ( $y_{test}$ ). Accuracy provides a quick baseline as it is easy to compute and understand; it tells you at a glance how well your model is performing overall. Accuracy works best with balanced datasets. However, in this Kaggle dataset, the data is slightly imbalanced, as shown in Section 3.2, where the "Structure" graph illustrates a skewed class distribution. Accuracy can also be quite misleading. For example, if 70% of the posts are classified as "normal" and the model predicts "normal" for everything, you'd get a 70% accuracy just by the model making a blind guess. For this reason, it's important to take into account other metrics such as F-1 score, precision, and recall to get a holistic understanding of the model's performance.

**Precision.** Measures how many of the items predicted as a certain class are actually correct. It answers the question: *When the model says a post belongs to this class, how often is it right?* High precision means fewer false positives. In the context of mental health detection, a high precision for "suicidal" means the model rarely mislabels non-suicidal posts as suicidal, reducing unnecessary alerts.

**Recall-** Measures how many of the actual items from a class the model successfully identifies. It answers the question: Of

all the real examples of this class, how many did the model catch? High recall means fewer false negatives. For this study, high recall is crucial for severe conditions like "suicidal" or "depression," because missing these posts could have serious consequences.

**F1-score.** The harmonic mean of precision and recall balances the two metrics into a single score. It's particularly useful when the dataset is imbalanced, because it ensures that both precision and recall are considered. In this study, a strong F1-score for critical mental health classes means the model is identifying important posts and doing so accurately.

## Results

This section compares the performance of the Scikit-learn Naive Bayes (Scikit-learn NB) implementation with the Custom Naive Bayes (Custom NB) model. Both models were evaluated using accuracy, precision, recall, F1-score, and confusion matrices.

### Accuracy:

The accuracy of classification using the Naïve Bayes classifier was 64%, which indicates a promising baseline.

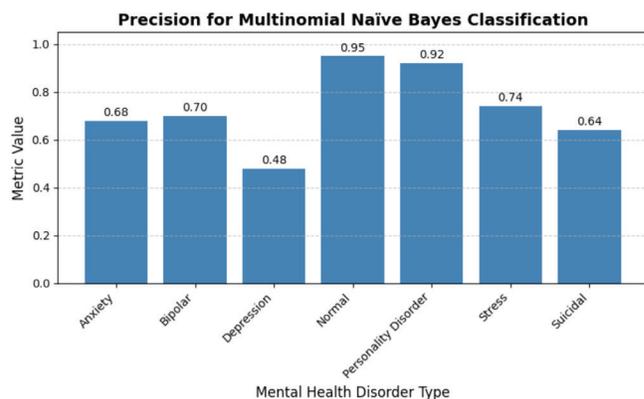
### Class-wise Metrics:

**Table 2:** Shows Class-wise Metrics for Scikit-learn.

Class	Multinomial Naive Bayes classification		
	Precision	Recall	F1
Anxiety	0.68	0.75	0.71
Bipolar	0.70	0.68	0.69
Depression	0.48	0.75	0.58
Normal	0.95	0.61	0.71
Personality Disorder	0.92	0.14	0.25
Stress	0.74	0.18	0.29
Suicidal	0.64	0.63	0.63

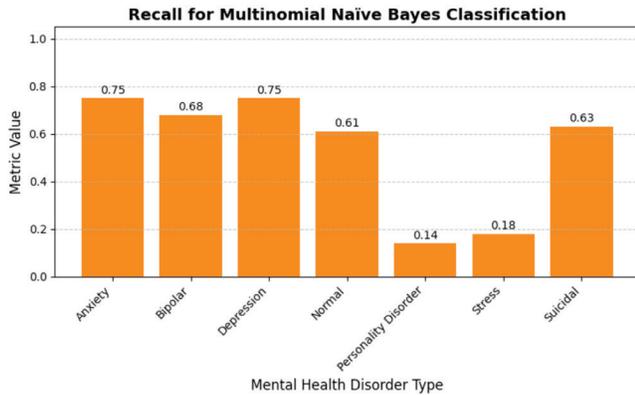
### Visual Comparison of Metrics:

*Model Precision for the different categories of mental health disorders:*



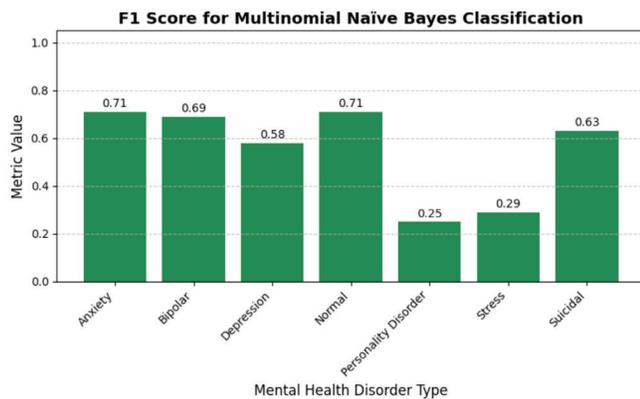
**Figure 2:** Bar chart showing the model Precision for various mental health disorders. Personality Disorder, Stress, and Bipolar Disorders have very less false positive results, as can be seen.

*Model Recall for the different categories of mental health disorders:*



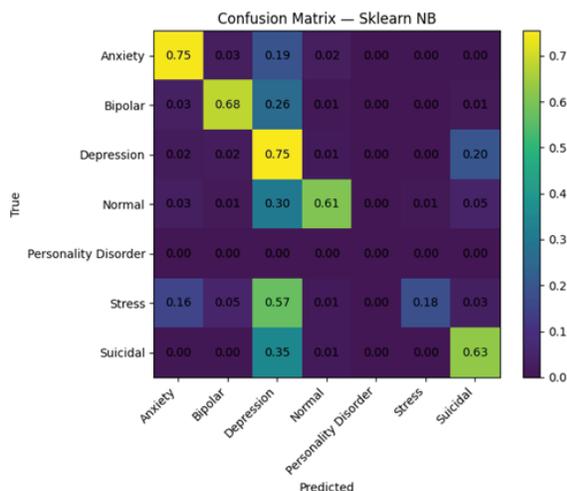
**Figure 3:** Bar Chart showing the model Recall for the various mental health disorders. Interesting to note that Personality Disorder & Stress have very poor Recall (unlike Precision), while Anxiety and Depression are detected with high Recall.

*F1-score (Balance of Precision and Recall):*



**Figure 4:** Bar Chart showing model F1-scores for the various mental health disorders. As can be seen, Anxiety and Bipolar disorders strike a good balance between Precision and Recall.

*Confusion Matrices:*



**Figure 5:** A Confusion Matrix that shows the correlation between the “True” Mental Health Disorders versus those predicted by Naive Bayes. “Anxiety” and “Depression” are the best predicted categories, whereas “Stress” and “Personality” disorders are very hard to predict from the chosen dataset.

*Conclusions:* The Scikit-learn Naive Bayes model achieves balanced performance across most classes, though misclassification occurs between related categories such as Anxiety and Depression (Figure 2). 19% of Anxious posts were classified as depressive, due to the similarity between the two categories.

*Conclusions:* The Custom NB model performed well in classifying Depression and Normal. However, the model struggled with classifying anxious posts, as 80% of the anxious posts were classified as depressive, similar to the similarities between the two categories. Additionally, more rare classes like bipolar got classified as depressive 96% of the time (Figure 3), highlighting that the Custom NB model struggles with rare classes.

*Interpreting the Confusion Matrix:*

These two matrices summarize how the two models’ predicted labels ( $y_{pred}$ ) against the true labels ( $y_{true}$ ) line up for each class.

- Diagonal values indicate correct predictions (the model guessed the right class)
- Off-diagonal values indicate mistakes (the model guessed the wrong class)

Bigger numbers in the wrong spots mean the model consistently mislabeled one class as another (Figure 5)

## ■ Discussion

*Remarks on Custom Implementation of Naive Bayes:*

*Pros of Naive Bayes (general):*

In general, Naive Bayes is fast, easy to implement, and useful for balanced datasets. It can also work well with small amounts of training data. Text data usually has thousands of features, and NB works well in this setting because it doesn’t get overwhelmed by large amounts of features.

*Cons of Naive Bayes (general):*

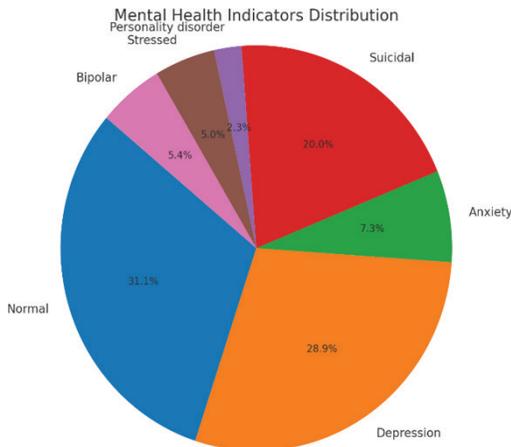
Naive Bayes assumes full independence of each word when computing probabilities. Usually, these mental health text samples are written in context, so assuming full independence of each word is not realistic. Additionally, classes that overlap each other (like stress vs anxiety) can be hard to distinguish. This is because these classes use similar words, so Naive Bayes won’t be able to tell the difference between these classes for the most part, leading to a lower F1-score (Figure 4). Lastly, if excessive nuanced language like sarcasm or slang is used, NB doesn’t adapt well.

*Limitations of Rule-Based Systems (NLP):*

Rule-based systems rely on fixed patterns, keywords, or handwritten rules to classify text. For this reason, these systems are rigid. They can work for specific cases, but they struggle when the language becomes complex, particularly when it involves slang, sarcasm, or new vocabulary. As shown in section 3.3’s example statements from the dataset, these mental health posts often use creative or emotional language, so a strict set of rules may struggle with identifying the meanings, which can lead to misclassification. Rule-based systems also require constant updating, which is not scalable for large or evolving datasets.

### Limitations of the Mental Health Kaggle Dataset:

The dataset itself also has limits. Many posts are short, unstructured, or written with spelling mistakes and slang, making it hard for models to understand them correctly. Also, a clear imbalance is shown with *bipolar* and *personality disorder* classes having fewer examples (Figure 6). This means that the model is less trained towards identifying these classes, which results in a low recall. Lastly, labels may not always be perfect, since posts were tagged by humans who could make mistakes or interpret them differently.



**Figure 6:** The pie chart illustrates the class imbalance present in the dataset. “Normal” and “Depression” are the majority classes, while “Personality Disorder” is a minority class, which is the reason why Naïve Bayes is unable to effectively model the probability distribution for this class.

### Conclusion

The main goal of the study was to explore the use of AI to measure early signs of mental health signals on social media platforms. To achieve this goal, a Naïve Bayes classifier was trained on a Kaggle dataset to classify social media posts into seven mental health categories: *depression*, *normal*, *anxiety*, *suicide*, *personality disorder*, *stressed*, and *bipolar* (Figure 1). The results show that the Scikit-learn implementation of Naïve Bayes achieves an accuracy of 64%. The study also highlighted limitations. The Naïve Bayes assumption of word independence makes it less effective for overlapping categories like anxiety and stress (Table 2). When a post is showing signs of anxiety, the model may classify the text as stressed since the two classes are similar and the model doesn’t use context clues to inform its decision. Additionally, the dataset itself was slightly imbalanced, with fewer examples for conditions such as personality disorder, which reduced recall for these classes. Short, informal, or slang-heavy social media posts also posed challenges for accurate classification. Future work could address these issues in several ways. First, collecting a higher-quality dataset with a more balanced representation across all mental health conditions would improve the overall metrics. Second, replacing simple count-based vectorization with TF-IDF could better capture the importance of words in context. Finally, expanding the label set to include more mental health categories could make the system more useful in real-world applications. Overall, this study demonstrates the potential of AI models in detecting mental health signals from social me-

dia. While Naïve Bayes has limitations, it serves as a strong foundation for beginners in machine learning and highlights both the positives and negatives of using AI for youth mental health support.

### Acknowledgments

The author would like to sincerely thank Mr. Phani Srikanth (Principal Applied Scientist, NetApp) for mentoring and providing invaluable feedback.

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### Author

11<sup>th</sup>-grade high school student, Riaan Kapoor, is aspiring to solve real-world problems through the use of cutting-edge technology.

# Underwater Drones: Advances in AUVs, ROVs, and Soft Robotics for Ocean Exploration

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**ABSTRACT:** The research paper examines the transformative role of the underwater drone. Including Autonomous Underwater Vehicles (AUVs) and Remotely Operated Vehicles (ROVs), in advancing ocean exploration. It examines the technological components driving these innovations, including propulsion systems, power sources, navigation tools, and underwater communication. A special focus is placed on the rise of soft robotics, highlighting their bio-inspired flexibility, low acoustic signature, and minimal ecological impact. The paper discusses how material selection, ranging from titanium to silicone elastomers, enables drones to withstand extreme underwater environments. Applications span marine biology, environmental monitoring, archaeology, and resource exploration, with examples from leading institutions like WHOI and Ocean Infinity. Challenges such as battery limitations, communication barriers, and pressure resistance are addressed alongside emerging trends like AI integration and swarm robotics. The study concludes by identifying the field's potential to inspire young interdisciplinary researchers, while emphasizing the vital role underwater drones play in protecting fragile ecosystems and expanding our understanding of the deep ocean.

**KEYWORDS:** Robotics and Intelligent Machines, Biomechanics, Autonomous Underwater Vehicles, Ocean Exploration, Soft Robotics.

## ■ Introduction

To understand the ocean, we need to study it and what it provides, as it serves as a life and regulatory resource for ocean resource management. There are multiple facets to the ocean we are exploring and experiencing, and experiences that would drive people to be motivated, sustainable stewards.<sup>1</sup> By exploring the ocean, we discover new resources, such as medicines, vaccines, food, and energy, that create sustainable thinking and creative ideas.<sup>2</sup> Participating in ocean discovery adds to our understanding of how environmental change (i.e., climate and weather) not only impacts the Earth, but also helps in understanding adaptation for natural disasters (i.e., earthquakes and tsunamis).<sup>3</sup> Science and technology have undergone radical shifts in the exploration and discovery of the ocean, enabling us to access and study the deep sea in a deeper, safer, and more precise manner than ever before.<sup>4</sup>

The devices being used today for ocean exploration are Autonomous Underwater Vehicles (AUVs) and Remotely Operated Vehicles (ROVs). AUVs, or autonomous underwater vehicles, are unmanned underwater vehicles that perform underwater surveys such as detecting and mapping underwater wrecks and obstacles. AUVs are classified as autonomous and do not require an operator, which does not surface prior to calculating the specific location of that vehicle for its data collection. Remotely operated vehicles, or ROVs, are unmanned underwater vehicles whose movements are completely tied to and connected to a host ship. ROVs can be directly controlled remotely and contain object recognition as one of their generic applications that acts as a substitute for diver assessments in situations where divers are not available or are not being performed due to safety concerns.<sup>5</sup>

**Table 1:** Differentiation between AUVs and ROVs applications.

Aspect	AUV (Autonomous Underwater Vehicle)	ROV (Remotely Operated Vehicle)
Control	Autonomous, pre-programmed	Remote-controlled via tether
Power	Battery-operated	Powered through cable from host ship
Communication	Limited, non-real-time	Real-time via tether
Mobility	Free-ranging	Range limited by cable
Main Applications	Mapping, surveying, data collection	Inspection, maintenance, object retrieval

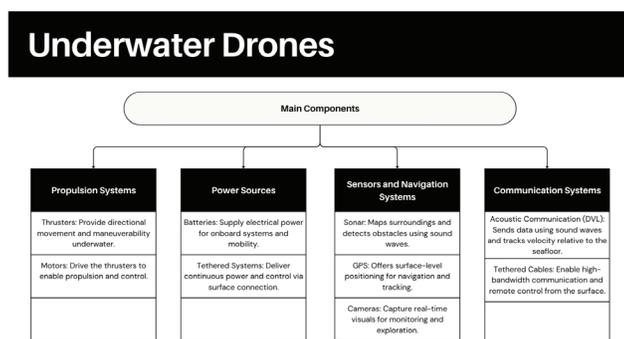
There have been tremendous advancements in underwater exploration technology over the years. In earlier decades, researchers built submersibles with reinforced hulls and ballast systems to achieve new ocean depths, such as the Bathyscaphe Trieste. When underwater science became more commonplace in the mid-1960s, sonar systems and underwater photography provided researchers with an unprecedented level of detail about the features and landforms of the ocean floor. In the late 1980s, remotely operated vehicles (ROVs) began to be widely used as well. ROVs were able to be outfitted with manipulator arms for property and sampling, video transmission, and a tethering system that enabled researchers to be miles away. Beginning in the early 2000s, autonomous underwater vehicles (AUVs) also began to be utilized. The value of the AUV was its pre-programmed autonomy on mission-based tasks dictated by GPS, inertial navigation, and onboard sensors. Today, the latest generation of underwater drones is as sophisticated and advanced as the terrestrial unmanned drones for land and aerial flights, as they are equipped with artificial intelligence, real-time data communication, and high-resolution, scaled 3D mapping. This technology represents a big evolution in deep-water research that makes for safer, more accurate, data-rich investigations.<sup>6</sup>

Drones are valuable tools in the marine environment because they can reach inaccessible places and depths without risking safety. There are many advantages to using drones, including: safety, data collection, cost effectiveness, use of space, environmental monitoring, and operations for search and rescue. In the marine environment, drones are a cost-effective and efficient means of exploration as opposed to fulfilling their goal of marine research, mapping of infrastructure, or environmental monitoring. Drones can capture high-definition images, sonar readings, and collect water samples, allowing for usable information to be located in the assessment of the ocean ecosystem and marine diversity.<sup>7</sup> Drones can support search and rescue operations and help increase situational awareness for emergency response teams during environmental emergencies by providing up-to-date information.<sup>8</sup> An example of a company is Ocean Infinity: a marine robotics company employing fleets of AUVs for deep sea mapping, subsea infrastructure inspection, and environmental monitoring. Ocean Infinity's "Armada" fleet has the ability to operate with a few crew members, and with controlled drone operations, safety is improved. Another company is WHOI (Woods Hole Oceanographic Institute): A global leader in research based on developing and deploying AUVs such as Sentry. WHOI has been involved in oceanographic exploration, climate research, and deep-sea missions, making a significant contribution to marine science.<sup>9</sup>

## ■ Result and Discussion

### *Technology Behind Underwater Drones:*

ROVs and AUVs are built to operate through different mechanisms for their intended functions. ROVs remain connected to a surface vessel, which supplies the ROV with continuous power and the ability to complete tasks, such as deep ocean sampling and inspection, in real-time.<sup>10</sup> AUVs operate independently of any support vessel and utilize pre-programmed instructions, together with onboard navigation systems, such as inertial navigation systems (INS) and Doppler velocity logs (DVL), to carry out their tasks autonomously. AUVs can operate in a manner to explore large areas, conduct mapping, and perform environmental monitoring projects. Although AUVs can travel and conduct successful projects in areas that may be cost-prohibitive for ROVs, their distinct advantages are the real-time control and human precision they can offer for a task.<sup>11</sup>



**Figure 1:** The main systems of an underwater drone and the components they consist of.

### *Components:*

To understand how an underwater drone works and its functioning, it is important to consider all of the components that make underwater navigation, data collection, and real-time control possible. Figure 1 briefly showcases the components in an underwater vehicle.<sup>12</sup>

**Propulsion System:** The propulsion system allows for the movement and control of underwater vehicles. Underwater vehicles contain thrusters that use electric motors that provide thrust to move in the water. Thrusters can be mounted in different configurations to achieve six degrees of motion (pitch, yaw, roll, transition in vertical, lateral, or longitudinal motion). Most thrusters use a brushless DC motor because of its advantages of being highly efficient, reliable, and long-lasting without brushes, thereby reducing friction and mechanical wear. They provide speed and torque control with great accuracy, while also being capable of operating smoothly and quietly at very low speeds. Motors are small and lightweight, providing lower heat, which enhances performance with limited space/thermal capacity, such as in electric vehicles, underwater robotics, and drones. More advanced drones have incorporated principles and systems for dynamic positioning, allowing the vehicle to almost hover and provide the accurate control needed for inspections or delicate tasks such as sampling. Dynamic positioning in underwater drones employs sensors (DVL, INS, acoustic systems) that observe position, movement, past position, etc. A control system processes the data collected from the sensors and controls the thrusters accordingly in a timely fashion to hold in a precise location and orientation, even with currents or turbulence present.<sup>12</sup>

**Power Source:** The power systems of underwater drones directly determine their operational range and operational time. Autonomous Underwater Vehicles (AUVs) are based on lithium-ion batteries. They typically use batteries as their power source due to lithium-ion batteries as their power source due to their high energy density, rechargeability, and light weight. Although battery management is critical for safety and sustainability, it is especially crucial for deep-sea operations, in these cases, due to high-pressure environments. Whereas Remotely Operated Vehicles (ROV) are, by definition, tethered power systems, this allows for a continuous power supply and continuous energy from a surface support vessel while tethered. The continuous power source allows for longer mission durations that have fewer limitations for the supplies onboard for the autopilots.<sup>12</sup>

**Sensors and Navigation Systems:** The accuracy of sensors and navigation approaches results in the efficacy of underwater drone missions. AUVs typically use an Inertial Navigation System (INS) for dead reckoning, which estimates position based on motion data. Due to accumulating errors (drift), it is common for INS to be aided by an independent sensor, such as Doppler Velocity Logs (DVL). The DVL measures velocity relative to the seafloor. AUV position fix is intermittent due to the inability of GPS signals to penetrate water. AUVs surface on a scheduled basis to acquire a GPS fix, permitting them to correct positional errors that occurred during submerged operation. For environmental mapping and situational awareness,

AUVs utilize sonar, including side-scan sonar to detect submerged objects and multi-beam sonar to map the seafloor in a high-resolution 3D format. AUVs are also equipped with integrated optical cameras and environmental sensors to monitor temperature, salinity, and depth. With images and environmental data collected in real-time, AUVs can be utilized in a range of applications, including scientific investigations and infrastructure inspections.<sup>12</sup>

**Communication Systems:** Underwater communication has unique difficulties. Acoustic communication systems (the most common type of system to use for data transmission), and in particular, a style of communication based on acoustic transmissions, or more specifically, underwater, is most common for data exchange, especially for AUVs, because the movement of radio/optical transmissions in water is limited. Acoustic communication systems support low-bandwidth, long-range communication. In contrast, ROVs work with tethered cables, encouraging high-speed, real-time transmission of video, sensor information, and control commands to and from surface operators.<sup>12</sup>

#### **Role of Soft Robotics in Underwater Drones:**

Soft robotics refers to the field of robotics that uses compliant materials to create machines that show locomotion that mimics a variety of real-life forms in nature. Soft robots can bend, twist, and deform because they can operate with gentler motions than stiff robots.<sup>13</sup>

With soft robotic underwater drones, soft robotics offers various benefits over traditional robotic designs, primarily in underwater manipulation, where the environment is hostile to grasping. Imagine a soft robotic arm or gripper that achieves effective, safe travel to fragile organisms, such as corals, jellyfish, or even starfish. Soft robotic arms can transform into variable shapes and can maintain a firm grip that does not harm other organisms under flowing water conditions.<sup>14</sup>

Considering the ocean's fluid and changing environment, soft robots with flexible parts have some advantages over traditional rigid robots with stiff parts. Soft robots can be less vulnerable to mechanical damage from impacting rocks and other debris. Their flexibility allows them to explore uncertain or dubious water. In addition, because the mechanical approaches used in soft robotics carry little noise, soft robots normally have low acoustic signatures, making them suitable for secret missions to collect biological information or military information. Incorporating soft robotics into underwater drones also highlights additional possibilities, giving drones more flexibility to adapt with an overall less negative impact on delicate ecosystems, while still enabling them to conduct difficult missions with little disturbance.<sup>15</sup>

Recent advancements in biomimetic soft robotics provide a snapshot of amazing technological evolution to mimic a natural organism in soft, flexible materials. It includes multiple features, flexible multi-degree-of-freedom actuators, and design inspiration from biological systems. The industry has transitioned from traditional synthetic rubber-making processes into new fabrication systems such as 3D printing of elastomers, mould casting, and thin-film layering. These methods

enable complex soft structures that resemble biological tissues in terms of compliance and flexibility. The range of actuation methods available has moved from hydraulic to electromechanical systems, and now to smart materials (electroactive polymers, shape memory alloys, and magnetic composites). The combination of high levels of design freedom with this selection of actuation methods means soft robots could provide smooth, fine, fluid three-dimensional motion similar to jellyfish, worms, and octopi. The vast array of possible applications for these soft robots has appeared on the market, including medical soft robots that can provide minimally invasive procedures; soft robots with sensors for marine life interactions; and adaptable systems for engineering capabilities in various industrial and operational approaches of the defense sector.<sup>16,17</sup>

#### **Materials Used in Underwater Drones:**

##### **Material Requirements:**

When designing underwater drones, material selection is important, especially given the environment they operate in while underwater. The challenges of underwater operation require these submersibles to meet quite tough performance requirements to function properly and remain safe. The primary requirements are:

**Pressure Resistance:** The deeper you go, the more the water pressure will increase exponentially. The materials used must be strong enough to withstand such great forces without bending, as you conduct exploration in the deep sea.<sup>18</sup>

**Corrosion Resistance:** Saltwater is extremely corrosive, and when a material is left for a while, it will erode. Costs need to be kept low and operational, so materials that can resist corrosion are important for carrying out any exploration or mission.<sup>18</sup>

**Durability in Extreme Temperatures:** Underwater drones often experience a wide range of temperature variation, particularly in the deep sea. The drones must be able to perform efficiently regardless of temperature fluctuation.<sup>18</sup>

##### **Commonly Used Materials:**

###### **Metals:**

**Aluminum Alloys:** Aluminum alloys tend to be one of the more popular materials used on a drone frame because of the unique strength-to-weight ratio and the corrosion-resistant qualities of aluminum. No, aluminum isn't the best material for the deepest dives, but an anodized aluminum structure will do well in the mid-depth situations.<sup>19</sup>

**Titanium:** Renowned for its incredible strength, plus its natural corrosion-resistant properties, titanium is the gold standard for deep-water operations. It is more expensive and difficult to fabricate and handle than aluminum, but it is unmatched for high-pressure, saltwater applications.<sup>19</sup>

###### **Plastics and Composites:**

**ABS Plastic:** Acrylonitrile Butadiene Styrene is lightweight, well-mouldable, and provides acceptable strength. It is found in the housings for typical underwater drones, allowing for variable operation at shallow to mid-depths. **Carbon Fiber Composites:** These are characterized by high tensile strength,

high rigidity, and very low weight. They are also very chemically stable, allowing for exposure over long periods of time, both in the long term and underwater. However, they are required to be combined into waterproof resins and sealed to prevent water intrusion.<sup>15</sup>

#### ***Flexible and Soft Materials:***

***Silicone Rubber:*** This is a commonly used material for soft robotic components because of its flexibility, resistance to chemicals, buoyancy, and its inert state, making it non-toxic to any animal species in the sea.<sup>18</sup>

***Elastomers:*** They can elongate and return to their original form. It is this extension, along with the strength of the elastic material, that lends itself to the design of actuators, soft-bodied designs, grippers, etc.<sup>18</sup>

***Benefits of Soft Materials in Soft Robotics:*** while flexible materials allow drones to mimic shapes of complex underwater environments, reducing damage upon interaction, flexible materials such as silicone and elastomers used in soft robotics allow designs to be inspired by nature, including a propulsor shaped like a tentacle or bell. These elements would not only be soft but would also enhance hydrodynamic efficiency, allowing for quieter and more efficient locomotion.<sup>15</sup>

Additionally, using soft materials decreases the failure rates of the drone in extreme environments. Soft robots can absorb and accommodate forces instead of resisting them, reducing structural stress. Buoyant materials reduce the total energy to remain afloat, a feature prominent in battery-powered systems. In conclusion, choosing materials, whether hard or soft, is the single most critical choice for the function and longevity of underwater drones. As the industry grows, we will start to see increasingly hybridized designs that focus on blending hard metals and soft polymers, finding an appropriate balance between durability, agility, and environmental considerations.<sup>20</sup>

**Table 2:** The suitable materials for each depth range with their examples.

Depth Category	Suitable Materials	Example Uses
Shallow (<100m)	ABS Plastic, Silicone Rubber	Consumer drones, educational submersibles
Mid-depth (100–500 m)	Anodized Aluminum, Carbon Fiber Composites, Elastomers	Survey drones, inspection robots
Deep-sea (>1000m)	Titanium, Carbon Fiber (sealed), Specialized Elastomers	Scientific exploration, oil & gas inspection, military drones

#### ***Applications of Underwater Drones in Ocean Exploration:***

Underwater drones, or Remotely Operated Vehicles (ROVs) and Autonomous Underwater Vehicles (AUVs), have renewed ocean exploration. AUVs and ROVs have also enabled access to previously inaccessible, fragile ecosystems and remote underwater areas for scientists. The flexibility of these devices has been based on several critically important sectors, ranging from biology to archaeology to sustainable resource management.<sup>21</sup>

Drones afford marine biologists a non-invasive opportunity to survey marine organisms. AUVs can come equipped with cameras and motion sensors that allow them to track the behavior of animals in the wild, as they allow researchers to track and observe migration, feeding, and social behavior, for endangered species such as deep-water jellyfish or sea turtles. Soft robotic equivalents have been implemented for these spe-

cies. Because these researchers employ a soft robotic gripper mode that imitates the soft-bodied animals, such as octopuses and squids, they can take samples while ecologically studying biodiversity.<sup>22</sup>

In environmental monitoring, drones are used to detect and monitor pollution in the world's oceans. For instance, machine vision and infrared sensors can see floating plastics, and detection sensors can characterize oil spills, etc. In monitoring coral reefs, the most fragile kinds of ecosystems in the ocean, drones fitted with a mixture of environmental sensors and soft robot arms permit researchers to inspect reefs very closely and sample organisms from the reef without an impact on the organisms. Close inspection of offshore reefs is critical when applying conservation and rehabilitation techniques.<sup>23</sup>

ROVs can help explore shipwrecks, sunken cities, and underwater caves that are either too dangerous to dive/or impossible to dive for most humans. ROVs had a significant role in documenting information about the RMS Titanic, while recently demonstrating their capabilities in uncovering antiquities from the Roman period off the coast of Croatia through similar geomorphology. Underwater drones can help scientists and researchers document fragile environments while taking and collecting samples with high-definition sonar, mapping, and robotic arms.<sup>24</sup>

Further, in the field of resource exploration, primarily in the mapping of the ocean floor, vessels that research minerals from the seabed can map for hydrothermal vent sources, etc. AUVs, with multi-beam sonar, will show a reference to resources that companies can use to correctly map future ocean mineral depletion, which have sustainability as needed; e.g., companies exploring manganese nodules, companies exploring rare earth elements, etc., can survey before they are exploitable. These demonstrable cases can be related to the NOAA which will use AUVs to monitor coral bleaching of corals, for example, in the Pacific, or the Schmidt Ocean Institute's SuBastian ROV - which has also helped discover new species and map explorable seafloor, while mapping unknown floors, all exemplify, how both underwater drones and ecosystems have pioneered ocean research taking into considerations of variations to soft robots, ocean opportunities, AI, etc.<sup>25</sup>

#### ***Challenges and Future Prospects of Underwater Drones:***

Despite their growing role in marine exploration, underwater drones face several key challenges that limit their full potential. One of the primary limitations is battery life; most AUVs and ROVs operate for limited durations due to the high energy demands of propulsion, lighting, and onboard systems. Depth restrictions are another concern, as increased pressure at deeper levels requires advanced, pressure-resistant housings and materials that can withstand extreme environments without failing. Furthermore, underwater communication is severely constrained; radio waves do not travel well through water, so drones must rely on slower acoustic signals or surface periodically for data transmission, affecting real-time control and coordination.

In terms of materials, maintaining durability under extreme pressure and corrosion remains an engineering challenge. Tra-

ditional rigid components often cannot cope with deep-sea dynamics, leading to increased interest in soft robotics. These systems, made from flexible, biocompatible materials, show great promise for the future, especially for delicate marine tasks such as coral monitoring or deep-sea sampling.

Emerging trends are helping address these issues. Artificial Intelligence (AI) is being integrated into AUVs to allow for autonomous decision-making, adaptive navigation, and environmental learning. Another innovation is swarm robotics, where multiple small drones cooperate, like schools of fish, to explore wide areas collaboratively and efficiently.

The future of soft robotics in underwater applications looks particularly exciting. Bio-inspired designs, such as those mimicking jellyfish or octopuses, offer enhanced maneuverability, silent movement, and better adaptability in complex terrains. These developments promise safer, more efficient exploration of previously unreachable ocean zones.

For young researchers and scientists, like myself, this is a field rich with opportunity—from robotics and marine biology to AI and materials science. As oceans remain largely unexplored, the next wave of breakthroughs will come from interdisciplinary minds pushing the boundaries of underwater innovation.<sup>26</sup>

## ■ Conclusion

In conclusion, by fusing robotics, material science, and artificial intelligence, underwater drones are transforming ocean exploration. These developments are opening up new avenues for marine research, from autonomous vehicles mapping the ocean floor to soft robotic grippers protecting fragile ecosystems. The quick development of soft robotics and AI-driven autonomy presents encouraging answers to problems like short battery life, communication limitations, and material durability. Looking ahead, future research is projected to increase the operational depth of underwater drones from the current average of 1,000 meters to over 6,000 meters within the next decade, while improving energy efficiency by up to 40% through advances in hydrodynamic design and adaptive AI navigation. The integration of next-generation composite materials could reduce overall drone weight by 25–30%, extending mission duration and reducing manufacturing costs. These quantified improvements will help underwater drones remain at the forefront of our exploration of the vast, unexplored ocean depths—empowering scientists, protecting biodiversity, and inspiring a new generation of researchers to better understand and preserve the last frontier of our planet.

## ■ Acknowledgments

I would like to extend my utmost gratitude to the Docent Research Program for the precious guidance, support, and constructive feedback in the process of composing this paper. I am equally thankful to my parents, Priyanka Jain and Gaurav Jain, for their steady support, tolerance, and trust in me, the cornerstone upon which my work has been built. Their support has not only inspired but also empowered me to develop my ideas in full confidence.

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## ■ Author

Aryaman Jain is an aspiring mechanical engineer with a deep fascination for the ocean and an even deeper curiosity about how machines can navigate its complexities. He envisions a future where soft robotic AUVs redefine underwater exploration. Aryaman is passionate about bio-inspired design, fluid dynamics, and autonomous systems, and is currently exploring how compliant materials and undulatory propulsion can revolutionize the stealth, efficiency, and scalability of subsea robotics. His long-term goal is to create a new class of Unconventional AUVs (UAUVs) that operate not against the ocean but with it.

# Reviewing: Monoclonal Antibody Therapies Targeting IL-6 & TNF-Alpha against Rheumatoid Arthritis

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**ABSTRACT:** Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by progressive joint damage with inflammation, significantly affecting the overall quality of life. Monoclonal antibody (mAbs) therapies have updated the management of RA by targeting key inflammatory pathways like tumor necrosis factor (TNF)- $\alpha$  and interleukin-6 (IL-6) due to their efficacy and safety profile compared to synthetic drugs. Thus, this review comprehensively investigates the mechanism of action, efficacy, and safety profile of various mAbs (TNF- $\alpha$  and IL-6 inhibitors). This review found significant therapeutic outcomes, like symptom alleviation, disease progression, remission rate, and safety profiles of TNF inhibitors (etanercept, adalimumab, and infliximab), and IL-6 inhibitors (tocilizumab and sarilumab). Furthermore, this review also explores adverse events and economic considerations associated with these mAbs, which can influence clinical decision-making. By synthesizing this evidence, clinicians can enhance RA management and tailor the treatment plan to individual needs.

**KEYWORDS:** Rheumatoid Arthritis, Monoclonal Antibody Therapy, TNF- $\alpha$  Inhibitors, IL-6 Inhibitors.

## ■ Introduction

Rheumatoid arthritis (RA), a multisystemic and multifactorial genetic autoimmune disorder, is primarily responsible for the damage to joints and later causes extra-articular manifestations.<sup>1</sup> RA not only causes damage to joints, but it also significantly damages other vital organs like the kidneys, heart, gastrointestinal tract, lungs, skin, eyes, and sense organs, and even causes mortality.<sup>2</sup> There are two main types of inflammation. First, acute inflammation is characterized by the infiltration of neutrophilic cells and then the monocytes. While in chronic inflammation, the presence of macrophages and lymphocytes at the inflammation site can be observed.<sup>3-5</sup> Although inflammation serves as the first line of the human body's defense by eliminating harmful stimuli, the repair of damaged tissues or organs requires the migration of leucocytes to the affected site, subsequently.<sup>6</sup> However, persistent acute inflammation leads to chronic inflammation, and this condition causes or escalates tissue damage.<sup>7</sup> The RA prevalence increased from 1990 to 2019 among adolescents and young adults from 34.11 to 36.34/100000 persons. The most significant increase and decrease in the number of cases were observed in 2000 and 2014, respectively,<sup>8</sup> and extra-articular manifestation also increased from 17.8% to 40.9%.<sup>9</sup> Similarly, an increase from 207.46 to 224.25 in disability-adjusted life years was also observed from 1990 to 2019.<sup>10</sup> Globally, it is estimated that by 2050, 31.7 million people will live with RA.<sup>11</sup> Therefore, its management is necessary as now monoclonal antibodies (mAb) are available for treating RA, particularly targeting tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL) pathways, which are required to be targeted by the inhibitors.<sup>12</sup>

Indeed, clinical trials of TNF inhibitors and IL-6 receptor antibodies have shown significant improvement in clinical outcomes, resulting in global approval for the treatment of moderate to severe RA, particularly in patients with inadequate

response to other synthetic drugs.<sup>13</sup> Furthermore, nucleotide polymorphisms are associated with the therapeutic response of mAbs, which target TNF, IL-6, and CD20 of B-cells. Similarly, numerous studies also reported the association between clinical responses of mAbs having various mechanisms, like IL-1, 7, 23, the receptor activator of nuclear factor-kappa B inhibition, and granulocyte-macrophage colony-stimulating factor.<sup>14</sup> These mAbs are generally designed to target inflammatory cells, molecules, and pathways that cause damage to the tissues in patients with RA.<sup>1</sup>

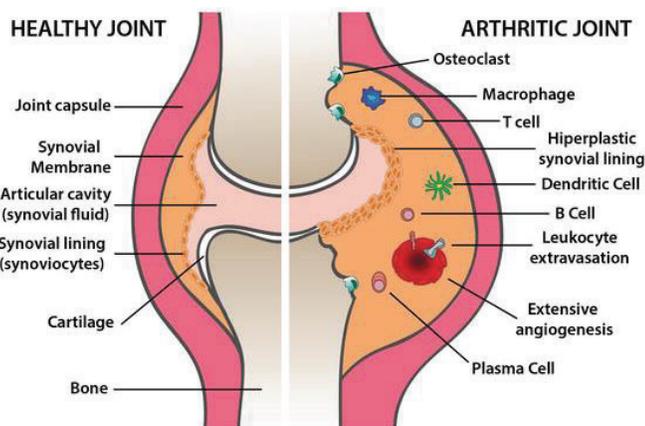
This literature review is a detailed analysis that aims to compare and contrast the mechanism of action, efficacy, and safety of the existing types of mAbs against RA. The first section of the paper explores the pathophysiology of RA, expands upon the mechanism and types of mAb therapies, and then delves into the detailed analysis of the varied TNF- $\alpha$  antagonists and IL-1, IL-6 inhibitors, as the main biological disease-modifying anti-rheumatic drugs (bDMARDs) of focus in this review. In the second section, this review draws a comparison among the mAb therapies to understand the relative advantages and disadvantages in terms of rate of remission, frequency of dosage, extent of potential side effects, financial accessibility, and overall drug efficacy.

## ■ Pathophysiology and cellular pathways of rheumatoid arthritis

Rheumatoid arthritis cannot be traced back to an individual factor, in fact. Initiation of the multisystemic autoimmune disease has been concluded as an interplay of multiple epigenetic and environmental triggers. Pathophysiology follows a complex trail of chain reactions concentrated in the synovium at a molecular level. In the first checkpoint, RA immunopathogenesis usually begins with autoantibody production (a process

in which the body's immune system mistakenly produces antibodies against its cells and tissues) against post-translationally modified proteins, in a process called citrullination. In the second checkpoint, tissue tolerance erodes, asymptomatic autoimmunity and progressive immune system remodeling take place, and failure occurs in protective joint-resident macrophages.<sup>16</sup>

In the third checkpoint, irreversible mechanisms occur with the transition of synovial stromal cells into auto-aggressive effector cells, which transfers the acute form of synovitis into destructive chronic synovitis.<sup>16</sup> Furthermore, before the clinical phase (pre-symptomatic or preclinical RA), despite not having overt disease, many people acquire autoantibodies, like anti-cyclic citrullinated protein/peptide antibody (ACPA) and rheumatoid factor (RF), which cause chronic joint destruction and systemic inflammation<sup>17,18</sup> as described in Figure 1.

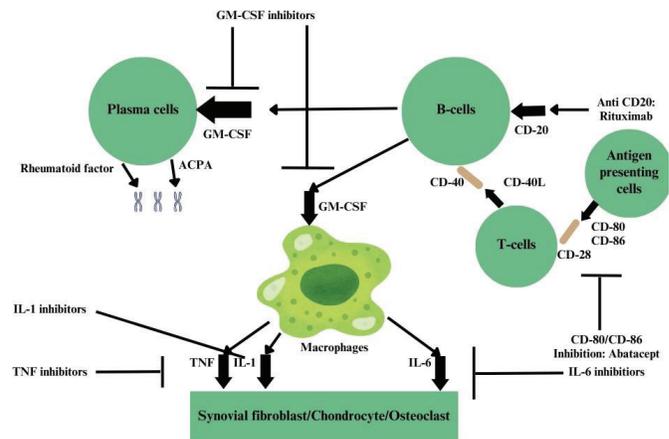


**Figure 1:** Comparison between healthy (left side) and RA (right side) infected joints.<sup>19</sup> The right side emphasizes the most critical clinical alterations and inflamed synovial membrane, and leukocytes infiltrate the synovial membrane, licensed under CC BY 3.0.

Meanwhile, RA susceptibility is strongly associated with genetic factors, like differences in human leukocyte antigen (HLA)-DRB1 alleles, particularly in RF and ACPA-positive RA patients.<sup>20,21</sup> The ACPA pathogenetic significance in RA patients is the outcome of their multidirectional biological activities, and two-thirds of positive patients have shown complexes that consist of ACPA and citrullinated fibrinogen. Stimulation of Fcγ receptors on macrophages also occurs in ACPA-positive RA patients.<sup>22,23</sup> In addition, alleles of HLA-DRB1 associated with RA encode a common sequence at 70-75 (QKRAA) amino acids in the β-chain third hypervariable section, which is known as the shared epitope.<sup>24-26</sup> The shared epitope in the HLA-DRB1 alleles generates citrullinated peptides, consequently leading to ACPA development.<sup>27</sup> Mainly, ACPA is categorized into two subtypes, such as ACPA-positive and ACPA-negative. Meanwhile, the citrullination process is catalyzed by the peptidyl-arginine deiminase (PAD) enzyme, which is helpful in the process of post-translational modification by changing a positively charged arginine to a polar but neutral citrulline.<sup>28</sup>

Furthermore, in the synovium, the antigen-presenting cells attract the modified or citrullinated foreign antigen, which is then transported to the lymph nodes for the activation of

CD4+ T helper cells, which further cause the co-stimulation of B cells.<sup>29,30</sup> As the first line of defense, these B cells propagate and differentiate into plasma cells by the expression of costimulatory cells and generate pro-inflammatory and anti-inflammatory cytokines like IL-17 and interferon-gamma, which are responsible for the drawing of macrophages into the joint spaces.<sup>31</sup> These activated macrophages further generate inflammatory cells, such as IL-1, 6, and TNF-α, which in turn activate FLS, which is required in the receptor activator for nuclear factor-κB ligand (RANKL) expression, leading to bone damage by activation of osteoclasts<sup>32,33</sup> and detailed pathophysiology is explained in Figure 2. Moreover, the abnormal signaling pathways in RA, which are essential for the diagnosis and treatment of RA, attracted the attention of researchers. The understanding of these pathways can explain complex diseases and be helpful in the development of RA-linked intervention targets and new advanced medication.<sup>34,35</sup>



**Figure 2:** Pathophysiology of rheumatoid arthritis (RA). Activated CD-T cells play an essential role in inflammatory responses in the synovial membrane, including inflammatory cytokine secretion (TNF, IL-1) by macrophages, autoantibody production by plasma cells, and highlighting key immune cell types and therapeutic interventions. This figure is created with the assistance of Canva (www.canva.com).

#### Different treatment modalities:

Different treatment modalities have shown promising outcomes, such as DMARDs, which particularly target inflammation and inhibit joint damage.<sup>12,36</sup> The three most important DMARDs are conventional synthetic DMARDs (sulfadiazine, hydrochloroquine, and methotrexate), biological DMARDs (inhibitors for TNF receptor, TNF-α inhibitors, IL-6, IL-6R, and B-cell depleting antibodies), and targeted synthetic DMARDs, like JAK ½ and pan-JAK inhibitors.<sup>12,37</sup> These DMARDs have the potential for the management of RA; however, they have significant side effects and high economic costs.<sup>12</sup> Likewise, non-steroidal anti-inflammatory drugs (NSAIDs) are also used for treating RA and have the ability to inhibit the biosynthesis of prostaglandins at the level of the cyclooxygenase enzyme.<sup>38</sup> However, their prolonged usage can lead to functional impairment.<sup>39</sup> Moreover, corticosteroids like prednisone, budesonide, and prednisolone also showed promising outcomes; however, corticosteroids showed serious adverse events.<sup>40</sup>

### Monoclonal antibody therapy:

Over the last three decades, due to advancements, the understanding of RA pathophysiology has transformed therapeutic approaches, with mAb therapies emerging as one of the most promising modalities and having encouraging outcomes in RA management.<sup>41</sup> The mAbs proved to be less toxic due to their target selectivity and binding to other targets and were specifically designed to target the TNF- $\alpha$  and IL-17 or more targets.<sup>42</sup> In addition, mAbs like adalimumab, rituximab, infliximab, and tocilizumab have a connection between the clinical response of these mAbs, having different mechanisms (IL-1, 17, and 23), the receptor activator of nuclear factor- $\kappa$ B inhibition, and granulocyte-macrophage colony-stimulating factor.<sup>14</sup> There is still no single established immunomodulator or any pharmacological preventive that is fully effective against RA.<sup>43</sup> However, certain drugs are approved and often used for RA treatment (see Table 1).

**Table 1:** Approved monoclonal antibody agents used for the RA.

mAbs	Dose	Structure	Target	FDA approval year
Actemra (Tocilizumab)	Every other week (132 mg sc) and then qw (wt<100) or 162 qw (wt >100)	Humanized mAb	IL-6R	2010
Cimzia (Certolizumab)	At weeks 0, 2, and 4 (400 mg sc) and then 200 mg/every other week	Humanized PEGylated fragment	TNF	2009
Enbrel (Etanercept)	Once a week (50 mg injection)	IgG fusion protein with TNF receptor	TNF	1998
Humira (Adalimumab)	Every other week (40 mg sc)	Humanized mAb	TNF	2002
Kezara (Sarilumab)	Every other week (200 mg sc)	Humanized mAb	IL-6R	2017
Remicade (Infliximab)	At weeks 0, 2, and 6 weeks (3 mg/kg)	Chimeric mAb	TNF	1999
Rituxan (Rituximab)	2 weeks apart (1000 mg IV)	Chimeric mAb	B-cells (CD20)	2006
Simponi (Golimumab)	Once a month (50 mg sc)	Humanized mAb	TNF	2009

Abbreviations: mAb: Monoclonal Antibody therapy, TNF: Tumor Necrosis Factor, IL: Interleukin, IV: Intravenous, SC: Subcutaneous

### ■ TNF- $\alpha$ inhibitors

A pro-inflammatory cytokine, TNF- $\alpha$ , is produced by activated macrophages, natural killer cells, and T lymphocytes.<sup>46</sup> Meanwhile, TNF- $\alpha$  abnormal production triggers synovial hyperplasia and generates matrix metalloproteinase and prostaglandin.<sup>47</sup> In addition, it stimulates bone cells to secrete the RANKL, which results in osteoclast formation.<sup>48</sup> The TNF- $\alpha$  inhibitors form a complex with the binding of the interface of the TNF- $\alpha$  dimer, which ultimately hinders the TNF- $\alpha$  receptors' binding, which stops inflammation and other signaling pathways in the TNFR1 and TNFR2 due to the activation of downstream signaling complexes.<sup>49,50</sup> These TNF- $\alpha$  inhibitors

have a chondroprotective impact on cartilage turnover, which helps in improving the type II collagen formation/resorption (C2C/PIICP) ratios in the assessment of the response of anti-TNF- $\alpha$  therapeutic effect in RA patients.<sup>51</sup>

Therefore, certain inhibitors such as etanercept, adalimumab, and infliximab have been approved for the treatment of RA, as these inhibitors help eliminate the TNF- $\alpha$  at the site of inflammation.<sup>52</sup> These inhibitors have been solidified as bDMARDs directed towards the control of TNF- $\alpha$  to instigate initiation and maintenance of remission in patients with RA. These bDMARDs may be registered subcutaneously (injection administered under the skin) or intravenously (drug administered through a needle or tube inserted into the vein).

### Etanercept:

Etanercept is the first biological TNF- $\alpha$  inhibitor that is authorized by the Food and Drug Administration (FDA) to treat RA. It showed its efficacy and safety when used as a monotherapy or combined with other drugs like methotrexate.<sup>45</sup> Structurally, it is a dimeric human TNF receptor p75-Fc fusion protein made of 75 kD (p75) TNFR attached with human immunoglobulin (IgG)-1 constant Fc portion,<sup>45,53</sup> and it contains 150 kDa of molecular weight and consists of 934 amino acids.<sup>54</sup> This dimeric structure of etanercept has the potential to improve the binding affinity with a short half-life mean time of 70 hours and comparatively has more inhibitory potential than monomeric soluble receptors.<sup>55</sup> Etanercept not only acts as a TNF inhibitor, but it also has effector activity against Fc, which triggers the production of complement-dependent cytotoxicity via triggering the complement pathway and also induces antibody-dependent cell-mediated cytotoxicity and target immune cell apoptosis.<sup>56</sup> It achieves this by having the TNF receptor bind to TNF- $\alpha$  and TNF- $\beta$  since etanercept prevents the binding of both TNF- $\alpha$  and TNF- $\beta$  to cell surface TNF receptors, which renders the TNF biologically inactive.<sup>57</sup> Due to its inhibitory effects, it disrupts this inflammatory cascade, resulting in the reduction of inflammation in the joints, bone erosion, and cartilage degradation. Meanwhile, clinically, it proves its efficacy. For instance, in a German research study, 824 RA individuals were treated with etanercept, and the patient's proportion achieving low disease activity or remission was 39% at 12 weeks and increased to 45% at week 24, and it further increased to week 52.<sup>58</sup> Similar outcomes were observed in another cohort study, which observed significantly lower disease activity after half a year of treatment. However, a higher rate of adverse events, including other serious infections and other central nervous system-associated events, was also recorded without a high rate of hospitalization.<sup>59</sup> Furthermore, when etanercept is combined with a gold standard DMARD methotrexate, its efficacy increases due to synergistic effects, leading to superior clinical response, a greater reduction in radiographic damage, and improved remission rates.<sup>60</sup>

### Adalimumab:

Adalimumab is a fully human recombinant immunoglobulin G1 (IgG1) with high affinity.<sup>61</sup> It binds with the cytokine TNF- $\alpha$ , inhibiting its interaction with TNFR1 and TNFR2,

and FDA-approved inhibitory mAb for the treatment of RA.<sup>62,63</sup> It consists of 1330 amino acids with a 148kDa molecular weight.<sup>64</sup> Adalimumab disrupts downstream signaling pathways, which are required for inflammation, joint destruction, and synovial hyperplasia.<sup>65</sup> It is also helpful in reducing the production of IL-1 and IL-6, inhibiting leukocyte recruitment and decreasing the FLS activation and osteoclasts.<sup>65,66</sup> Moreover, it is helpful in downregulating serum matrix metalloproteinases (MMP) 1 and 3, and this inclusive impact causes the TNF inhibitor effectiveness in RA.<sup>67</sup>

When adalimumab is used as monotherapy, it has demonstrated significant efficacy in treating RA, and patients showed significant, sustained, and rapid progress in their disease activity and physical function.<sup>68</sup>

For RA individuals who persistently used adalimumab for one year, 67% of individuals demonstrated low disease activity and showed clinically improved functions.<sup>69</sup> Similarly, significant improvement was noticed in week 4. After 12 weeks, 15.3% of RA individuals achieved clinical remission, and 28.9% had low disease activity.<sup>70</sup> Moreover, when adalimumab is combined with methotrexate, it promotes the efficacy of adalimumab by inhibiting the formation of anti-drug antibodies, and this combination demonstrated better clinical and radiological outcomes than when used alone.<sup>71</sup> In short, studies have endorsed the benefits of adalimumab in promoting quality of life, work efficiency, and safety, and in how it is well-tolerated, and its ability to solve sleep problems.<sup>72</sup>

### ***Infliximab:***

Infliximab, a chimeric mAb, has been widely used to treat TNF- $\alpha$ -associated diseases for a decade and binds with the human IgG1 Fc region, and a further mechanism involves the overlapping with the TNF- $\alpha$  receptor interface, indicating its pivotal role in the E-F loop.<sup>73</sup> It can further bind to free types, and inhibition can take place for the cytokines binding and other associated receptors, with complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity effects superior to etanercept.<sup>15</sup> In addition, it is also helpful in reducing IL-6 and CRP concentrations in the blood after its administration<sup>74</sup>, and it provides evidence for the reduction in IL-1 and soluble TNF receptors, two main types of anti-cytokines regulated by TNF- $\alpha$ .<sup>75</sup> Moreover, it can successfully inhibit the formation of osteoclasts and activity in vitro and suppress RANKL expression by blocking TNF.<sup>76</sup> Another important mode of action is regulating the immune responses by restoring the altered regulatory T cell function in RA, which is an efficient method to treat RA.<sup>77</sup> Furthermore, CD4+ and CD25+ Treg functions regulate immune response and the suppression of auto-reactivity, which is often dysregulated in RA patients.<sup>78</sup> The efficacy of infliximab is evident in numerous studies. For instance, RA patients were treated with infliximab for 36 months, and patients demonstrated significant and sustained improvement in all parameters associated with the disease. Meanwhile, <2% of patients showed adverse events, like arthralgia, headache, upper respiratory tract infection, pruritus, infection, nausea, rash, fatigue, back pain, bronchitis,

dizziness, pyrexia, and dyspnea.<sup>79</sup> Key characteristics of these three TNF inhibitors are mentioned in Table 2.

**Table 2:** Summary of key characteristics of TNF- $\alpha$  inhibitors.

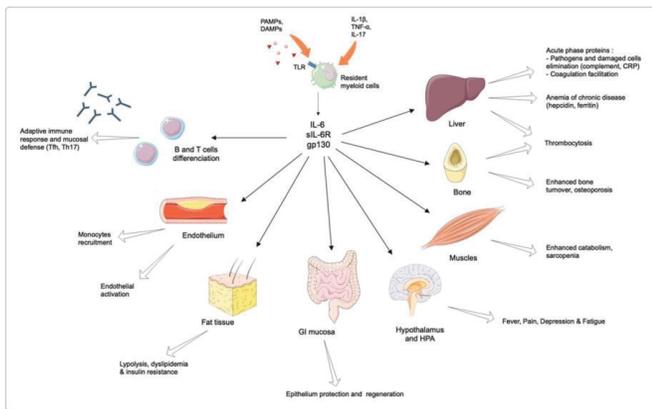
Key variables	Etanercept	Adalimumab	Infliximab
Molecule	IgG fusion protein with TNF receptor <sup>80</sup>	Humanized mAb <sup>64</sup>	Chimeric mAb <sup>81</sup>
Mode of action	Soluble TNF receptor binding to TNF- $\alpha$ <sup>82</sup>	Inhibition of the TNF- $\alpha$ binding <sup>64</sup>	Blinds both soluble and membrane-bound TNF- $\alpha$ <sup>83</sup>
Administration route	SC and injected <sup>84</sup>	IV and SC <sup>85</sup>	SC and IV <sup>86</sup>
Indication	Ankylosing spondylitis, plaque psoriasis, psoriatic arthritis, RA, juvenile idiopathic Arthritis <sup>84</sup>	RA, psoriasis, psoriatic arthritis, juvenile idiopathic arthritis, Crohn's disease <sup>62</sup>	Ankylosing spondylitis, RA, psoriatic arthritis, Crohn's disease <sup>87,88</sup>
Dosage	50 mg once a week <sup>84</sup>	40-80 mg every 2 Weeks <sup>67</sup>	3 mg/kg <sup>76</sup>
Storage and stability	25 °C $\pm$ 2 °C for up to 1 Month <sup>89</sup>	2 °C $\pm$ 8 °C for 36 months and 25 °C for 14 days <sup>90</sup>	Can be stored at 4 °C over 6 weeks <sup>91</sup>
Use	Monotherapy or in Combination <sup>92</sup>	Monotherapy and in Combination. <sup>71</sup>	Monotherapy and in Combination. <sup>76</sup>
Efficacy	Effective monotherapy & in combination <sup>92</sup>	Effective <sup>68</sup>	Effective <sup>79</sup>
Safety or Adverse events	Serious infections (viral, fungal, and bacterial) are mostly in upper respiratory tract <sup>92</sup> , and injection site reactions (pain, itching, erythema, bleeding, swelling, and bruising), and central nervous system related adverse events <sup>93</sup>	Safe and well-tolerated <sup>70</sup> , adverse events like reactivation of tuberculosis can be seen <sup>94</sup>	Adverse events were reported: lower and upper respiratory symptoms, urticarial/angioedema, <sup>95</sup> bacterial pneumonia <sup>96</sup>
Cost-effectiveness	High acquisition cost (61,552 \$)/year <sup>97,98</sup>	Higher cost (91,695 \$/year) <sup>99</sup>	Lower cost (79,518 \$/year) <sup>99</sup>

### ■ IL-6 inhibitors

Interleukin-6 is transiently and promptly produced in the infection or tissue injury response. However, the expression of IL-6 is managed by posttranscriptional and transcriptional mechanisms, while irregular IL-6 synthesis plays a critical role in the pathogenesis of chronic inflammation.<sup>100</sup> It is a small polypeptide of 6 kDa molecular weight.<sup>101</sup> In 1986, human B cell stimulatory factor 2 was successfully cloned, and due to its multiple activities, later numerous names were used; however, now all those names are unified and are called IL-6.<sup>102</sup> Furthermore, biological activities exerted by IL-6 are through two molecules, gp130 and IL-6R. A high functional receptor complex comprising IL-6, IL-6R, and gp130 is formed when

IL-6 binds with the membrane-bound type of IL-6R.<sup>103</sup> Initially, the interaction between IL-6 and IL-6R $\alpha$  takes place, and later, this interacting pair forms a complex with gp130. This high affinity of the complex of these three subunits is associated with a second high-affinity complex, which further forms a hexameric complex and is essential to induce signal transduction.<sup>104</sup>

Furthermore, IL-6 exerts its impact via two primary signaling pathways, such as classic signaling and trans-signaling, and both pathways involve IL-6R and gp130.<sup>105</sup> Overall, IL-6 promotes joint destruction and inflammation and causes pain, morning stiffness, and fatigue.<sup>106</sup> In addition, the pleiotropic impact of mAbs (IL-6) on the other body organs is also described in Figure 3.



**Figure 3:** The pleiotropic impact of IL-6 on different body parts. The complexes formed by IL-6, by glycoprotein (gp) 130, and its soluble receptor (sIL-6R) can induce various systemic effects distant from the inflammation's initial site,<sup>107</sup> under Creative Commons CC BY-NC-ND 4.0 license. Mainly, there are two types of IL-6 inhibitors, including tocilizumab and sarilumab, which have been approved to treat RA and are discussed below.

### *Tocilizumab:*

Tocilizumab is the first anti-IL-6R humanized mAb approved for treating RA alternative to methotrexate and helps inhibit the cis and trans signaling cascades, which involves the activator of transcription pathway and Janus kinase signal transducer.<sup>108</sup> It prevents the IL-6 interaction with the signal transducer gp130 and IL-6R by targeting both membrane-bound and soluble IL-6R.<sup>109</sup> It also decreases B-cell hyperactivity, induces expansion in regulatory B-cells, and decreases peripheral memory B-cell numbers.<sup>110</sup> This action reduces inflammation, modulates the function of immune function, and is also helpful in mitigating tissue damage, making it a powerful treatment option for RA patients. Moreover, it also prevents joint destruction progression by the inhibition of bone or cartilage resorption and is also helpful in improving hematological abnormalities, like high levels of autoantibodies, hypergammaglobulinemia, and elevation of ESR and acute phase proteins.<sup>111</sup> Notably, it improves life quality by inhibiting symptoms like anemia, fever, fatigue, and anorexia.<sup>111</sup> In terms of efficacy, it is effective from as early as week 2 after its administration, and its effectiveness increases with time.<sup>111</sup>

Two efficacy studies, SATORI and SAMURAI, were performed with adult moderate-to-severe RA patients, and tocilizumab was administered as monotherapy, and patients

receiving a dose every 4 weeks. At weeks 24 and 52, excellent ACR and remission rates were observed.<sup>112,113</sup> Similarly, when used in combination with methotrexate, the efficacy was maintained with no patient demonstrating a decrease in effectiveness.<sup>114</sup> Adverse events associated with tocilizumab are presented in Table 3.

### *Sarilumab:*

Sarilumab, a humanized IL-6R mAb, binds both soluble and membrane-bound human IL-6R with a high affinity, which blocks the cis and trans inflammatory signaling cascade.<sup>115</sup> Its effectiveness in inhibiting IL-6 signaling depends on the dose and is also helpful in reducing CRP levels.<sup>116,117</sup> This blockage suppresses phase reactants, reduces systemic and local inflammation, and mitigates immune cell recruitment to the synovium, which is helpful in halting the progression of joint damage and promoting life quality.<sup>118</sup> Furthermore, it also decreases bone resorption by inhibiting osteoclast differentiation, reducing radiographic evidence of joint erosion and narrowing.<sup>119</sup> It was developed using animal models for the production of human antibodies with an affinity of 20-fold greater than tocilizumab for the human IL-6R.<sup>120</sup> Clinical trials using sarilumab as monotherapy or combined with other DMARDs demonstrated that it is effective either alone or in combination.<sup>121</sup> Similarly, the MOBILITY<sup>122</sup> and MONARCH<sup>123</sup> studies have shown functional and symptomatic improvement in RA patients with intolerance or inadequate responses to conventional DMARDs like methotrexate. The key characteristics of these two IL-6 inhibitors, including adverse events, are described in Table 3.

**Table 3:** Summary of key characteristics of IL-6 inhibitors.

Key variables	Tocilizumab	Sarilumab
<b>Molecule</b>	Humanized mAb <sup>108</sup>	Humanized mAb <sup>120</sup>
<b>Mode of action</b>	Bind with both soluble and membrane bound IL-6R <sup>109</sup>	Bind with both soluble and membrane bound IL-6R <sup>115</sup>
<b>Administration route</b>	IV and SC <sup>124</sup>	SC <sup>120</sup>
<b>Use</b>	Monotherapy and in combination <sup>125</sup>	Monotherapy and in combination <sup>126</sup>
<b>Dosage</b>	8 mg/kg <sup>111</sup>	Every two weeks, 150 or 200 Mg <sup>126</sup>
<b>Storage and stability</b>	2 - 8 0C or room temperature and should be protected from light	2 - 8 0C, once removed from refrigeration, use with 14 days <sup>120,127</sup>
<b>Indication</b>	Systemic juvenile idiopathic arthritis, giant cell arteritis, juvenile idiopathic	Acute moderate to severe RA <sup>120,127</sup>
<b>Efficacy</b>	Effective <sup>112</sup>	Effective <sup>115</sup>
<b>Safety or adverse events</b>	Adverse events were reported (infections [cellulitis, herpes zoster, tuberculosis, pneumonia]), <sup>128</sup> skin and subcutaneous tissue disorders, and eye abnormalities <sup>129</sup>	Serious adverse events like cardiovascular events, upper respiratory tract infections, injection site erythema, malignancies, and venous thromboembolism are rare <sup>130</sup>
<b>Cost-effectiveness</b>	More cost-effective compared to other DMARDs (ICER=1301 \$) <sup>131</sup>	Compared to adalimumab, sarilumab is more cost-effective with total cost of 492,000 \$ <sup>132</sup>

## ■ Discussion

Monoclonal antibody therapies have revolutionized RA management by targeting specific molecules involved in the disease pathogenesis, offering more effective and precise alternatives to conventional therapies. Despite having small molecule therapies, mAbs offer a more targeted approach, specifically blocking key inflammatory cytokines that play an essential role in the pathogenesis of RA, leading to more precise and potent effects on the reduction of inflammation. Therefore, this comprehensive literature review aimed to compare mAbs used to manage RA.

In the present literature review, we discussed two types of mAbs, such as TNF- $\alpha$  and IL-6 inhibitors, and the literature highlights their efficacy when compared to other drugs used for the management of RA individuals. The efficacy of TNF- $\alpha$  inhibitors is well established as RA patients were treated between 2004-2014. EULAR moderate and good treatment responses (69% and 40%) were achieved at 6-month follow-up.<sup>133</sup> Likewise, the clinical response of three TNF inhibitors (etanercept, adalimumab, and infliximab) was evaluated for 6 and 12 months of treatment. The adalimumab had the highest number of patients (32 and 39) with remission rates.<sup>134</sup> Similar findings were observed in the SAMURAI trial, which included individuals with inadequate response towards sDMARDs, and patients were treated with IL-6 inhibitor (tocilizumab) and found superior with ACR 20 and lower disease activity 28 at 24 weeks compared to the control groups.<sup>113</sup> Similarly, another study also observed a 55.3% remission rate in the tocilizumab group.<sup>135</sup> Furthermore, RA individuals treated with either TNF or IL-6 inhibitors, 10-50% of patients achieve remission in 6-12 months.<sup>136,137</sup> Likewise, randomized phase 3 and 4 trials demonstrated better remission rates and superiority when RA individuals with high disease activity were treated with tocilizumab and sarilumab.<sup>123,138</sup> In contrast, RA individuals were treated with baricitinib and compared with sarilumab, although sarilumab induced similar improvement in disease activity. However, at week 24, the baricitinib-treated group significantly improved the clinical disease activity index. At 52 weeks, both groups had similar outcomes.<sup>139</sup>

In addition, the efficacy of these mAbs is dose-dependent. For instance, the tocilizumab standard dose is 8 mg/kg every 4 weeks (q4W) for treating RA individuals. However, for patients with high disease activity and high body weight, which resulted in high levels of IL-6, the effectiveness of tocilizumab at the current rate is inadequate, so reducing the interval between the administration of tocilizumab to once a week can be beneficial in the management of the disease.<sup>140</sup> Similarly, another randomized trial demonstrated that adequate effectiveness can be achieved when RA patients with a weight of >100 kg are treated weekly with tocilizumab SC.<sup>141</sup> In contrast, TNF inhibitors did not significantly improve the efficacy with increasing doses.<sup>142</sup> Likewise, in RA patients with low levels of IL-6, tocilizumab SC or IV prolonged dose interval can sustain remission.<sup>143,144</sup>

Even though mAbs demonstrate effectiveness, they are not free from adverse events or complications. For instance, adali-

mumab is associated with 63 unique adverse events, such as vascular dementia, systemic lupus erythematosus rash, and ovarian cancer. Similarly, etanercept has 180 unique adverse events, including chondrolysis, ankle and finger deformity, and joint warmth. Meanwhile, infliximab demonstrated 60 unique adverse events, such as metastatic neoplasm and Hodgkin's disease.<sup>145</sup> In addition, meta-analysis observed an increased risk of cancer with 1.36 OR (95% CI, 1.20-1.653;  $p < 0.00001$ ) and severe infections with 1.72 OR (95% CI, 1.56-1.90;  $p < 0.00001$ ) in TNF TNF-treated group.<sup>146</sup> Furthermore, three (4%) patients reported neurological adverse events when treated with adalimumab, etanercept, and infliximab.<sup>147</sup> Similarly, 612 RA individuals who were treated with tocilizumab reported adverse events, and 27.5 events/100 patients were reported every year, with 5.7 serious infections/100 patients/year.<sup>135</sup> In another study, five cases of malignancy with an incidence rate of 1.36/100 patients per year, and an incidence rate for major adverse cardiovascular events of 0.83/100 patients per year, were reported in patients treated with IL-6 inhibitors.<sup>148</sup>

Meanwhile, for every effective drug, cost-effectiveness should also be considered. TNF inhibitors and IL-6 inhibitors like IL-6 had lower costs with higher quality-adjusted life years (QALYs) than rituximab, and TNF inhibitors showed the most cost-effective treatment modality.<sup>149</sup> Similarly, tocilizumab had a lower cost with higher QALYs than rituximab.<sup>149</sup> Furthermore, when tocilizumab was compared with adalimumab, tocilizumab was found to be more cost-effective with 76% probability, with higher 4.24 QALYs gained compared to adalimumab.<sup>131</sup> In contrast, a Swedish study reported a higher cost per QALY for three different types of TNF inhibitors, and the cost ranged from €50,000-120,000.<sup>150</sup> Similarly, another pragmatic randomized trial documented that rituximab therapy was significantly more cost-effective, compared to TNF inhibitors, over a willingness to pay ranging from \$0-80,000.<sup>151</sup>

A primary limitation should be considered, and that is the meta-analysis was not performed due to the nature of the study, as meta-analyses are necessary for better comparison of dosing regimen, age-wise impact, and comparison of treatment groups with control. They also calculate the heterogeneity level among the studies as well as the source of heterogeneity. Future research should include meta-analyses for a statistically better comparison between treatment and control groups.

## ■ Conclusion

The present review aims to compare and contrast the relative advantages and disadvantages of the existing forms of mAbs for the management of RA patients. These therapies have revolutionized disease management by targeting specific immune pathways, and the scope for further innovation remains significant. Overall, these mAbs (TNF [etanercept, adalimumab, and infliximab] and IL-6 inhibitors [tocilizumab and sarilumab]) demonstrated efficacy. These mAbs have transformed clinical outcomes for many patients by offering effective, targeted strategies to mitigate progression of the disease and improve quality of life, but they have also raised uncertainty regarding cost-effectiveness and association with adverse events, in-

cluding increased susceptibility to infections. Notably, using antibody fragments can further enhance tissue penetration, tailor delivery mechanisms, and reduce immunogenicity. Overall, this review provides insights into the importance of ongoing research to refine therapeutic approaches and bring improvement in the patient's associated outcomes. Future longitudinal and multicenter studies are required to further validate the findings of the present review.

## ■ Acknowledgments

I would like to wholeheartedly thank Dr. Hamidreza Shaye for his patience and guidance as a mentor during my writing, as well as for laying my foundation in genetics. I am massively grateful to my parents for empowering me and supporting me each step of the way, and especially Dr. Ashish Singh, who inspired me to research this important topic and mentored me throughout. I am indebted to my mentors and my parents, who have fueled the inspiration for my academic endeavors.

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## ■ Author

Lavanya Singh is a high-school student at the Hazeley Academy, United Kingdom. She is deeply driven towards studying biology and hopes to pursue her academic passions as a career in medicine. This paper, therefore, captures a subject of great personal significance as well as her intellectual curiosity in molecular biology and genetics.

# Magnetic Nanoparticles in Targeted Drug Delivery Systems: Overcoming the Blood-Brain Barrier for Central Nervous System Therapeutics

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**ABSTRACT:** The blood-brain barrier (BBB) presents one of the most substantial and drastic challenges in delivering therapeutic agents to the brain. Iron oxide nanoparticles have emerged as a promising strategy for overcoming this barrier due to their nanoscale, tunable surface properties, and potential for targeted drug delivery. These nanoparticles play a crucial role in delivering effective treatments to the brain, particularly by traversing the BBB via processes like magnetic hyperthermia, where an alternating magnetic field (AMF) disrupts tight junctions, improving the permeability of nanoparticles without any lasting impact. Moreover, external magnetic fields, such as the AMF, can direct iron oxide nanoparticles to localized regions in the brain for efficient drug delivery. Additionally, surface modifications and coatings have drawn attention to improving cellular uptake, targeting efficiency, and drug release. Guiding iron oxide nanoparticles using an external magnetic field enables precise delivery, minimizing side effects and toxicity, thereby enhancing the effectiveness of medicine. To explore this, experiments involving ferrofluid—a substance composed of iron oxide nanoparticles—were conducted to observe how magnetic fields affect nanoparticle mobility. This research highlights the potential of iron oxide nanoparticles to revolutionize neurological disease treatments by enhancing drug delivery across the BBB and ensuring effective, targeted therapies.

**KEYWORDS:** Biomedical and Health Sciences, Cell, Organ, and Systems Physiology, Central Nervous System, Drug Delivery, Magnetic Nanoparticles.

## ■ Introduction

The blood-brain barrier (BBB) presents numerous challenges related to its high selective permeability for medical treatments, particularly in the form of drugs and other therapeutic substances. To overcome this barrier, specifically engineered nanoparticles could be used. The use of magnetic nanoparticles, particularly iron oxide nanoparticles, for drug delivery across the blood-brain barrier has been studied and proposed as an effective method for enhancing brain permeation. Most studies focus on the prospect of various nanoparticles simply transporting medicine across the BBB and eventually mitigating negative brain disorders. However, these nanoparticles must efficiently travel across the BBB to deliver medicine and target the brain parenchyma without massive disturbance to other bodily functions. Subsequently, further studies should be conducted to explain the effects of iron oxide nanoparticles in the human body. The use of various fields to improve the drug delivery process, such as implementing external magnetic fields (EMFs) outside of the brain to guide magnetic nanoparticles, has also remained a highly recognized theory. Data is needed to demonstrate the correlation between EMFs and iron oxide nanoparticles for drug delivery.

Additionally, drug delivery of iron oxide nanoparticles using external magnetic fields has been tested in the reticuloendothelial system, including organs such as the liver and spleen, where significant nanoparticle accumulation demonstrates promising therapeutic potential for treating tumor areas in this system.<sup>1</sup>

According to a study, the application of an external magnetic field in mice facilitated magnetic nanoparticles to cross the BBB with no apparent damage to its integrity and functionality, which is the key to maintaining homeostasis.<sup>2</sup>

The use of the EMF for the specific targeting of malicious cells in the brain parenchyma was investigated in a study. Researchers have concluded that magnetic nanoparticles, guided by an external magnetic field, can deliver drugs to any poorly accessible site of inflammation, such as the brain, and can be used for the treatment of various neurological diseases. The EMF can drive magnetic nanoparticles toward damaging cells, delivering medicine to the proper place in the brain.<sup>3</sup>

The high surface area-to-volume ratio of the magnetic nanoparticles allows for the attachment of various molecules, making them suitable for drug delivery applications. Additionally, the various coatings and surface modifications, such as liposomes, can increase their biocompatibility, improve permeation through the blood-brain barrier, and increase the ability to control drug release. The surface charge of the magnetic nanoparticles can also be modified to tune their properties and functions.<sup>4</sup>

This study is meant to showcase the extensive ability of magnetic nanoparticles for drug delivery across the challenging BBB to treat life-changing neurological disorders. While these nanoparticle methods are certainly useful for biomedical applications, they may pose significant health risks and safety concerns by accumulating in organs within the body and serv-

ing an unintended purpose. We discuss the effects of external magnetic fields, surface charges, and surface coatings on the targeted drug delivery applications of iron oxide nanoparticles.

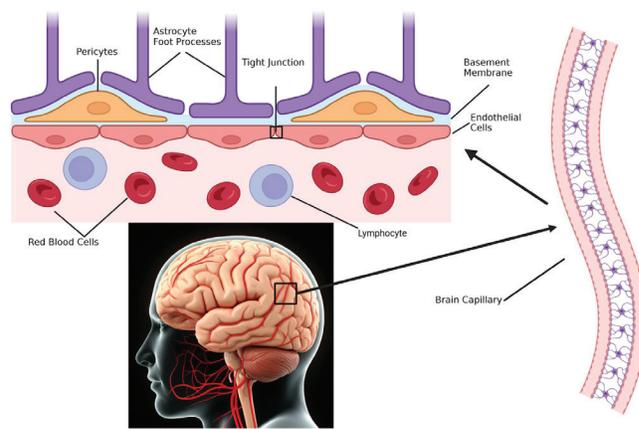
## ■ Literature Review

### *The Blood-Brain Barrier:*

The blood-brain barrier (BBB) is a natural, semipermeable, and selective membrane that prevents pathogens and toxic substances from crossing into the central nervous system (CNS) and into the brain (Figure 1). The BBB's function could be a significant disadvantage for potential drug remedies and treatments since it recognizes them as toxins. Large compounds, such as proteins and drug molecules, are unable to permeate the BBB due to their larger size compared to other molecules. A recent study estimates that the BBB excludes approximately 98% of small-molecule drugs and nearly all large-molecule therapeutics.<sup>5</sup> Generally, only lipid-soluble (lipophilic) molecules with a low molecular weight (under 400–600 Da) can cross the BBB. In contrast, the rest of the molecules require certain cell endogenous transport systems, such as carrier-mediated transport, receptor-mediated transport, or absorptive-mediated transport.<sup>6</sup> That poses a major challenge for the administration of vital medicine into the brain because the BBB rejects the drugs, and it is instead released into the bloodstream. The blood-brain barrier is an extremely important factor to consider when determining treatments for different neurological diseases, since the disruption of the BBB can lead to severe pathology observed in many different diseases, but also because crossing the BBB is an essential consideration in the development of CNS-acting therapeutics.<sup>7</sup>

To effectively provide beneficial drugs and biopharmaceuticals into the brain, the development of methods to bypass the BBB has become a necessity. However, some strategies may have limited drug penetration, potential toxicity, and difficulty in targeting specific areas of the brain, emphasizing the need for effective drug delivery methods and prompting scientists to continue formulating and improving the methods of delivery. Similarly, insufficient drug delivery into the brain leads to low therapeutic efficacy as well as aggravated side effects due to the accumulation in other organs and tissues.<sup>8</sup> Mainly, there are two primary pathways for substances to cross the BBB: paracellular transport, which involves passing in between the endothelial cells, and transcellular transport, which involves passing across the luminal side of the endothelial cell, crossing the cytoplasm, and then passing across the abluminal side of the endothelial cell into the brain interstitial fluid.<sup>9</sup>

The BBB mainly consists of capillary endothelial cells, tight junctions, pericytes, astrocytes, and basement membranes that serve as primary parts of the BBB situated inside the brain capillaries (Figure 1). Specifically, the property of low permeability is controlled by tight junction (TJ) protein complexes that seal the passageway between opposing brain microvascular endothelial cells.<sup>10</sup> For specific substances like water and small molecules to enter the brain, they must pass the tight junctions and travel into the bloodstream through the BBB, which is highly restrictive.



**Figure 1:** Diagram of the blood-brain barrier. The location of the brain capillaries inside the brain, a representation of the brain's capillary (enlarged), and parts of the blood-brain barrier (enlarged), including endothelial cells, pericytes, astrocyte foot processes, tight junctions, basement membranes, red blood cells, and lymphocytes, are depicted within this image. A visual representation of the parts of the blood-brain barrier is shown, which work together to maintain the passageway into the brain. On the inside, there are red blood cells, lymphocytes, water, and other substances that get distributed throughout the brain from capillaries. The figure was created using BioRender.

### *Nanoparticles for Drug Delivery:*

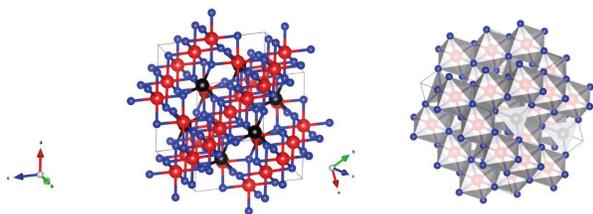
Nanoparticle drug delivery has been proposed as an effective method of brain permeation within the scientific community and continues to be researched extensively. Nanoparticles have various advantages for drug delivery across the BBB because of their small size (1 to 100 nm), tunable surface properties, and the ability to act as carriers.<sup>11</sup> For nanoparticles to carry drugs and medicine into the brain, they must pass through the blood-brain barrier (BBB) properly and efficiently.

Due to their small size, nanoparticles can penetrate the tissue system, facilitating the easy uptake of the drug by cells, permitting efficient drug delivery, and ensuring action at the targeted location.<sup>12</sup> A study examining the use of nanoparticles for drug delivery states that the nanoparticles also possess enhanced bioavailability, an additional ability to cross the blood-brain barrier (BBB), and can be absorbed through the tight junctions of the endothelial cells.<sup>13</sup>

Magnetic nanoparticles, particularly iron oxide nanoparticles, are important nanoparticles for drug delivery and imaging applications. Iron oxide nanoparticles are nanoscale forms of iron oxides with unique magnetic and surface properties. Since these nanoparticles are typically less than 100 nanometers, they are small enough to cross the BBB. When synthesized using a variety of complex methods, iron oxide nanoparticle size can range from 3 nanometers to 11 nanometers, which would make them less recognizable by the BBB.<sup>14</sup>

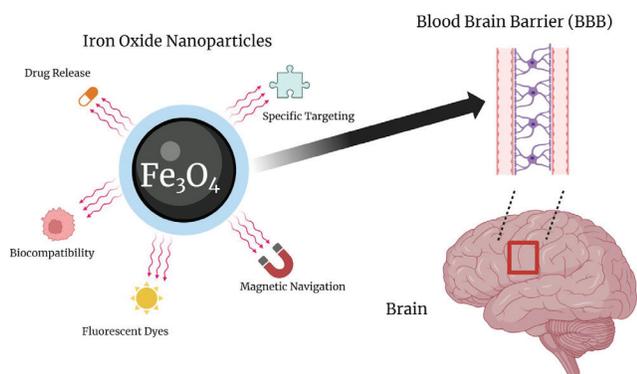
The iron oxides magnetite ( $\text{Fe}_3\text{O}_4$ ) and maghemite ( $\gamma\text{-Fe}_2\text{O}_3$ ) exhibit magnetic properties, which make them capable of magnetic navigation. The crystal structure of magnetite is shown in Figure 2. Magnetite ( $\text{Fe}_3\text{O}_4$ ) and maghemite ( $\gamma\text{-Fe}_2\text{O}_3$  or  $\text{Fe}_{8/3}\text{O}_4$ ) are the reduced and oxidized end-members of a spinel solid solution.<sup>15</sup> Stoichiometric magnetite  $\text{Fe}_3\text{O}_4$  has an inverse spinel structure with one  $\text{Fe}^{3+}$  per formula unit on tetrahedral sites and  $\text{Fe}^{2+}$  and the remaining  $\text{Fe}^{3+}$  randomly distributed on the octahedral sites. Maghemite (magnetite –

hematite),  $\gamma\text{-Fe}_2\text{O}_3$  or  $\text{Fe}_{8/3}\text{O}_4$ , has a defect spinel structure with one-third mole of cation vacancies per four moles of oxygen, compensating for the oxidation of  $\text{Fe}^{2+}$ .<sup>16</sup>



**Figure 2:** Crystal structure of magnetite. On the left side: black atoms –  $\text{Fe}^{2+}$ , and the red ones –  $\text{Fe}^{3+}$ . The blue atoms represent oxygen. On the right side: octahedral and tetrahedral sites. Source: American Mineralogist Crystal Structure Database CIF data. VESTA software was used for visualization.

Iron oxide nanoparticles are also biocompatible. However, it is important to note that factors such as dosage, cellular effects, and long-term exposure can lead to the accumulation of these nanoparticles in various organs, potentially disrupting homeostasis. Like most nanoparticles, iron oxide nanoparticles can be coated or functionalized with various agents to aid in the drug delivery process and improve the nanoparticles' efficiency in transporting drugs to the BBB (Figure 3).



**Figure 3:** Iron oxide nanoparticle abilities for drug delivery purposes. The various abilities that iron oxide nanoparticles have are depicted in this image. These properties are all necessary when the iron oxide nanoparticle enters the brain and crosses the blood-brain barrier. A visual representation showing the particle traveling towards the blood-brain barrier with these helpful properties emphasizes the potential of iron oxide nanoparticles to improve disorders within the brain. Drug release, specific targeting, biocompatibility, fluorescent dyes, and magnetic navigation all impact iron oxide nanoparticles for drug delivery. The figure was created using BioRender.

#### *External Magnetic Field Technology:*

Iron oxide nanoparticles offer the enhanced benefit of magnetic targeting due to their ability to respond to magnetic forces. When an external magnetic field (EMF) is applied outside the human body, the resonating magnet draws iron oxide nanoparticles towards localized areas and targets, releasing the drugs, which then target pathogens and invasive cells within the brain (Figure 4).

Apart from the normal drug delivery process, utilizing an EMF reduces the negative drug side effects of the nanoparticle drug delivery, drawing nanoparticles towards localized areas and minimizing nanoparticle concentrations in unwanted regions of the brain, ensuring drugs are shifted towards the proper location. Furthermore, nanoparticles can release

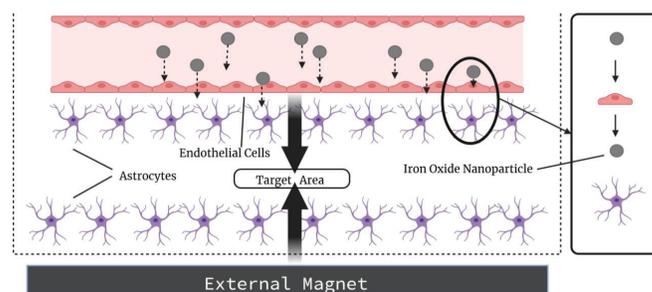
amounts of toxicity, causing inflammation and cell death, by drugs that may distribute in healthy tissues, causing them to die. While iron oxide nanoparticles have less toxicity than other nanoparticles, using an EMF to draw iron oxide nanoparticles away from susceptible areas is better for the body and maintains brain homeostasis.

In addition, the EMF's strength can manipulate iron oxide mobility depending on the magnetic field intensity. Low or high magnetic attractions can change the location and the distance of the iron oxide nanoparticles traveling inside the brain. Furthermore, a typical EMF has an intensity barely sufficient to control a moving nanoparticle but is usually stronger than a low-intensity magnet. If the EMF is too strong, then the surrounding tissues will be damaged by the magnetic field. As stated, when an external magnetic field is present, a force is exerted on the magnetic nanoparticles, effectively pulling them towards the magnet, as shown in Figure 4. The formula represents the magnetic force of an external magnet:

$$F_{mag} = (\chi_1 - \chi_2)V \frac{1}{\mu_0} B(\nabla B)$$

Where  $V$  is the volume of the magnetic particle,  $\mu_0$  is the magnetic permeability of free space,  $B$  is the magnetic field strength,  $\nabla B$  is the magnetic field gradient, or how fast the strength or direction of the magnetic field changes over a given distance,  $\chi_2$  is the magnetic susceptibility, or how much magnetic nanoparticles will respond to the applied magnetic field,  $\chi_1$  is the magnetic susceptibility of the medium, which is very small in comparison to  $\chi_2$  and is therefore disregarded in biological systems.

Based on this information, the important parameters for considering the capture of the magnetic nanoparticles are the magnetic properties, volume of the nanoparticles, the magnetic field strength, and the magnetic field gradient. It is clear that as the external magnetic field strength decreases, the ability of the magnet to capture the nanoparticles decreases.<sup>17</sup>



**Figure 4:** An external magnetic field affects the mobility of iron oxide nanoparticles. Iron oxide nanoparticles are traveling across the BBB to the target area. The iron oxide nanoparticles must pass the endothelial cells and astrocytes before reaching the target site. Then, the EMF may be pulled away when the nanoparticles reach the target, and the nanoparticles can release medicine to cure cognitive diseases. The external magnetic field is key for magnetic targeting, since it allows iron oxide nanoparticles to successfully penetrate the blood-brain barrier and reach the target area. The figure was created using BioRender.

#### *Alternating Magnetic Field Technology:*

Another important magnetic field helping magnetic nanoparticles target harmful cells within the brain is the al-

ternating magnetic field (AMF). The AMF is a specific type of external magnetic field that produces heat, where only the magnetic components alternate and the direction of the alternating magnetic field changes constantly, resulting in vibrations and heat. The alternating magnetic fields (AMFs) amplitudes vary in time, causing vibrations and secured motions towards a certain place or target.<sup>18</sup> The AMF can be defined with characteristics such as differing alterations, frequency, and strength. Depending on their frequencies, they may have low or high amplitudes, which can control vibrations. These magnets are commonly used in technological applications, particularly for manipulating electrical and magnetic particles.

The AMF can be used for nanoparticle interactions, influencing materials that have magnetic properties like a common EMF. However, the AMF plays a different role for these magnetic nanoparticles within the brain. When an AMF is projected at iron oxide nanoparticles within the brain, they start to rotate and vibrate as a result of the amplitudes directed by the AMF. This causes the iron oxide nanoparticle to heat up as it travels across the BBB, resulting in temporary structural changes in the endothelial cells. Due to the AMF's alterations and vibrating motion, the tight junctions and tight junction proteins in the endothelial cells, which are necessary to secure the BBB, lose their structural integrity temporarily. In the tight junctions, vital proteins such as claudin and occludin lose their function, causing openings in the BBB. This method of penetration through the BBB is called magnetic hyperthermia, which is an applicable method that may be implemented specifically for iron oxide nanoparticles. This makes the BBB more permeable and, in turn, aids iron oxide nanoparticles in reaching the brain without potential backlash.<sup>19</sup>

The AMF can be implemented to assist the drug delivery targeting malicious cells, particularly tumors and similar abnormal cells, using iron oxide nanoparticles. The iron oxide nanoparticles engineered for drug delivery are typically coated with specific molecules to enhance the process's efficiency and facilitate binding to harmful cells within the brain. In fact, they can be conjugated to antibodies that bind to specific antigens expressed on the membranes of targeted cells.<sup>20</sup> Via this means, cells in suspension, like blood samples, can be specifically targeted, and upon applying a low magnetic field gradient, the target cell population can be separated from all other cells. This means that iron oxide particles can bind to target cells, ultimately releasing drugs to eliminate harmful molecules. Moreover, the AMF's function can cause iron oxide nanoparticles to vibrate and rotate, thereby creating heat that propels them toward target cells, interferes with their functions, and eventually kills them. Examples typically refer to tumor and cancerous cells within the brain that get immobilized and killed due to magnetic hyperthermia.<sup>19</sup>

#### ***Surface Charges and Surface Functionalization:***

Nanoparticle surface charge relates to the electrical charge that exists on the surface of the nanoparticle. It usually results from interactions with particles in environments. Surface charges can be introduced on nanoparticle surfaces by attaching charged molecules to the nanoparticle surface. These

charges affect the stability, cellular interactions, and chemical reactivity of a specific nanoparticle. Surface charges typically interact with charged particles or other materials that have opposite charges. For example, positively charged iron oxide nanoparticles exhibit stronger interactions with negatively charged biomolecules, while negatively charged iron oxide nanoparticles interact more strongly with positively charged biomolecules. Both positively charged iron oxide nanoparticles and negatively charged nanoparticles have different properties and can be utilized for various applications, such as drug delivery across the BBB.

In fact, magnetic effects are caused by movements of particles that have both mass and electric charges. This includes magnetic fields like EMFs, surface charges, and other important iron oxide characteristics.<sup>21</sup>

It is important to note that specific coatings may be applied to iron oxide nanoparticles to change the nanoparticles' surface charge and overall functionality. The preparation of surface functionalized MNPs using iron oxide NPs (MNPs), poly (lactic-co-glycolic acid) (PLGA), and sodium alginate via co-precipitation, emulsification, and electro-spraying, respectively. Additionally, the surface functionalization of MNPs with selective polymers alters the surface chemistry to improve biocompatibility. To test the utmost functionality of iron-oxide nanoparticles for drug delivery, it may be coated or functionalized on its surface to ensure that the iron oxide has more capability for drug delivery and crossing the BBB.<sup>22</sup>

Positively charged iron oxide nanoparticles have numerous advantages for overcoming the BBB and performing the drug delivery process efficiently. After a study evaluated the surface charge of biological membranes within the blood-brain barrier (BBB), it was found that the surface charge of brain endothelial cells forming the blood-brain barrier (BBB) is highly negative due to phospholipids in the plasma membrane and the glycocalyx.<sup>23</sup> Additionally, positively charged nanoparticles are known to be more easily internalized than neutral or negatively charged nanoparticles.<sup>24</sup> This is because positively charged nanoparticles respond better with negative counterparts like the brain's endothelial cells, which are key for the BBB crossing. As a result, the positively charged iron oxide nanoparticles are preferred in drug delivery over negatively charged ones due to their specific advantages. The advantages and disadvantages of negatively and positively charged iron oxide nanoparticles (Table 1) regarding their potential for drug delivery are notable. Notably, the positively charged nanoparticles are most popular among experiments pertaining to drug delivery.

On the other hand, negatively charged iron oxide nanoparticles facilitate safer interactions with molecules, have better stability, but are less efficient as drug delivery nanocarriers. In addition, they induce a lower cytotoxic response compared to positively charged nanoparticles and neutral charged nanoparticles.<sup>25</sup> However, the negatively charged nanoparticles could cross the BBB effectively and complete the process with appropriate surface modifications and functionalization. In a study considering the surface charge of negatively charged nanoparticles and evaluating other surface coatings, it was deduced that negatively charged nanoparticles targeted with an RVG

peptide can also penetrate across a human BBB model effectively.<sup>26</sup> The pros and cons of surface charges used in iron oxide nanoparticle drug delivery are listed in Table 1.

**Table 1:** Advantages and Disadvantages of Iron Oxide Nanoparticle Drug Delivery. A comparison of iron oxide nanoparticle drug delivery across the BBB. The advantages of positively charged iron oxide nanoparticles, negatively charged iron oxide nanoparticles, and neutrally charged iron oxide nanoparticles are depicted. Positively charged iron oxide nanoparticles were identified as the best candidates for drug delivery to the brain.

	Advantages	Disadvantages
<b>Positively Charged Iron Oxide Nanoparticles (+)</b>	Enhanced Cellular Uptake	Higher Rates of Toxicity
	Can Target Specific Cells for Drug Release	More Instability and Aggregation
	Bioavailability	
	High Interaction with Magnetic Targeting	
<b>Negatively Charged Iron Oxide Nanoparticles (-)</b>	Lower Rates of Toxicity	Lower BBB penetration
	Better Stability in Blood Circulation	Lower Cellular Uptake
<b>Neutrally Charged Iron Oxide Nanoparticles</b>	Better Interactions with Molecules	Weaker Interactions with Magnetic Targeting comparatively
	Low Inflammatory Response	
	Bioavailability	Low Cellular Uptake
	Better Stability	Little Targeting Efficiency
	Lower Rates of Toxicity	Difficulty with Functionalization

Note: Iron oxide nanoparticles typically need coatings, ligands, or other materials for full optimization and improvement. This is especially required for neutral (no charge) iron oxide nanoparticles.

The BBB is a vital component of drug delivery, since it hinders the ability of therapies to travel effectively into the brain. Iron oxide nanoparticles have been proposed as effective nanocarriers because of their magnetism, which allows for effective BBB permeation using EMFs. The potential of these nanoparticles for facilitating effective drug delivery has been recognized. However, the lack of *in vitro* BBB models to test iron oxide nanoparticles for predictive outcomes has limited accurate results, and this gap in experimental modeling continues to make magnetic targeting difficult for translation into real-world applications such as drug delivery. In this study, we conducted multiple experiments to simulate BBB permeation and assessed whether iron oxide nanoparticles could react to external magnets properly.

## ■ Methods

The goal of this paper is to evaluate the effect of magnetic nanoparticles, and particularly the iron oxide nanoparticles, for drug delivery across the blood-brain barrier. This paper assesses the efficacy and effect of these magnetic nanoparticles.

Two separate experiments were conducted to determine the application of iron oxide nanoparticle properties for drug delivery, focusing primarily on mobility and the presence of a magnetic field. All three trials in the second experiment were maintained for 24 hours, so that the results could be compared under consistent time conditions.

## Experiment 1: Evaluating the Magnetism of Iron Oxide Nanoparticles using Ferrofluid:

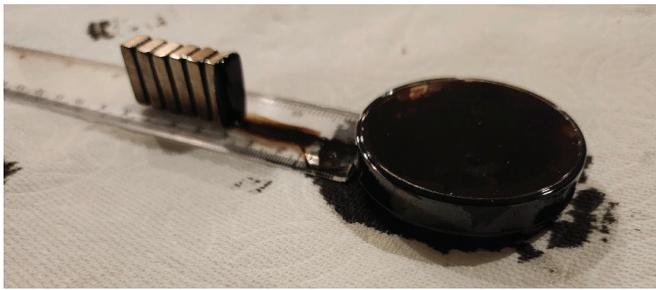
The first experiment was conducted to measure how ferrofluid (an iron-oxide-based liquid that is attracted to the poles of a magnet) reacts to a magnetic field and then use those measurements to create a distance versus magnetic field graph, uncovering the scope of iron oxide magnetism in real-life scenarios. During this experiment, a magnetic field was applied to determine how the amount of ferrofluid, which is made up of iron oxide nanoparticles, responds to an external magnetic field. We measured how the ferrofluid properties changed, collected data, and plotted them on a graph to see the common relation between field strength and nanoparticle behavior, proving that iron oxide nanoparticles have sustained magnetism and can be applied for drug delivery purposes.

Initially, a Gauss meter was used to measure the magnetic field strength (H), which is a measure of the intensity of a magnetic field produced by an external source such as the neodymium magnet (5mm height x 20mm length x 10mm width) used in this experiment. The magnet was positioned at distances ranging from 20 centimeters to 2 centimeters from the ferrofluid, which had been measured beforehand, and the corresponding magnetic field strength (in mT) was recorded at each point using a Gauss meter. Starting from 20 centimeters, the neodymium magnet was moved closer by 2 centimeters to 10 centimeters, where it was then moved closer by 1 centimeter onward for consistent results. The ferrofluid was contained within a petri dish to avoid inconsistent results, including prior magnetization. The data collected from the setup in Figure 6 were then analyzed to observe the relationship between the distance and magnetic field strength of the standard iron oxide nanoparticles. For all the materials used in this experiment, see Figure 5.

This step-by-step experiment was recorded for varying amounts of ferrofluid, including 5 mL, 8 mL, and eventually 12 mL of ferrofluid, for the magnetic field strength (H) to compare results and test different ferrofluid inputs.



**Figure 5:** Materials used in experiment 1 to represent the magnetism of ferrofluid. Ferrofluid for testing, a Gauss meter to measure in Tesla (mT), a ruler for measuring distances, two petri dishes to contain the ferrofluid, and a neodymium magnet as the active source of magnetic force were all used in testing and described in the visual.



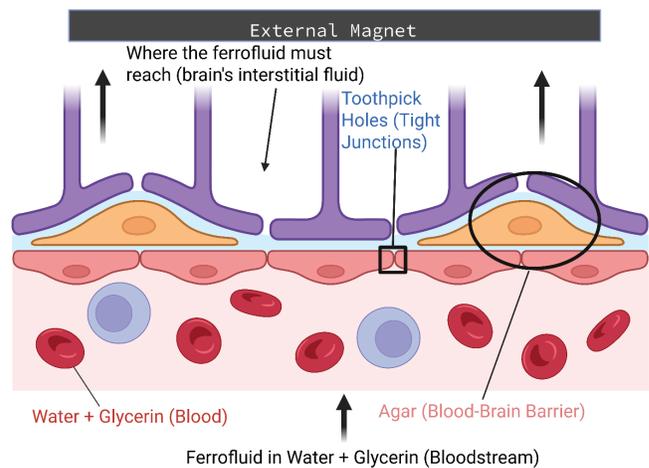
**Figure 6:** Experimental setup and material utilization as depicted in Figure 5. A small neodymium magnet was placed on the ruler at centimeter marks in increments, and ferrofluid, acting as the magnetic substance used for measuring magnetic field strength, was placed at the end of the ruler. From the experiment, the magnetic field strength exponentially grew as the neodymium magnets moved closer to the ferrofluid.

### **Experiment 2: Conducting Systematic Trials to Simulate Iron Oxide Nanoparticle Permeability Across the Blood-Brain Barrier:**

Three trials were conducted to evaluate the extent to which iron oxide nanoparticles could penetrate the blood-brain barrier (BBB) effectively, with each covering an aspect vital to iron oxide nanoparticle permeation past the BBB and into the brain's cerebrospinal fluid, enabling drug release.

The materials used within each trial remain constant; however, the amount of each substance used varies per trial to progressively test multiple scenarios. Throughout all 3 trials, the amount of ferrofluid remained 8 milliliters to ensure consistent results. The materials included within the 3 trials are agar (agarose gel), water, and glycerin (mixed in a 3:1 ratio, respectively), a toothpick, ferrofluid (8mL), a neodymium magnet (5mm height x 20mm length x 10mm width), plastic containers and glass cups, a ruler, and a syringe.

For all trials, agar (agarose gel) was used to simulate the blood-brain barrier because it acts as a semi-permeable medium; water and glycerin (mixed 3:1, respectively) were used to mimic blood consistency within the human body. The tiny, punctured holes (made with a toothpick 1.5 mm in diameter) were used to replicate tight junctions (TJs) in the agar (representing the blood-brain barrier) (Figure 7). The agar or the blood-brain barrier in this case served as a benchmark that the ferrofluid had to reach past to travel into the outside interstitial fluid (under the agar).



**Figure 7:** Modeling Magnetically Guided Transport Across an In Vitro Blood-Brain Barrier Model. The materials used in experiment 2 correspond to a function of the blood-brain barrier process. Water and glycerin mimic blood, while agar represents the blood-brain barrier due to its matrix-like formation, with toothpick holes that form tight junctions. Ferrofluid, which contains iron oxide nanoparticles, is used, and an external magnetic field acts as a pull factor for the ferrofluid. The ferrofluid successfully passed through the toothpick holes and past the agar using the external magnet. The figure was created using BioRender.

### **Trial 1: Ferrofluid Diffusion Through Simulated Blood-Brain Barrier with Pierced Tight Junctions (without magnet):**

In the first trial, ½ inch of agar (agarose gel), ½ inch of water and glycerin, 8 mL of ferrofluid, and toothpicks were the materials used. Initially, the agar (representing the blood-brain barrier) was placed on the bottom level, and water and glycerin (representing blood) were placed directly above the agar. Five evenly spaced toothpick holes were pierced into the agar to act as tight junctions for the iron oxide nanoparticles to follow through. Then, 8 mL of ferrofluid was carefully measured using a syringe and dropped into the glass cup containing water and glycerin. This solution was left alone for approximately 24 hours and kept at a constant room temperature (70°F). This will serve as the control, from which the following trials will build upon.

### **Trial 2: Ferrofluid Diffusion Through Simulated Blood-Brain Barrier with 5 Pierced Tight Junctions (with magnet):**

The materials used in the second trial were ½ inch of agar (agarose gel), ½ inch of water and glycerin, 8 mL of ferrofluid, a neodymium magnet (5mm height x 20mm length x 10mm width), and toothpicks to pierce 5 evenly spaced holes. The neodymium magnet is an addition to this trial, compared to the previous trial, to test the relation between the strong magnet and the ferrofluid within the blood-brain barrier or agar in this case. Following a similar procedure, the agar (representing the blood-brain barrier) was placed on the bottom level, and water and glycerin (representing blood) were placed directly above the agar. Five evenly spaced toothpick holes were pierced into the agar to act as tight junctions for the iron oxide nanoparticles to follow through. Then, 8mL of ferrofluid was carefully measured using a syringe and dropped into the glass cup containing water and glycerin. The neodymium magnet was placed at the bottom of the cup or below the agar, where it could pull

the ferrofluid through the pierced holes. This solution was left alone for approximately 24 hours and kept at a constant room temperature (70°F).

***Trial 3: Ferrofluid Diffusion Through Simulated Blood-Brain Barrier with 1 Pierced Tight Junction (with magnet):***

In the third trial, the materials used were 1 ½ inches of agar (agarose gel), ½ inch of water and glycerin, 8mL of ferrofluid, a neodymium magnet (5mm height x 20mm length x 10mm width), and a toothpick to pierce 1 hole in the center of the agar. As stated, the neodymium magnet was used in this trial to compare whether an external magnet has a significant impact on the movement of the ferrofluid in the cup. Once again, the agar (representing the blood-brain barrier) was placed on the bottom level, and water and glycerin (representing blood) were placed directly above the agar. Only one center hole was pierced to impede the ferrofluid compromise under the agar. Then, 8mL of ferrofluid was carefully measured using a syringe and dropped into the glass cup containing water and glycerin. The neodymium magnet was placed at the bottom of the cup or below the agar, where it could pull the ferrofluid through the one pierced hole. This solution was left alone for approximately 24 hours and kept at a constant room temperature (70°F).

## ■ Results and Discussion

Based on our literature review, we suggest that iron oxide nanoparticles with smaller sizes, a positive surface charge, and a biocompatible coating should be the most suitable nanocarriers for BBB permeation and targeted drug delivery. They can be synthesized using the method suggested in a study, which results in nanoparticles with a diameter of around 1 nm.<sup>27</sup> The product will be a thermodynamically stable solid solution between magnetite and maghemite, i.e., partly oxidized magnetite with a formula  $Fe_{3-x}O_4$  where  $x$  is between 0 and 0.33.<sup>15</sup> The primary method of verifying the phase purity and crystal structure will be powder X-ray diffraction with Rietveld refinement to determine the lattice parameters.<sup>28,29</sup> The average particle size will be determined from the surface area measured using the five-point Brunauer-Emmett-Teller (BET) technique, a standard method for nanoparticles.<sup>28-30</sup> The particle size distribution and morphology will be determined using Transmission Electron Microscopy (TEM), a standard technique for characterizing nanomaterials.<sup>28,29,31</sup> Fourier-transform infrared spectroscopy (FTIR) will be used to determine the type of organic molecules adsorbed on the surface.<sup>28</sup> The magnetic moment will be measured on MPMS superconducting quantum interference device (SQUID) magnetometers.<sup>27</sup>

The rate of cellular uptake can be increased by altering the surface charge of the nanoparticles. We suggest using positively charged nanoparticles to confer a higher internalization rate in the brain cells. They can be obtained using a method that describes the process of getting positively charged iron oxide nanoparticles.<sup>32</sup> Iron oxide nanoparticles were synthesized with designed charged ligands via reversible addition fragmentation chain transfer (RAFT) polymerization for stability. A positive charge was obtained by stabilizing the iron oxide nanoparticles

with a catechol-derived dopamine ligand along with polyethylene glycol (PEG), carboxylic acid, and amine groups. The tertiary amine group ((N-[3-(dimethylamino)propyl]acrylamide)) was used as a positive charge. The zeta potential of the positively charged iron oxide nanoparticles after completing this process was +32mV, respectively.<sup>32</sup>

Lipids will coat the iron oxide nanoparticles to ensure effective drug delivery for the BBB influx. A study suggests that lipid-based magnetic nanocapsules with a 12 wt.% iron oxide content exhibit magnetic behavior, making them suitable for biomedical applications that require external magnetic fields (EMFs).<sup>33</sup> Moreover, the lipid coating showed increased biocompatibility against human cerebral endothelial cells and pericytes. Guided by an EMF, the coated iron oxide nanoparticle displayed an enhanced targeting ability across the BBB. Additionally, a lower exposure time was required for this targeted delivery to pericytes, demonstrating targeting efficiency.<sup>33</sup>

Adding an external magnetic field will further improve properties by driving iron oxide nanoparticles towards localized areas to reduce minimal effects, disrupting healthy cells, and generally enhancing the therapeutic potential of the drug delivery system. We suggest using an alternating magnetic field (AMF), which causes localized heating and disrupts the tight junctions between endothelial cells. The spatial distribution of the iron oxide nanoparticles can be controlled by modulating the strength of the magnetic field.

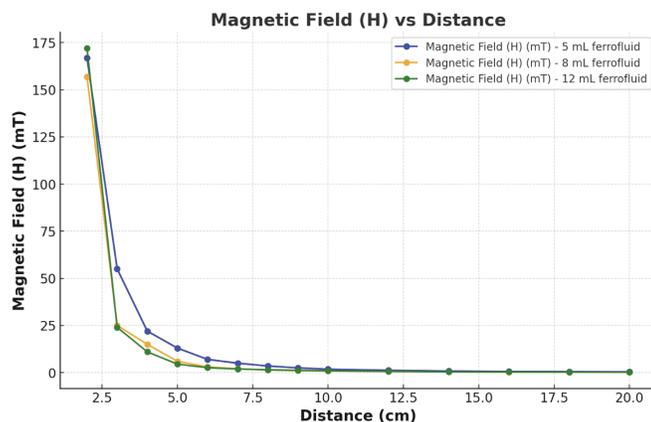
### ***Experiment 1:***

In the first experiment, where a magnetic field was introduced to iron oxide nanoparticles, we found that the closer the magnet approached the ferrofluid, the more the iron oxide nanoparticles within the ferrofluid began to react. Data were collected by measuring the distance and observing the magnetic strength of the field using a Gauss meter (Tables 2 and 3). The closer the magnet was, the more intense the magnetic field displayed by the ferrite iron oxide nanoparticles and the magnet. All three samples of ferrofluid (5mL, 8mL, and 12mL) showed relatively the same exponential increase. As the magnet inched closer to the ferrofluid, there was an exponential increase in the magnetic field intensity during the experiment (Figure 8), meaning the closer the magnet traveled, the more magnetic intensity was put on the ferrofluid. This experiment demonstrates the prominence of a magnetic field when iron oxide nanoparticles are exposed to magnetic objects, specifically magnets. External magnets seem to demonstrate a strong correlation with the magnetic nanoparticles and most likely can be used to control iron oxide nanoparticles for drug delivery applications.

Based on the experiment conducted, iron oxide nanoparticles show promising results for drug delivery, crossing the blood-brain barrier, and enhancing treatments for individuals with neurological disorders, particularly when an external magnet is used.

**Table 2:** Recorded Results for Magnetic Field Strength (H) in Experiment 1. Data on how distance correlates to the magnetic field using 5 mL, 8 mL, and 12 mL samples of ferrofluid. As the distance between the neodymium magnet and the ferrofluid decreased, a more magnetic field intensity occurred in all of the samples.

Distance (cm)	Magnetic Field (H) (mT) - 5 mL ferrofluid	Magnetic Field (H) (mT) - 8 mL ferrofluid	Magnetic Field (H) (mT) - 12 mL ferrofluid
20	1.02	0.9	0.94
18	1.07	1.09	1.03
16	1.14	1.13	1.18
14	1.25	1.17	1.2
12	1.4	1.27	1.48
10	1.75	1.52	1.64
9	2.2	2.05	2.26
8	3.0	2.52	2.75
7	4.36	3.24	3.28
6	6.18	4.31	4.48
5	12.41	5.36	6.93
4	20.5	12.56	11.52
3	55.53	25.3	23.25
2	164.46	155.57	168.39



**Figure 8:** Distance vs. Magnetic Field (H) in Experiment 1 of the 5, 8, 12 mL Samples. Based on the results, there is a prominent exponential relationship between distance and the magnetic field for all 5 mL, 8 mL, and 12 mL ferrofluid samples. As the distance reduces and the magnet slowly approaches the ferrofluid, more magnetic field (H) exposure is available.

### Experiment 2:

In the second experiment, which involved numerous trials to gather information on the mobility of iron oxide nanoparticles in concentrated solutions and through obstructing barriers like the blood-brain barrier (BBB), we found that iron oxide nanoparticles were able to pass through the BBB via the tight junctions successfully.

#### *Trial 1: Ferrofluid Diffusion Through Simulated Blood-Brain Barrier with Pierced Tight Junctions (without magnet):*

The ferrofluid containing the iron oxide nanoparticles was able to pass through the selective tight junction areas scattered in the agar during the 24 hours; however, this process was gradual, with the ferrofluid being immobilized for long periods of time. All three ferrofluid samples were successful.

#### *Trial 2: Ferrofluid Diffusion Through Simulated Blood-Brain Barrier with 5 Pierced Tight Junctions (with magnet):*

The ferrofluid containing iron oxide nanoparticles was able to pass through the selective, tight junction areas scattered in

the agar almost instantly over the 24 hours. Once the magnet was centered under the tight junctions, it passed through immediately. Using external magnets to divert and mobilize iron oxide nanoparticles is key in the drug delivery process, so brain regions affected by harmful cells are targeted.

#### *Trial 3: Ferrofluid Diffusion Through Simulated Blood-Brain Barrier with 1 Pierced Tight Junction (with magnet):*

The ferrofluid containing iron oxide nanoparticles was able to pass through the selective tight junction areas scattered in the agar almost instantly over the 24 hours. Once the magnet was centered under the tight junctions, it passed through immediately again. This trial was conducted to make it significantly harder for ferrofluid to pass through the punctured holes, even by using a magnet. However, the external magnet's force was strong enough to bring the ferrofluid past the agar, deeming this trial successful.

In the second experiment, which included each of the trials, we found that the iron oxide nanoparticles were able to pass through the agar via the toothpick holes or tight junction formations; however, the ferrofluid took hours to drift past the agar without any immediate result (Trial 1). Upon applying an external magnetic force, such as a neodymium magnet, the ferrofluid swiftly entered the tight junctions and traversed the agar or blood-brain barrier within minutes (Trials 2, 3). On a larger scale, iron oxide nanoparticles are considered effective drug delivery carriers, as they are small enough to pass through tight junctions and exhibit magnetic properties, allowing for individual control over their movement.

## Conclusion

In conclusion, magnetic nanoparticles, particularly iron oxide nanoparticles, are promising carriers for drug delivery that can cross the blood-brain barrier (BBB). The BBB limits the efficiency of many therapeutic drugs attempting to enter the brain, as it serves as a membrane that shields the brain from substances like drugs and pathogens. The nanoparticles provide significant improvement for drug delivery systems due to their small size, unique properties, and tunable surface. External magnetic fields can control the iron oxide nanoparticles due to their magnetic properties. The surface charges of iron oxide nanoparticles hold distinct properties like biocompatibility, which proves beneficial in drug delivery applications across the BBB. Positively charged iron oxide nanoparticles are typically used for drug delivery compared to other surface charges because their properties are mainly tailored towards drug delivery. Surface charges on iron oxide nanoparticles allow for clinical variability in specific situations and are important for delivering drugs across the BBB.

Advancements in the magnetic nanoparticle-based drug delivery have opened new pathways for surface functionalization and coatings, which ultimately aid in the delivery process because of their ability to enhance iron oxide nanoparticle abilities and the permeability of the BBB. Furthermore, surface coatings like other therapeutic ligands can assist iron oxide nanoparticles in the drug delivery process across the BBB,

thereby enhancing the performance of these nanoparticles. With surface charges, surface functionalization can additionally be added to iron oxide nanoparticles to boost separate properties that are hindered by the specific surface charge. For example, positively charged nanoparticles may have instability for drug delivery across the BBB; however, adding extra coatings will improve stability rates.

Additionally, magnetic fields may be implemented in the drug delivery process to help iron oxide nanoparticles pass through the BBB and deliver drugs in the brain. External magnetic fields (EMFs) help draw iron oxide nanoparticles towards target sites in the brain, capturing the iron oxide magnetic properties, and reducing side effects in the body. Since the magnetic field controls iron oxide nanoparticle mobility, it ensures effective drug usage in the brain and throughout the bloodstream, which also minimizes harmful side effects. The alternating magnetic field (AMF), a type of external magnetic field, uses the iron oxide nanoparticle properties of magnetism to mobilize iron oxide nanoparticles across the BBB by disruption. In addition, techniques involving the use of BBB disruptors, such as magnetic hypothermia, which effectively allow iron oxide nanoparticles to pass through the BBB, make the delivery process more accessible in clinical applications. These magnetic fields may be amplified based on clinical settings and prove to be significant elements able to aid in iron oxide nanoparticle drug delivery across the BBB.

Despite ongoing research on iron oxide nanoparticles as promising candidates for drug delivery across the blood-brain barrier (BBB), limitations must be considered when evaluating their primary clinical success. As mentioned, external magnets present challenges related to optimization and standards-based trials for drug delivery. Therefore, accuracy in medical situations might prove to be significantly difficult, especially since iron oxide nanoparticles can be immensely enhanced by using external magnets. More experiments to address the effect of specific molecules in the brain, mainly for prolonged exposure, are essential for proper performance. In this study, multiple experiments were conducted to simulate BBB diffusion using ferrofluid; however, the biological complexity of the BBB is not explicitly represented through these experiments. Research addressing the roles of iron oxide nanoparticles and the BBB in relation to each other should be considered. In addition, more research targeting aspects and limitations contributing to the iron oxide drug delivery process must be evaluated to provide safe and effective treatments for neurological diseases. One major limitation is the inconsistent ability to predict results in preclinical studies, making it challenging to predict outcomes in human trials. The size, surface charge, and functionalization of iron oxide nanoparticles complicate the generalization of results across studies. Furthermore, external magnetic field (EMF) manipulations are difficult to translate into clinical experiments due to the limited information surrounding their overall functionality and factors involving their magnetism.

## ■ Acknowledgments

This paper was completed with the help of two guiding professors, Professor Kristina Lilova and Professor Virgel Torremocha. I am tremendously grateful for their support and understanding throughout this research paper.

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# UroBuddy: Enhancing Dignity And Comfort For Bedridden Geriatric Patients Through Smart Diaper Technology

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**ABSTRACT:** Many older people suffer from urinary incontinence, a prevalent illness that causes both physical and emotional anguish. To give caretakers real-time notifications, we created "UroBuddy," a clever, non-invasive moisture monitoring technology built inside diapers. An ESP32 microcontroller and a moisture sensor based on copper tape are part of the hardware, and the system uses Firebase Cloud Messaging to sync data with a mobile app. When moisture levels are above 50%, family members and caregivers are notified so that prompt action can be taken. The gadget is inexpensive, reusable, and simple to attach with magnets. The companion Flutter app facilitates user interactions and the logging of health data. This method uses useful, reasonably priced technology to address issues in elder care.

**KEYWORDS:** Biomedical and Health Sciences, Geriatric Monitoring, Smart Diapers, Moisture Detection, Real-Time Alerts.

## ■ Introduction

Although aging is a normal part of life, many older people find it extremely difficult to perform even basic tasks, particularly if they are bedridden or have long-term conditions like Parkinson's disease. Urinary incontinence, which affects more than 50% of residents in long-term care facilities worldwide, is one such problem.<sup>1-5</sup> In addition to discomfort and health hazards such as skin and urinary tract infections, it has a significant emotional cost. It frequently results in feelings of shame, worry, and diminished self-worth.

Urinary incontinence (UI), particularly in immobile elderly people, is a social and emotional problem in addition to a medical one.<sup>4</sup> Between 2015 and 2050, the proportion of the world's population over 60 years will nearly double from 12% to 22%.<sup>5</sup> Many bladder health management applications and devices are too clinical or require manual data entry, making them inconvenient for older adults to use.<sup>6</sup> This study presents UroBuddy, a smart diaper system that combines mobile notifications and real-time sensing to provide effective and respectful senior care. The device is compact and easy to use, with copper conductive tape for improved moisture detection coverage and magnets for a smooth connection. The system is enhanced with a mobile app built with Flutter that provides family members and caregivers with specific interfaces, as shown in Figure 5. The software includes a history tab that allows caregivers to track moisture and pH levels over time. For pH monitoring, a litmus paper is used, and the caregiver simply observes the colour change and manually enters the pH value into the app. A built-in chat feature facilitates communication between caregivers and family members, ensuring coordinated care.

For dependable notification delivery, real-time synchronization, and safe data storage, Firebase Realtime Database and Firebase Cloud Messaging (FCM) are used. At a 50% saturation threshold, experimental results show reliable and accurate detection. The system is an economical and efficient way to

care for the elderly because it is reusable after cleaning and costs less than ₹2000.

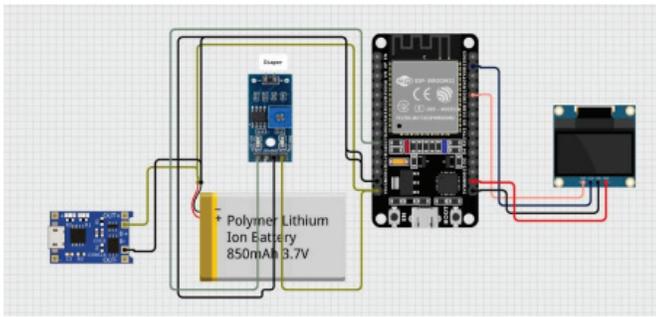
## ■ Methods

### *Hardware Components:*

The UroBuddy project's hardware device is based on a small, effective electronic setup intended for incontinence monitoring in real time. The central processing unit is an ESP32 microcontroller, which was chosen for its tiny size, low power consumption, and integrated Bluetooth and Wi-Fi. It receives information from a moisture sensor that uses conductivity changes to detect wetness and is based on flexible copper tape. After using the diaper, the device can be easily removed and reused. However, the copper tape acts as a one-time sensor layer and should be replaced with a fresh strip for the next use. When moisture reaches a predetermined threshold, the signal is conditioned using an LM393 comparator to provide a dependable digital output.

Power is supplied by a 3.7V, 850mAh Li-ion rechargeable battery, managed by a TP4056 charging and protection module, which allows safe recharging and extended usage of up to six hours per charge. And the battery required around 50 minutes to charge fully. To ensure convenience and portability, the entire circuitry is housed in a 3D-printed enclosure with openings for charging, a power switch, and a slot for a small 0.91-inch OLED display that shows real-time readings, as shown in Figures 2 and 3.

For ease of integration with diapers, the system incorporates neodymium magnets that allow the enclosure to be attached and detached outside the diaper without causing discomfort or exposure of the electronics to moisture. The lightweight and skin-safe enclosure ensures comfort for prolonged use, while smooth edges prevent irritation.



**Figure 1:** Circuit Diagram.

- **ESP32 Microcontroller:** Wi-Fi enabled for real-time data transmission.
- **Moisture Sensor:** Built with copper conductive tape and LM393 comparator.
- **OLED Display:** A 0.91" screen connected via I2C interface.

This sensor is composed of parallel copper strips that are secured within the diaper to function as electrodes. The other side is connected to LM393. When liquid penetrates the diaper, the resistance between these strips diminishes, resulting in a change in the sensor's output. UroBuddy system operates at a 3.3V logic level and draws minimal current (typically below 150 mA), which poses no risk of electric shock or discomfort to the user. All conductive parts are fully enclosed within the casing, and the diaper-integrated copper strips carry only low-signal sensing current (<1 mA).

To process this signal, the sensor was connected to an LM393 comparator module, which transformed the variable resistance into a clear analog voltage signal that the ESP32 can interpret.<sup>6,7</sup> The LM393 facilitated threshold adjustments through an on-board potentiometer,<sup>7</sup> enhancing the stability of the readings and minimizing noise compared to relying solely on raw copper tape, as shown in Figure 1.

#### **Experimental Setup:**

- Fluids tested: water and honey at volumes of 50 ml and 100 ml.
- Procedure: Each liquid was introduced into the diaper, and the sensor's response was documented in both analog (percentage moisture) and digital (wet/dry threshold crossing) formats.

#### **Moisture Calculation:**

The analog output from the LM393 module (spanning from 0–3.3V) was sampled by the ADC of the ESP32.<sup>7</sup> A dry baseline (0%) and a fully saturated reference (100%) were established. The percentage moisture was computed using:

$$\text{Moisture \%} = ((\text{Sensor Value} - \text{Dry Value}) / (\text{Wet Value} - \text{Dry Value})) \times 100$$

The estimated moisture content in ml was then calculated as:

$$\text{Moisture Content (ml)} = (\text{Moisture \%} / 100) \times \text{Fluid Volume (ml)}.^{11}$$

**Table 1:** Moisture content calculation across fluids.

Sample	Volume (ml)	Moisture (%)	Moisture Content (ml)
Water	50	63	31.5
Water	100	70	70
Honey	50	5	2.5
Honey	100	6	6

Tests were carried out throughout the UroBuddy project's experimental phase to assess the moisture sensing system's accuracy and reactivity in various scenarios. To test the ESP32 microcontroller's sensitivity and dependability, the moisture sensor was subjected to a range of fluid types and quantities. Parameters, including the fluid type, sample volume in millilitres (ml), system-detected moisture percentage, and associated computed moisture content, are all listed in Table 1. The ability of the copper tape-based sensor to discriminate between pertinent fluids—such as urine, which has a conductivity similar to that of water—and irrelevant ones—such as thicker, low-conductivity liquids—was validated with the aid of this structured data in Table 1.

With 100 ml of water yielding a near-saturation value of 70%, the findings in the table amply illustrated the sensor's strong sensitivity to conductive liquids like water, where bigger volumes correlated with higher moisture percentages. Conversely, even at higher volumes, viscous and less conductive materials like honey showed extremely low moisture percentages (5–6%). This demonstrates how the technology can prevent false alarms from non-urinary fluids, guaranteeing precise incontinence detection. As a result, the experimental Table 1 confirms the sensor's appropriateness for actual aged care applications in addition to highlighting its accuracy.

The device has not yet been tested on human subjects. All evaluations were conducted in a controlled environment under normal conditions. The testing process involved manually applying measured amounts of water and honey onto the diaper to observe the sensor's response and verify the device's functionality.

#### **Data Transmission:**

The ESP32 transmitted the processed values to Firebase,<sup>8</sup> and the UroBuddy Flutter app provided real-time alerts when the moisture surpassed the designated threshold ( $\geq 50\%$ ) as shown in Figure 4. This mechanism ensured prompt notifications for caregivers while differentiating between various fluid types.

#### **CAD Design:**

The system housing is modelled in SolidWorks, featuring a 0.91-inch OLED display in a rectangular slot that lets developers or caregivers see the device's moisture information. The device enclosure was 3D printed using PLA (Polylactic Acid) material, which is biocompatible, non-toxic, and skin-safe. Additionally, it has specific apertures for programming and charging, making it simple to access the ESP32. The shell included a push-on power button that allowed for easy on/off.

The enclosure's measurements were roughly 55 mm × 40 mm × 30 mm. The design of UroBuddy prioritizes comfort and ergonomics. The sensor strip is flexible and thin, seamlessly integrating into the diaper lining without adding noticeable bulk. And the device position is easily adjustable using magnets, so that it won't affect sleep.



Figure 3: CAD design.



Figure 4: Compact design for user comfort.

### Mobile Application (Flutter):



Figure 4: Real-time alerts.



Figure 5: Logging features for pH and moisture.

## Results and Discussion

When the moisture level rises beyond 50%, the ESP32 uses Firebase Functions to start a real-time notification, as shown in Figure 4. These serverless features provide the basis for communication without the need for a dedicated backend server, ensuring automated and seamless alert delivery. Authentication tokens, which Firebase Functions manage, verify, and authorize each request, guaranteeing secure and reliable access. Additionally, unique device tokens are maintained under the unique patient ID, allowing notifications to be sent to the right family member or caregiver. This systematic strategy ensures timely alarms, secure data handling, and personalized communication for effective senior care management.

Notifications were sent within 10–15 seconds of moisture detection. Firebase synchronization updated app data within 5–10 seconds. While the UroBuddy system proved effective, certain limitations were identified during testing. The durability of the copper tape-based sensor may be reduced over extended use or repeated washing, potentially affecting long-term reliability. Additionally, the system is susceptible to occasional false alerts triggered by factors such as sweat, high humidity, or accidental spills of water, which can mimic urinary moisture. Another limitation is its dependency on Wi-Fi

connectivity, as the current version does not support offline operation or Bluetooth Low Energy (BLE), restricting its usability in areas with poor internet access.

## Conclusion

The UroBuddy project successfully demonstrated that an affordable, reusable smart diaper system can enhance dignity, hygiene, and comfort for bedridden geriatric patients while easing the burden on caregivers. By integrating a copper tape moisture sensor with an ESP32 microcontroller and a Flutter mobile app, the system reliably provided real-time alerts and data logging. UroBuddy addressed the project's original aim of creating a low-cost, user-friendly, reusable system that improves quality of life for patients and simplifies caregiving. Beyond its technical achievements, it highlights how empathetic, user-centered design can transform eldercare. Future work should involve direct caregiver and patient trials and improved sensor durability.

## Acknowledgments

The Innovation Story in Mumbai, where I worked on UroBuddy's development, provided the resources and support that made this idea possible. I received invaluable guidance from my mentors, Lavkush Chaudhary and Janhavi Karande, in electronics, CAD design, and programming. Their unwavering support, insightful advice, and openness to discussing ideas played a major role in the project's success. I am also deeply grateful to Dr. Samir S. Shirodkar, Consultant Urologist, whose insightful comments helped shape the project's relevance and usefulness for senior incontinent patients. His knowledgeable observations ensured that the system aligned with real medical needs, and I sincerely appreciate his help and encouragement.

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# A Review of Microplastic Contamination in Wastewater-Derived Sewage Sludge

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**ABSTRACT:** Microplastics, which account for 90% of plastic pollution, pose serious physical and toxicological threats to the environment and, by extension, human health. A significant amount of research has been conducted to assess microplastics in various types of water and wastewater treatment plants. However, there is limited knowledge about microplastics in the byproduct of wastewater treatment plants, sewage sludge. Wastewater treatment plants are moderately effective in reducing microplastics in effluent water. Paradoxically, the removed microplastics become concentrated in the sewage sludge. This is often discharged to the land, used as fertilizer for agriculture, or used for land refill, and poses a critical environmental problem. Sewage sludge discharge can be a significant point source of land contamination, thereby impacting terrestrial ecosystems, crop health, and human health, which warrants further study. Addressing microplastic pollution in sewage sludge is a complex challenge due to several factors, including a lack of standardized methods for detecting and monitoring microplastics, the need for long-term studies on the soil and the pathways of spread, the absence of current treatment technologies to eliminate microplastics, and a lack of public awareness and policy. This paper aims to present an overview of microplastics in sewage sludge.

**KEYWORDS:** Earth and Environmental Sciences, Environmental Effects on Ecosystems, Microplastics, Sewage Sludge, Wastewater Treatment.

## ■ Introduction

Since the 1970s, the rate of plastic production has grown faster than any other material, with over 9.1 billion tons of plastic produced since the 1950s.<sup>1</sup> Most of the plastics are made from fossil-fuel-based hydrocarbons. It is estimated that approximately 4.8 to 12.7 million tons of plastic waste enter the oceans from coastal countries each year.<sup>2</sup> Microplastics are plastic particles that are smaller than 5 mm in size.<sup>2</sup> They can be classified as arising from primary or secondary sources of microplastics based on whether they were intentionally made at this size or if they have degraded from larger plastics to this size. Primary microplastics can come from microbeads, nurdles (plastic pellets), tire wear, synthetic textiles, and many other sources. Primary microplastics include polymers such as polyethylene, polypropylene, and polystyrene particles, which are found in cosmetic and medical products, as well as in sludge from the washing and wearing of synthetic textiles and fibers.<sup>3</sup> The wear from tires and road markings may also cause microplastic pollution into rainwater runoff, which is a pathway for carrying tire and road wear particles to surface waters.<sup>3-5</sup> These enter the wastewater and are processed along with the wastewater as it enters wastewater treatment plants. Secondary microplastics originate from the degradation of plastics, including plastic litter, fishing gear, packaging materials, and other sources of plastic waste.<sup>6</sup> Secondary microplastics originate from activities such as littering and waste disposal processes.<sup>3</sup> These can also enter wastewater streams and are processed in wastewater treatment plants.

Microplastics are important to understand due to the direct and indirect risks they pose to marine/aquatic animals, as well as humans. The direct injury is caused by the small size and

density of microplastics, which allows them to be consumed/absorbed by various microorganisms and animals.<sup>7,8</sup> They can interfere with the food source and consumption, leading to obstruction and a lack of feeding, which can lead to the eventual death of marine creatures. Indirect damage from microplastics can occur when microplastics adsorb heavy metals and other organic pollutants, such as polycyclic aromatic hydrocarbons (PAHs), chlorinated organic pesticides, and poly-chlorinated biphenyls (PCBs), referred to as persistent organic pollutants (POPs).<sup>7,8</sup> These create long-term consequences like cancer, immune-related problems, and endocrine disruption. According to the Organization for Economic Cooperation and Development, nearly 1.7 million tons of plastic waste enter the oceans, while an additional 6.1 million tons are discharged into various water bodies.<sup>9</sup>

The purpose of this review article is to understand the presence and importance of microplastics in sewage sludge.

## ■ Discussion

### *Waste Water Treatment Plants (WWTP):*

Wastewater Treatment Plants (WWTPs) are facilities that receive wastewater and treat it through physical, chemical, and biological processes to remove contaminants before reintroducing the treated water back into the environment. The inflow to a wastewater treatment plant includes domestic sewage, industrial wastewater, rainwater runoff, and groundwater leakage into sewers.<sup>2</sup> These waters often contain tirewear microplastics, microplastic fibers from the washing of synthetic fibers, overflow water in extreme weather, etc. These waters are treated in a WWTP, and the effluents are discharged into wa-

ter bodies or used for non-potable water requirements, or they can be discharged into the soil to supplement it.

Microplastic removal for most wastewater treatment ranges from 84% to 99%.<sup>10</sup> The removal rate from wastewater varies by the WWTP and can depend on the microparticle size, shape, density, and surface characteristics. Additionally, the rates of reported removal may vary significantly due to the techniques used for sampling, particle size detection, removal methods, sample volume, and other factors.

#### ***Microplastics in Solid Sewage Sludge (SeS):***

The microplastics removed from the water are concentrated in the solid material that forms the solid sewage sludge (SeS). SeS is a byproduct of WWTP. Microplastics found in the wastewater must be removed from the water before it is used for non-potable purposes or discharged into water bodies. Sludge treatment varies significantly but typically involves processes such as thickening, stabilization, aerobic and anaerobic digestion, conditioning, dewatering, and heat drying. The purpose of the sludge treatment is to reduce water and eliminate microbes and gases. Microplastics can survive the sludge treatment and remain in the SeS. SeS can be used as landfill, incinerated, or applied to agricultural land as a fertilizer. In some cases, SeS can be accidentally disposed of on the land.<sup>11</sup> Since microplastic removal from SeS is not the aim of the sludge treatment, microplastics will be present and can become concentrated in this sludge, and can persist in the soil for long periods.<sup>12</sup> SeS can be a significant source of microplastic contamination into soil, soil animals, microbes, and plants.

#### ***Importance of Microplastics in Sewage Sludge:***

Despite the high number of microplastics in the wastewater influent to WWTP, most of the wastewater treatment processes are effective in removing 84 to 99% of microplastics from the wastewater.<sup>13,14</sup>

This relatively high efficiency of removal necessitates that these "removed" microplastics concentrate in sludge, a byproduct of wastewater treatment. Sludge consists of primary sludge (fecal matter) and post-treated activated secondary sludge (biosolids).

20% to 60% of a WWTP's total operating costs are related to sludge management, which is disproportionately high considering that sludge accounts for only 1 to 2% of the effluent volume.<sup>15</sup> SeS is rich in organic material, phosphorus, and nitrogen, and can be very effective in improving soil quality. The most common method used to handle sludge is its application to agricultural lands.<sup>16</sup>

Utilizing SeS as a soil fertilizer or in landfills is one way to recycle waste materials in a circular manner. Due to the presence of microplastics in SeS, the use of SeS in agricultural lands can introduce microplastics into the soil, contaminating terrestrial ecosystems. These particles may also be transported into nearby water bodies through surface water runoff and can also impact aquatic environments. These microplastics can also be ingested by animals in the soil and introduced into the food chain, potentially affecting crop production, crop health, and human health.

They can create long-lasting contamination of agricultural lands, reducing the overall productivity of these lands in growing crops and raising concerns about maintaining agricultural growth and food security.

#### ***Treatment of Sewage Sludge:***

Several processes can be used to treat sewage sludge, including stabilizing, thickening, drying, dewatering, composting, anaerobic digestion, lime treatment, and thermal treatment. Conventional sludge treatment methods have not demonstrated a reduction in microplastic concentration. Composting has been shown to alter the surface structure and reduce the number of microplastics in sewage sludge in China.<sup>17</sup> Composting is aerobic biodegradation of organic material, which is exothermic. This raises the temperature in the sludge to over 70°C, which can cause degradation and fragmentation of microplastics within the sludge.

#### ***Concentration of Microplastics:***

It is proposed that the concentration of microplastics in the influent to a WWTP determines the concentration of microplastics in the SeS.<sup>18</sup>

In recent review articles on this topic, a wide range of concentrations has been observed, varying by country of origin and specific WWTP (Table 1). Microplastics can range anywhere between 0.37 and 495,000 microplastic particles per gram of SeS. Since the weight of microplastics can be difficult to measure, a quantitative method of counting particles per fixed weight of the sewage sludge is used and is described as MP/g (number of microplastics/gram of sewage sludge). Other reasons for microplastics concentration variability in wastewater and SeS include differences in urbanization, population density, plastic use, and the number of surrounding industries. Regions with advanced waste management practices have lower microplastic loads compared with regions with inadequate waste management practices. Warm seasons are associated with higher concentrations of microplastics in influent water and, consequently, in sewage sludge.<sup>19</sup>

Repeated sludge applications are associated with higher concentrations of microplastics in agricultural soil, which persist over time.<sup>20</sup>

In addition to these reasons, the variability in microplastics in sewage sludge may be related to improper or inconsistent sampling and differences in testing methods.<sup>21</sup> There is a need for standardized procedures for collecting, pretreating, and testing microplastics in SeS.

**Table 1:** Concentration of reported microplastics as the number of Microplastics per gram of sewage sludge from review articles showing the range of microplastic concentration detected.

Author	Title	Journal	Minimum MP/g	Maximum MP/g	Number of sites
Charles Rolsky <i>et al.</i> <sup>22</sup>	Municipal sewage sludge as a source of microplastics in the environment	Current Opinions in Environmental Science and Health	0.45	113	67
Maliheh Arab <i>et al.</i> <sup>23</sup>	Microplastics in Sludges and Soils: A Comprehensive Review on Distribution, Characteristics, and Effects	Chemical Engineering	510	495,000	22
Sarra Hechni <i>et al.</i> <sup>24</sup>	Soil contamination with microplastics (MPs) from treated wastewater and sewage sludge: risks and sustainable mitigation strategies	Discover Environment	18	240,300	23
Fahir Hassan <i>et al.</i> <sup>25</sup>	Microplastic contamination in sewage sludge: Abundance, characteristics, and impacts on the environment and human health	Environmental Technology & Innovation	0.37	169,000	13
Daisy Harley Nyang <i>et al.</i> <sup>26</sup>	Investigation and analysis of microplastics in sewage sludge and biosolids: A case study from one wastewater treatment works in the UK	Science of the Total Environment	0.51	169,000	65

**Size and shape of microplastics:**

As shown in Table 2, more than 80% of microplastics in sewage sludge are less than 500 micrometers in size.<sup>27</sup> It is thought to be related to the quick adsorption of small microplastics onto sewage. Larger microplastics can interfere with flocculation and sludge dewatering.<sup>28</sup>

Conesa and Ortuno classified the types of microplastics into different shapes, including microfibers, fragments, spheres, and granules.<sup>29</sup>

The most common types were microfibers, followed by fragments and films. These account for 60 to 85% of all microplastics in sewage sludge.<sup>23</sup>

**Table 2:** The size and shape of microplastics in sewage sludge.

Author	Title	Journal	Year	Purpose	Size Distribution Findings	Type (Fiber/Fragment)
H Kang <i>et al.</i> <sup>30</sup>	Occurrence of microplastics in municipal wastewater treatment plants	Environmental Health and Toxicology	2018	Assess microplastic presence in WWTPs' sludge	>80% of microplastics <500 µm; most commonly detected: 20–300 µm	Fragments are dominant; some fibers
X Li <i>et al.</i> <sup>31</sup>	Microplastics in sewage sludge from wastewater treatment plants in China	Water Research	2018	Study microplastic occurrence and risks in sewage sludge	60–90% <500 µm; particles <100 µm dominated sludge samples	Fibers are dominant, followed by fragments
Corradini <i>et al.</i> <sup>20</sup>	Evidence of microplastic accumulation in agricultural soils from sewage	Science of the Total Environment	2019	Examine microplastic transfer via sludge to soil	Peak particle size range: 100–300 µm; particles <1 mm comprised >90%	Fibers and fragments were both found; fibers are more prevalent
NB Turan <i>et al.</i> <sup>32</sup>	Microplastics in wastewater treatment plants: Occurrence, fate, and identification	Process Safety and Environmental Protection	2021	Review detection methods and fate of microplastics in WWTPs	~70–85% of detected microplastics in sludge <500 µm	Fibers standard; fragments also observed
Ziajahromi <i>et al.</i> <sup>33</sup>	Wastewater treatment plant effluent as a source of microplastics: review of the fate, chemical interactions, and potential risks to aquatic organisms	Water Science & Technology	2016	Review WWTPs as microplastic sources	The majority of microplastics in sludge are <1 mm; fine particles are prevalent.	Fragments and fibers both reported
Liu <i>et al.</i> <sup>34</sup>	Effects of microplastics on the properties of different types of sewage sludge and strategies to overcome the	Science of the Total Environment	2023	Characterize sludge microplastics and assess removal strategies	>50% of microplastics were <300 µm; larger particles (>1 mm) were rare	Fragments are slightly more common; some films and fibers
Gatidou <i>et al.</i> <sup>35</sup>	Review on the occurrence and fate of microplastics in Sewage Treatment Plants	Journal of Hazardous Materials	2019	Review the distribution, fate, and management of microplastics in WWTPs	75–95% of particles <500 µm; distribution varies based on treatment technology	Fibers are dominant in most samples

**Microplastics Polymer Composition in Sewage Sludge:**

The most common type of polymers seen were the polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), and polyamide (PA), followed by polyester, polystyrene (PS) and polyvinyl chloride (PVC) as shown in Table 3.<sup>23</sup> This maybe because polyethylene and polypropylene are the most

used plastics, and polyester and polyamide are frequently released with washing synthetic clothes.<sup>27</sup>

**Table 3:** Polymer composition of microplastics seen in sewage sludge on different articles.

Author(s)	Title	Journal	Year	Polymer Composition Findings
H Kang <i>et al.</i> <sup>30</sup>	Occurrence of microplastics in municipal wastewater treatment plants	Environmental Health and Toxicology	2018	PE, PP, and PS are the most common; some PET and PVC
X Li <i>et al.</i> <sup>31</sup>	Microplastics in sewage sludge from wastewater treatment plants in China	Water Research	2018	PET, PE, PP dominant; trace PS and PA
Corradini <i>et al.</i> <sup>20</sup>	Evidence of microplastic accumulation in agricultural soils from sewage	Science of the Total Environment	2019	PET and PE are prevalent; also detected are PP and PS
NB Turan <i>et al.</i> <sup>32</sup>	Microplastics in wastewater treatment plants: Occurrence, fate, and identification	Process Safety and Environmental Protection	2021	PE and PP are dominant in sludge; PET is also found
P Kay <i>et al.</i> <sup>36</sup>	Wastewater treatment plants as a source of microplastics in river catchments	Environmental Science & Pollution Research	2018	Varied: PE, PP, PET, PS, PVC in different proportions
Liu <i>et al.</i> <sup>34</sup>	Effects of microplastics on the properties of different types of sewage sludge and strategies to overcome the inhibition: A review	Science of The Total Environment	2023	PP and PE major types; some PS, PET, PA
Hu <i>et al.</i> <sup>37</sup>	Current research trends on microplastic pollution from wastewater systems: a critical review	Environmental Science and Technology	2019	PET and PE are the most reported; regional differences affect composition

\*PET = polyethylene terephthalate, PE = polyethylene, PP = polypropylene, PS = polystyrene, PVC = polyvinylchloride, PA = polyamide

### *Surface morphology of microplastics in sewage sludge:*

The surface of microplastics in sludge demonstrates varying degrees of deterioration.<sup>28</sup> This may be the result of the sludge treatment process and causes these microplastics to have scratches, folds, and aggregated structures.<sup>31</sup> These are thought to be areas where microplastics can readily trap heavy metals and other organic pollutants. Eventually, these microplastics will fragment along the folds or scratches, forming even smaller microplastics.

### *Effects of microplastics on soil and plant growth:*

Microplastics have been shown to decrease soil bulk density and increase the water-holding capacity of soils. These changes mimic the effects of waterlogging, which can result in increased susceptibility to pests/diseases, root decay, and a reduction in soil oxygen levels.<sup>38</sup> Microplastics can also increase microbial activity in the soil, which can have long-term consequences on the soil.

Research indicates that the effect on plants is complex, with an increase in root length and root surface area but a decrease in shoot length and shoot biomass.<sup>39</sup> The increased root growth may be due to altered soil porosity and increased water in the soil due to the presence of microplastics. These findings suggest an adverse effect on overall plant health due to the negative impact on shoot growth, which indicates a decrease in nutrition to the shoots.

Microplastics have also been shown to absorb persistent organic pollutants (POPs) such as pesticides and dioxins and furans (byproducts of combustion and incineration). These are carried by microplastics from the soil to the plants, where they desorb and get deposited in the plants. In addition, polycyclic aromatic hydrocarbons can be absorbed by microplastics, increasing their bioavailability to plants and other organisms. This process of bioavailability and bioaccumulation of chemicals such as POPs and PAHs can threaten our entire ecosystem by affecting both plant life and animal life.<sup>40</sup>

## ■ Conclusion

Wastewater treatment plants have improved their efficiency in removing microplastics from wastewater. This efficiency has paradoxically shifted the burden of microplastics onto one of its byproducts, sewage sludge. Sewage sludge is routinely applied to agricultural land and landfills and can be accidentally discharged into the environment, introducing microplastics into both soil-based and aquatic ecosystems. Most microplastics are fine fibers less than 500 µm in size, as discussed earlier. Microfibers may get concentrated in sludge due to their long and thin shape, which allows them to bypass some filters. Commonly found microplastics in sewage sludge include polyethylene, polypropylene, and polyethylene terephthalate. Sewage sludge is an often-overlooked source of microplastic contamination in agricultural lands, which can impact the soil, soil animals, crop growth, and human health. The number of microplastics in agricultural lands increases with the number of sludge applications. It can be reasonably surmised that microplastic concentrations in agricultural lands are and will become an even more serious problem. Microplastics have a

myriad of adverse effects, including their impact on plants and their ingestion by microorganisms and other animals, and their profound effect on the health of animals and humans. This underscores the need for standardizing global methods for detection and quantification, implementing mandatory regulations related to microplastics monitoring in WWTPs and sewage sludge output, improving existing treatment of sewage sludge with proper disposal procedures to reduce and prevent microplastics' entry into the environment, and investing in innovative technologies to degrade and minimize microplastics in sewage sludge.

## ■ Acknowledgments

I want to thank Dr. Ramphul and Dr. Sharma for their mentorship and support throughout this research project.

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# Reframing ADHD: How Attention Shapes Adult Dynamics

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**ABSTRACT:** Attention Deficit Hyperactivity Disorder, commonly known as ADHD, is a prevalent psychiatric disorder. There are three different subtypes of ADHD: hyperactive, inattentive, and combined. This paper examines the impacts of ADHD on interpersonal relationships, particularly focusing on communication, social skills, and emotion regulation. Evidence suggests that studies involving children are far more detailed than those involving adults, leading to a notable gap in understanding adult development. This study analyzed data obtained from a survey developed to assess the experiences of adults with ADHD. As shown, ADHD has a remarkable influence on countless lives. By examining the obstacles adults with ADHD face, researchers can better understand how to support the development and maintenance of healthy relationships over time. The data revealed that individuals with combined-type ADHD may experience greater difficulties in their relationships compared to the other subtypes. The analysis further revealed that individuals with ADHD recognize social cues and body language, but experience difficulty in responding appropriately.

**KEYWORDS:** Intrapersonal Relationships, ADHD, Emotions, Communication, Regulation.

## ■ Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is prevalent among children, and studies have determined that it persists into adulthood in over half of the cases. ADHD affects a person's ability to focus and manage their impulses. From an early age, the ability to form and maintain relationships is greatly restricted in patients with ADHD. Extensive research has been conducted on children over the past years; however, research on adult relationships is minimal. ADHD has proven to significantly impact relationships from a very young age.<sup>1</sup> This paper aims to analyze the issues adults with ADHD face in social settings while focusing on their understanding of social cues by gathering data through a survey with real-life examples.

## ■ Literature Review

ADHD directly impacts overall well-being, including the ability to organize tasks and make decisions. This disorder is relatively common, as around 5-7 percent of the population globally is affected by ADHD.<sup>2</sup> It can significantly impact daily living tasks, mainly through difficulties with maintaining focus and frequent forgetfulness. ADHD is separated into three different subtypes: inattentive, hyperactive, and combined. Hyperactive symptoms include constant excessive movement, difficulty remaining seated, and making impulsive decisions or actions. Inattentive symptoms include difficulty paying attention, organizational issues, or time management. Combined symptoms include difficulty paying attention, impulsive decisions, struggles with organization, and constant movement.<sup>3</sup>

One similarity among the three subtypes is their significant impact on relationships. Relationships are the basis of human nature; individuals thrive on relationships, and without stability, life can become less fulfilling. Research suggests that ADHD impacts the ability to converse in public settings, as individuals

with ADHD often get distracted.<sup>4</sup> For example, during conversation, those with ADHD lack the ability to pay constant attention, which leads to ignorance or interruption throughout the discussion.<sup>4</sup> A common issue faced by those with ADHD is impulsivity and a deficiency in understanding non-verbal signals. This includes a tendency to interrupt conversations, which, due to impulsivity, can discourage others from wanting to pursue relationships or friendships.<sup>5</sup> Children with ADHD often face challenges in building and sustaining friendships with their peers, with 50 to 60 percent affected.<sup>6</sup> Often lack of understanding and awareness impacts their understanding of social cues.<sup>4</sup> This creates challenges in relationships, as those with ADHD struggle to comprehend emotional dynamics and the feelings of others.

Additionally, those living with ADHD sometimes lack the emotional connection necessary to understand the viewpoints of others, which can be limiting. Because of this disconnect, individuals without ADHD may find the behavior of those with ADHD, especially those at a younger age, slightly off-putting, as their interpretation of society is different from their neurotypical counterparts. Those experiencing ADHD often overestimate their understanding of interpersonal skills from a very young age.<sup>7</sup>

ADHD can make it extremely difficult for one to grasp their environment, as they are often preoccupied with their attention deficit issues, leaving them less proficient at receiving nonverbal signals than others. Individuals are often hyper-focused on the completion of certain tasks, resulting in forgetfulness or completely ignoring their surroundings.<sup>4</sup> This can, in turn, cause them to overlook social cues entirely, as even focusing on the conversation itself becomes a challenge.

While these findings may carry into adulthood, research is primarily conducted on children. Current studies on adults with ADHD are significantly scarcer than studies on children;

thus, research examining how ADHD impacts adults in social roles is virtually nonexistent. This paper targets the complications that adults with ADHD face in social settings and their understanding of social cues.

It is crucial to understand how ADHD affects social relationships in adults, as it is currently known to hinder their ability to function effectively and engage in conventional social events. Understanding the challenges that individuals with ADHD face is necessary so that we can effectively intervene and help them create meaningful connections. Building on previous studies, this study hypothesizes that adults with ADHD face significant challenges in relationships and friendships due to difficulties in analyzing cues and regulating emotions.

## Materials

A laptop was the source for data collection and analysis. Google Forms was used for constructing and administering the survey for convenience. To get an appropriate and relevant population, the survey was distributed via CHADD (Children and Adults with Attention-Deficit/Hyperactivity Disorder), a non-profit organization for people with ADHD. This engaged a population that was credible.

## Methods

For data collection, an online-based survey was developed based on existing literature. Participants included adults formally diagnosed with ADHD and those without a formal diagnosis but whose symptoms were consistent with ADHD. The survey consisted of individuals with all three subtypes: hyperactive, inattentive, and combined. It also consisted of individuals who were diagnosed but were unsure of their subtype.

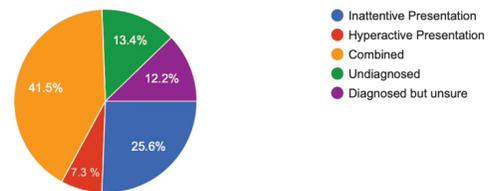
The survey was distributed to people via CHADD (a non-profit ADHD organization that requires membership for access to the survey). A convenience sample was taken, which resulted in a sample size of 81. To qualify for the study, participants must have been 18 or older and identified as having ADHD. The survey included 14 questions, including both multiple-choice and open-ended questions for primarily qualitative data. Participants completed a consent form prior to completion of the survey, ensuring complete privacy and confidentiality. The questionnaire was reviewed and approved by the Institutional Review Board (IRB) prior to starting the study. The questions were primarily based on the biggest social issues that those with ADHD face, including their feelings when encountering a new group of people or their understanding of social cues.

The survey included participants residing in the United States. The participants had a mean age of 45.61 years old ranging from 19 to 79.

**Table 1:** Survey Questions Presented to Participants

Item No.	Survey Question
1.	Please state your age.
2.	Are you currently taking any kind of medication for ADHD?
3.	Do you work in the healthcare field?
4.	Which type of ADHD have you been diagnosed with?
5.	If you choose undiagnosed or diagnosed but unsure in the question above, please choose the answer that describes you the most.
6.	What are the biggest social challenges you face, please give examples?
7.	Do you feel you have a good understanding of social cues, similar to those without ADHD? Please give examples.
8.	Do you find yourself constantly wanting to interrupt conversations and/or difficult to not zone out of a conversation? Please give examples.
9.	Do you find yourself approaching a new group of people by trying to fit in, or rather gaining their attention?
10.	Do you find yourself reacting in situations, that those (who do not have ADHD) around you completely disregard?
11.	Do you find it difficult being empathetic in situations that it is necessary? Please give examples.
12.	How do you view friendship, like someone you can rely on or someone who you can only have a good time with? Please give examples.
13.	Do you feel like your peers treat you differently than others?
14.	Do you find that having ADHD affects your relationships significantly? Please give examples.

Which type of ADHD have you been diagnosed with?



**Figure 1:** Displays the responses for each subtype, including those who are undiagnosed as well as those who have been diagnosed but remain uncertain. The survey consisted of individuals with all three subtypes: hyperactive, inattentive, and combined, along with individuals who were diagnosed but were unsure of their subtype, and eight individuals were undiagnosed. This ensured that the data was not skewed or biased.

### Broader Social Interactions:

The survey focused on social interactions and real-life examples of issues that people with ADHD face. It included questions about social challenges they encountered and what they understood about social cues. The questions gave individuals the ability to share what they considered to be the most significant issue or challenge based on their personal experiences. These were designed to be open-ended and lacked specific guidelines, unlike other questions in the questionnaire.

### Specific Impairment:

Some questions were detailed and specific, including asking about the urge to constantly interrupt conversations, how to approach a new group of people, or the ability to be empathic. Those questions focused on a different impairment of ADHD. While each question may have been fixated on a specific topic, the responses were open-ended and varied based on experiences.

### ***Relationships:***

Other questions concentrated on the dynamics of relationships, involving those who had ADHD. Including how they viewed friendship, how they were treated by peers, and the impact of ADHD on relationships. These questions were directed to the influence of ADHD on all adult relationships.

### ■ **Analysis**

The data from each question were qualitatively analyzed. It was separated based on theme, and then each theme was analyzed. Similar answers were then further dissected, and summaries of each answer were written in a chart. Key examples were identified and included in this paper. Where relevant, quantitative sums were used to determine how many out of 80 dealt with the same issues or had the same beliefs.

### ■ **Results**

The analysis suggested three primary themes: social challenges, social cues, and relationships. ADHD is known to significantly hinder the ability to maintain relationships and thrive in social environments.

### ■ **Social Challenges:**

Common social challenges included time management, being on time, procrastination, and only completing a task when necessary. A recurring theme among many of the participants was issues with time, as their thought processes differed substantially from those of the average person. Their brain was often overwhelmed with the thought of completing the task; they felt lethargic and unable to move. One participant noted, "I can't sit down to complete tasks or assignments unless there's an insane amount of pressure to complete something quickly". This highlights the difficulty of completing a task when there is no intense motive.

In addition, the survey suggested that many faced difficulties paying attention during conversations in loud areas with music. They were so focused on filtering out the music that it impaired their ability to concentrate on the conversation. A constant fear was that, because of this difficulty, participants would not be able to fit in, and many noted anxieties about this situation. For example, 60 out of 80 participants stated a constant sense of concern about not fitting in. Contrary to popular knowledge, individuals with the disorder were aware of their tendency to overshare.

Organization was also a prevalent issue among most individuals, as the simple thought of organization completely exhausted the mind. The situation overwhelmed them; this led to stress over where to start and which task to tackle next, instead of focusing on cleaning. This generated the idea of seeming unorganized and messy to their peers. One participant explained, "They see my disorganization, and I feel that they get frustrated and want to take over some tasks. It usually makes me feel insecure". The data showed the individuals were aware of their disarray and even felt others were judging them for it. Responses from peers made it extremely difficult to properly communicate or create a lasting relationship.

Furthermore, many overthink responses during a conversation in large groups, forcing them to stay silent. Large groups were another struggle for individuals as they required a lot more energy, shifting between topics with no clear flow, which made it difficult to create a flow in conversation from beginning to end. Sensitivity also made conversations very demanding, as a participant recognized, "I am very sensitive and tend to view negative things as being said about me." Even if the negative comment was not made about them, they have shifted to a reactive mode, no longer staying focused on the topic. The participants noted that they were well aware of their difficulty in conversation and therefore assumed people spoke negatively about them. This provoked their ability to pay attention and properly engage in the conversation.

### ***Social Cues:***

Around 50% of the individuals agreed to understanding social cues, while the other half mentioned struggling with such cues. For example, one participant mentioned, "I get confused by the social cues, and at times I need to ask the other person what they mean exactly." While another participant noted, "Yes, I'm good at reading people". These two quotes symbolize the difference in functional challenges those with ADHD face. It reveals that each individual can face completely different issues having the same mental disorder. Many endorsed correctly interpreting facial expressions, tone of voice, reading a room, analyzing the emotions of people, and seeing if someone around them was nervous or anxious. However, understanding these emotions, many found themselves surrounded by too many emotions to cope with. They reported that reacting was extremely difficult, as they were overwhelmed. Each participant struggled with some portion of a relationship, whether that may have been reading a room or finding the correct words to communicate. Although their basic problem may be different, they caused the same result of not being able to properly maintain a relationship.

Most individuals without ADHD believe those with the disorder lack emotional understanding.<sup>8</sup> However, they lack the understanding of how to respond to the situation. For example, one participant mentioned, "While I can pick up on social cues, such as subtle gestures or tones of voice, I often overanalyze them or feel overwhelmed by how to respond". Individuals get anxious, constantly feeling judged by their surrounding peers, who seem to have no understanding of the event taking place. ADHD can lead to social anxiety, as the participants noted, due to previous experiences and trauma from their past. The primary issue was that, by the time they determined how to respond, the time to respond had already passed. Comprehending emotions was not difficult, but it was rather difficult to stop oneself from obsessively rereading the behavior. From an external perspective, it might seem like individuals with ADHD struggle to understand emotions. However, in some cases, they may simply find it difficult to respond appropriately, often feeling judged or misunderstood.

### **Relationships:**

A common theme among the participants was that relationships often felt like a task, rather than a source of enjoyment and reliability. While friendship was seen as someone to rely on and enjoy, staying in contact was difficult. They often went prolonged periods without contact, such as forgetting to respond to texts or calls, as they struggled with communication. This then led to overcompensation, followed by a realization of the complexities of maintaining a relationship. For instance, one participant explained, "I might randomly reach out to everyone I've ghosted and check in and overcompensate for my absence; however, the second that the active engagement is expected of me or the nice favors and effort I put in becomes expected, I suddenly cannot do it." This forces the creation of a never-ending cycle, as preserving relationships feels like a job with a constant, overwhelming feeling.

Another typical misunderstanding was that individuals with ADHD did not realize when they were interjecting in conversations.<sup>9</sup> However, participants from the survey noted that they interrupted conversations, as they felt the need to express their thoughts before they were forgotten. Frequently concentrating so hard on remembering what to say, rather than paying attention to the actual conversation, makes them seem uninterested or ignorant. While many of these participants resorted to excessive talking, many believed they were unaware of what they were doing. However, they were well aware and even comprehended the body language cues that indicate discomfort from others. Despite understanding the situation, they simply could not regulate their speech due to impulsiveness.

Overall, over 88% of participants agreed that ADHD significantly affected social roles. Participants explained that relationships felt emotionally draining and extremely overwhelming. They found it difficult to fully connect with others due to the significant expectations that come with it. One participant explained, "I also feel like I am analyzing people or expecting the worst in people (like expecting them to fail me)." Partially because of previous experiences and their existing fear of past awkward social relationships, the data suggested many were unable to trust those around them. This forced a wedge between each relationship, whether it was desired or not. Participants also suggested that a supportive, dependable partner is extremely important, as many require a person to rely on.

### ■ **Discussion**

The purpose of the survey was to understand how ADHD affects adult social relationships and to what extent. The data suggested that most participants believed that ADHD significantly impacted relationships in several ways. Most participants noted that they often felt surrounded by expectations that felt impossible to uphold. The research also suggested that some participants understood everything happening around them, while simply unsure of how to express themselves, forcing them to stay silent. Individuals also stated that they were aware of interjecting statements but were simply unable to stop themselves. The respondents experienced a sense of judgment for each response they gave, which made them overthink everything. Based on the data, an inference is made regarding

how individuals can be helped. People need to understand that individuals with this disorder require blunt and unambiguous answers. Peers, family members, and psychologists all need to create an environment that shows them that their answers and behavior are not constantly being judged. Others must treat these individuals' emotions similarly to the way they treat everyone else. Treating individuals with ADHD with patience and respect when they struggle to adapt or respond will help reduce their social anxiety.

### *Limitations:*

Some limitations include the limited sample size, with a little over 80 responses, but this is a respectable sample for qualitative analysis. Due to the limited sample size, the three different ADHD presentations did not have an even split. Since there was not enough information, a clear conclusion about the differences among presentations could not be drawn. Several of the participants were undiagnosed, and the responses were limited to the United States. However, removing the undiagnosed participants would not have led to a different conclusion. Other limitations include a lack of physical communication, as the survey was completed online. Due to the survey being conducted online, any misunderstandings regarding the questions or responses provided by participants could not be addressed, confusing both sides. This survey was completely anonymous and collected little data about the actual participants' protection; participant gender and race were not assessed. While this approach minimized potential risks, it also limited the ability to examine demographic differences or assess the representativeness of the sample. Given that this study has a small sample size, some limitations are an increased risk of bias, higher variability, and limited generalizability. In subsequent studies, a greater sample size, along with deeper questions specifically focusing on social relationships, would be useful. Future studies should aim to study the differences in behavior among the three different presentations and the gender differences.

### ■ **Conclusion**

The results suggested that neurotypical individuals often doubted the level of understanding of those with ADHD, as some can comprehend social cues, and most understand the emotions experienced. However, they are simply unsure about what to do with the information they just grasped. Many individuals refrain from responding in conversations due to a fear of being judged based on their answers, often overanalyzing the situation long after the moment has passed. This may lead to these individuals appearing uninterested, shy, or unaware. This is especially true during extremely emotional situations, as the overload of emotions makes it difficult for them to respond and focus on each feeling. When emotionally intense situations arise, individuals with ADHD may stay quiet, not reply, or overreact. While neurotypical individuals may view this as being apathetic, they do not understand that the person with ADHD may be experiencing a sensory overload. It may be useful for family members, peers, and others interacting with individuals with ADHD to explicitly state how they want them to react or what they need from that person.

## ■ Acknowledgments

I would like to express my gratitude to all those who have supported me throughout this research project. I am deeply grateful to CHADD (Children and Adults with Attention Deficit/Hyperactivity Disorder) organization for allowing me to spread my survey among their members. Without their constant support, the data for this project would not have been collected. I would also like to thank Olivia Losiewicz for her invaluable guidance and insights. Finally, I would like to thank my family and friends for their constant encouragement throughout this process.

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## ■ Authors

Veda Sheth is a high school student whose experiences living with a neurodivergent individual inspired an interest in learning about ADHD and supporting those who live with it. This passion prompted her to create a project aimed at helping adults with ADHD by providing access to resources and community support. Veda is also the host of the Instagram podcast ADHDconnects, which explores how industrial-organizational psychology can improve the workplace for individuals with ADHD. Through these initiatives, Veda strives to promote inclusivity and create a community where everyone can thrive.

# Sex Differences in Trimethylamine N-Oxide Levels as Cardiovascular Disease Risk Factors

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**ABSTRACT:** Trimethylamine N-oxide (TMAO), a gut microbiota-derived metabolite, has been linked to various cardiovascular diseases (CVDs) and is an important component of understanding cardiovascular health. Despite being an important marker of cardiovascular health, the influence of sex-related differences on TMAO levels remains poorly understood. The primary objective of this review is to analyze sex-based hormonal influences on TMAO production, while also looking at interlinked mechanisms and contributors. This review reveals that increased TMAO levels are linked to greater risks of atherosclerosis, endothelial dysfunction, dyslipidemia, and inflammation; it discusses the variability of sex-based findings and emphasizes the need for further research to fill these gaps. An exhaustive review of available literature suggests TMAO as a promising biomarker and therapeutic target in cardiovascular health in male and female populations, with a potential for further studies into its advancement and health interventions.

**KEYWORDS:** Biomedical and Health Sciences, Genetics and Molecular Biology of Disease, Cardiovascular Disease, Gut Microbiota, Trimethylamine N-Oxide.

## ■ Introduction

Cardiovascular disease (CVD) is a collective term used to refer to a group of diseases affecting the heart (“cardio”) and blood vessels (“vascular”). The most common types of CVDs include Coronary Artery Disease (CAD), Myocardial Infarction (MI), and Stroke.

CAD is caused by the buildup of atherosclerotic plaques in the coronary arteries, leading to narrowed blood vessels that restrict blood flow. This can cause chest pain (angina) and increase the risk of a heart attack (myocardial infarction) due to plaque rupture and clot formation. CAD remains a major cause of death worldwide.<sup>1,2</sup> MI, more commonly known as a heart attack, occurs when blood flow to a part of the heart is obstructed, typically due to a blood clot in the coronary artery, leading to damage to the heart tissue. MIs can lead to the sudden onset of cardiac arrest, depending on the extent of the damage.<sup>3</sup> Stroke occurs when blood flow to the brain is disrupted, either due to a blocked artery (ischemic stroke) or a vessel rupture and bleeding in the brain (hemorrhagic stroke). Stroke can lead to long-term disability or death, depending on the affected brain area and its severity.<sup>4</sup>

The prevalence of CVDs differs greatly by region and is affected by the environment, lifestyle, genetics, and healthcare access. In high-income countries, such as the United States, Canada, and Western European countries, CVD has long been a leading cause of death.<sup>5</sup> Although CVD rates have decreased in recent years due to advanced healthcare and prevention strategies employed in these countries, CVD remains significant in countries with rising rates of obesity, diabetes, and hypertension due to lifestyle and diet. Additionally, in low-middle-income countries, such as India, China, and many in Sub-Saharan Africa, the prevalence of CVDs is sharply rising due to urbanization, changes in diet, and other increas-

ing risk factors.<sup>6</sup> These regions face challenges in access to healthcare, early detection, and treatment strategies, leading to significantly delayed diagnosis and disease management. In Asia, countries such as Japan and South Korea have an increasing rate of CVD, mainly due to westernized lifestyle factors.<sup>7</sup> Southeast Asian countries, such as Indonesia and Vietnam, are also victims of this increase in CVDs due to urbanization. A primary contributor to this is the Western diet and high-fat diets, which are characterized by a high intake of red meat, processed foods, refined sugar, and saturated fats.<sup>8</sup>

Ongoing research explores the role of the gut microbiome as a primary factor in the pathophysiology of cardiovascular diseases. The gut microbiota is a vast microbiological community, full of millions of microorganisms, that plays an important role in the metabolism of dietary compounds. Dysbiosis, which is an imbalance in gut microbiota composition, has been related to systemic inflammation, metabolic disorders, and endothelial dysfunction.<sup>9</sup> Additionally, certain metabolites produced by the gut microbiome can have direct impacts on human health.<sup>10</sup> Trimethylamine N-oxide (TMAO), a metabolite of gut microbiota derived from dietary precursors such as choline, phosphatidylcholine, and carnitine, has come to light for its link with increased cardiovascular risk.<sup>11</sup> These precursors, typically found in foods like eggs, red meat, and dairy products, are metabolized by the gut microbiota into trimethylamine and subsequently oxidized to TMAO by flavin monooxygenase 3 (FMO3) in the liver.<sup>12</sup> In recent years, research has focused on sex-specific differences in gut microbiome, specifically TMAO production and its impact on CVD.<sup>13</sup> However, this area remains incompletely understood. Research suggests that differences in hormonal activity, metabolic pathways, and lifestyle choices may drive these sex-based differences in the generation of TMAO. This review will first discuss the metabolism

of TMAO, subsequently explore its involvement in cardiovascular disease, and finally examine the sex-linked differences associated with the effects of TMAO.

## ■ Mechanisms of TMAO Production and Dietary Sources

### Mechanisms:

Trimethylamine N-oxide (TMAO) is synthesized through a series of metabolic stages involving microbial activity in the gut and hepatic oxidation with the aid of host enzymes. TMAO production starts in the gastrointestinal tract, where specific strains of bacteria metabolize dietary precursor compounds such as choline, L-carnitine, lecithin, phosphatidylcholine, and betaine into trimethylamine (TMA).<sup>14</sup> Bacterial strains that produce TMA through choline metabolism are present in nearly all individuals.<sup>15</sup> These include *Anaerococcus hydrogenalis*, *Clostridium asparagiforme*, *Clostridium hathewayi*, *Clostridium sporogenes*, *Escherichia fergusonii*, *Proteus penneri*, *Providencia rettgeri*, and *Edwardsiella tarda*.<sup>16</sup> The genomes of these, and other bacteria that utilize alternative substrates, encode specific enzymes listed in Table 1, which are necessary for converting TMA-containing compounds to TMA.

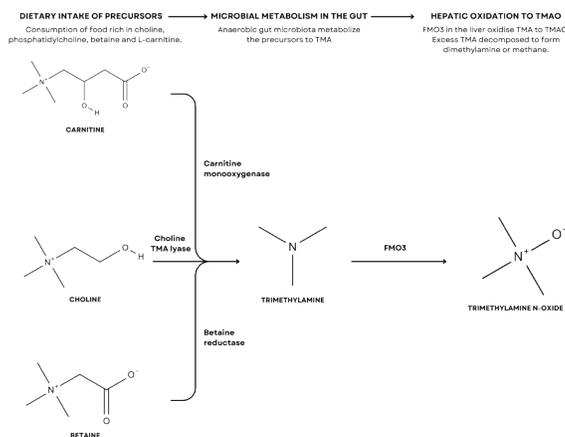
**Table 1:** These are the primary microbial enzymes involved in the production of TMA. This table outlines the key microbial enzymes and their associated strains that are involved in the conversion of dietary precursors to TMA. The recurrence of specific strains, such as *Escherichia coli*, suggests their integral role in the pathway.

Enzyme	Common Strains	Gene	Substrate	Function	Reference
Choline-TMA lyase	<i>Clostridium sporogenes</i> , <i>Lachnospirillum</i> , <i>L. saccharolyticum</i>	cutC/D	Choline	Converts choline to TMA by catalyzing the C-N bond cleavage	17
Carnitine monoxygenase	<i>Escherichia coli</i> , <i>Ralstonia eutropha</i> , <i>Clostridium sporogenes</i>	cntA/B	Carnitine	Converts carnitine to TMA	18
Betaine reductase	<i>Clostridium sporogenes</i> ,	Unknown	Betaine	Reduces betaine to TMA	19, 20
TMAO reductase	<i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i>	Unknown	TMAO	Reduces TMAO to TMA	21
YeaW/X (homologous to cntA/B)	<i>Escherichia coli</i> , <i>Acinetobacter baumannii</i>	yeaW/X	Various substrates (including choline, carnitine)	Utilizes a variety of substrates to promote TMA synthesis	22

The composition of TMA-producing bacteria varies significantly between individuals and is heavily influenced by external factors,<sup>23</sup> particularly sex, diet, age, and health. Diets rich in red meat, eggs, and dairy, which are sources of choline and L-carnitine, are associated with greater TMAO levels due to the increased TMA precursors and a gut microbiota composition favoring TMA-producing bacteria. Studies also suggest that dietary controls (such as plant-based diets) can effectively reduce TMAO production, suggesting that individuals at high risk for TMAO-related diseases may benefit from reducing their intake of TMA precursors.<sup>23,24</sup> This is supported by studies which show that the production of TMA and thereby TMAO is beneficial for the survival of several strains of bacteria, such as *Escherichia coli*.<sup>25</sup>

TMAO has the chemical formula (CH<sub>3</sub>)<sub>3</sub>NO and is a tertiary amine oxide, a colorless organic compound that forms following the oxidation of the amino group of TMA.<sup>26</sup> After TMA is produced, it enters the hepatic portal and is transported to the liver, where it is oxidized to TMAO. This is catalyzed

by hepatic flavin-containing monooxygenase enzymes, specifically FMO3, which oxidizes TMA to form TMAO. The excess TMA decomposes into dimethylamine or methane. This pathway by which TMAO is produced from dietary precursors of TMA is illustrated in Figure 1.



**Figure 1:** Summary of the primary pathway by which TMAO is produced. This figure represents the pathways followed in the conversion of dietary precursors to TMA by gut microbial enzymes, followed by the subsequent hepatic oxidation of TMA to TMAO via FMO3. This is an original figure created using Canva and MolView.

There are strong correlations between FMO3 activity and plasma TMAO levels.<sup>27</sup> Genetic variation influences the activity of the FMO3 enzyme, which is highly polymorphic.<sup>28</sup> Certain polymorphisms in FMO3 can lead to either a loss of function or increased enzyme activity, thereby altering the production of TMAO and giving rise to primary trimethylaminuria (TMAU), a disorder where the sweat, breath, saliva, and urine of a person smell like rotten fish. While this disorder does not have major health consequences, it can cause the mental health of the individual to deteriorate.<sup>29,30</sup>

The role of L-carnitine in TMAO synthesis is also a significant area of study; Koeth *et al.* shed light on an alternative pathway for TMAO production, in which L-carnitine is initially converted to an intermediate metabolite,  $\gamma$ -butyrobetaine (GBB), and then into TMA, which is then converted to TMAO by oxidation in the presence of FMO3.<sup>22</sup> This pathway may be relevant in individuals with high dietary L-carnitine intake, as it could be associated with increased TMAO levels beyond those produced through direct TMA synthesis from choline and other dietary precursors.

Sex also plays a significant role in TMAO production because of the differences in male and female hormones. Estrogen has been observed to increase FMO3 expression, and thereby TMAO metabolism. In one study, researchers administered 0.5mg estradiol pellets, a type of estrogen, in a murine model and compared the subsequent plasma TMAO levels to control groups, they found that the group with estradiol administration showed higher TMAO levels.<sup>27</sup> This is backed by a study that showed that plasma TMAO levels are reduced in menopausal women.<sup>31</sup> However, some studies suggest that males have higher TMAO levels than females, possibly due to hormonal levels, dietary habits, and microbiome composition.<sup>32</sup>

Age is another factor in TMAO metabolism, as it impacts gut microbiota composition and enzyme activity. Older adults often experience shifts in their gut microbiome and changes in liver and kidney function, which can both influence TMA production and TMAO elimination. One study found that increased age was associated with higher TMAO levels in males,<sup>31</sup> and proposed hormonal influences on the expression of the FMO3 gene as a potential explanation for this difference.

Once produced, TMAO is either excreted through renal elimination or accumulates in tissues where it acts as an osmolyte, helping maintain cellular homeostasis under osmotic stress.<sup>33,34</sup> The contribution of TMAO to cardiovascular conditions remains an active area of study, with many studies suggesting enhanced cholesterol deposition in arterial walls, promoted platelet hyperreactivity, and impaired endothelial function as potential mechanisms.

#### **Dietary Precursors:**

Diet is a determinant of TMAO production as it supplies key precursors such as choline, L-carnitine, lecithin, phosphatidylcholine, and betaine.<sup>35-37</sup> Choline is an essential nutrient that is abundant in animal products such as eggs, meat, and fish. Phosphatidylcholine, a major component of cell membranes, is a key source of choline and is found in foods like egg yolks, soybeans, and organ meats.<sup>38</sup> High plasma TMAO concentrations, above 2 - 5  $\mu\text{M}$ , have been linked with high choline intake.<sup>39</sup> However, some research shows that chronic intake of choline does not alter plasma TMAO levels, challenging the assumption that high choline consumption could directly contribute to CVDs. In Lemos *et al.*, researchers supplemented the diet of 30 adults with 400 mg/day of choline through either 3 eggs or choline bitartrate; they observed no significant changes in fasting plasma TMAO levels. Researchers did, however, observe a significant increase in plasma choline after 4 weeks of consumption of 3 eggs daily without changes in TMAO levels. This suggests that a great proportion of phosphatidylcholine was absorbed before reaching the colon for microbial conversion to TMA,<sup>40</sup> highlighting the significance of metabolism and absorption rates in the production of TMAO.

L-carnitine, primarily found in red meat, poultry, and fish, is also a major dietary precursor of TMAO. It is synthesized endogenously from methionine and lysine.<sup>41</sup> While most individuals obtain sufficient amounts of L-carnitine through their diet and endogenous synthesis, vegetarians and vegans typically have much lower intakes, which contributes significantly to lower plasma TMAO levels when compared to omnivores.<sup>42</sup> In a study conducted by Samulak *et al.*, supplementation with 1500 mg of L-carnitine L-tartrate for 24 weeks in healthy older women significantly increased plasma TMAO levels compared to a placebo group.<sup>43</sup> However, despite this increase, there were no significant changes in several markers of oxidative stress. In a follow-up study conducted 4 months after discontinuation, a reduction in plasma TMAO levels was observed, while lipid levels remained stable. These findings suggested that plasma TMAO concentrations can be

modulated by L-carnitine supplementation and cessation, yet its direct impact on lipid metabolism and cardiovascular health remains uncertain.<sup>44</sup>

Fish and shellfish, which contain preformed TMAO, lead to the highest concentrations of TMAO among dietary sources.<sup>45</sup> In a study by Cho *et al.*, fish consumption resulted in plasma TMAO levels that were ~50 times higher than eggs or beef.<sup>36</sup> A high-fat diet can influence plasma TMAO levels, with certain studies indicating that high-fat meals lead to increased postprandial TMAO levels. In one study involving healthy men, postprandial TMAO levels rose significantly after a high-fat meal, which consisted of 55% fat, with 50% of the fat being saturated.<sup>46</sup> This suggested that the fat content, particularly the saturated fat, may contribute to the observed rise in TMAO levels. In recent years, the Western diet (WD) has increasingly resembled high-fat diets, characterized by an excessive intake of processed foods, refined carbohydrates, and unhealthy fats.<sup>47</sup>

## **■ The Role of TMAO in Cardiovascular Diseases**

Several clinical studies indicate that elevated plasma TMAO levels correlate with a higher risk of cardiovascular events, providing evidence for TMAO as an independent biomarker of cardiovascular risk.<sup>48-52</sup> Outlined below are the mechanisms by which TMAO plays a role in cardiovascular diseases, summarized visually in Figure 2.

#### **Endothelial Function Disruption:**

TMAO disrupts endothelial function by inhibiting endothelial cell proliferation during the G1 phase of the mitotic cell cycle and has cytotoxic effects on circulating endothelial progenitor cells.<sup>53,54</sup> This impairs the regenerative processes of blood vessels, contributing to vascular dysfunction.

#### **Pro-Inflammatory Mechanism:**

A substantial body of research has shown that TMAO can accelerate atherosclerosis by diverse mechanisms that have been systematically investigated. Atherosclerosis is a condition where plaque buildup in blood vessels leads to narrowing of blood vessels and loss of the necessary elasticity needed to withstand and maintain blood pressure. TMAO can induce the up-regulation of CD36 and SR-A1 receptors on macrophages, which are responsible for lipid uptake into cells. This induces increased accumulation of lipids within macrophages, causing them to transition into foam cells.<sup>55</sup> The excessive lipid levels trigger the release of inflammatory cytokines that lead to vascular inflammation and subsequent damage to blood vessels.<sup>56</sup>

TMAO is known to activate nuclear factor kappa B (NF- $\kappa$ B), a transcription factor that up-regulates production of pro-inflammatory proteins,<sup>57</sup> and therefore induces the expression of adhesion molecules like vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1). These molecules cause the accumulation of leukocytes on the endothelium of vessels and contribute to the development of plaque formation.<sup>58-60</sup>

### Mitochondrial Dysfunction, Oxidative Stress, and Vascular Physiology:

Mitochondria are the sites of oxidative phosphorylation; however, the superoxide radicals produced during this process are extremely reactive and dangerous. Mitochondrial superoxide dismutase 2 (SOD2) is responsible for converting free radicals into less harmful hydrogen peroxide. However, elevated TMAO levels have been found to interfere with this by suppressing the activity of mitochondrial sirtuin 3 (SIRT3), which is responsible for the activation of SOD2.<sup>61</sup> This results in elevated superoxide levels and increased oxidative stress.<sup>61</sup> Oxidative stress impairs endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) activity.<sup>62,63</sup> eNOS and iNOS are two essential enzymes for the production of nitric oxide (NO), a signaling molecule that is important for vasodilation and vascular health, in both murine cells and human endothelial cells.<sup>64,65</sup> Reduced bioavailability of NO causes vascular stiffness, resulting in hypertension.

Increased ROS triggers the release of calcium ions ( $\text{Ca}^{2+}$ ) from intracellular stores through inositol 1,4,5-trisphosphate receptors (IP3Rs) on the endoplasmic reticulum. This change in calcium balance causes an impairment in the contractile response of the vascular endothelial cells and promotes vascular stiffness and atherosclerosis.<sup>66,67</sup>

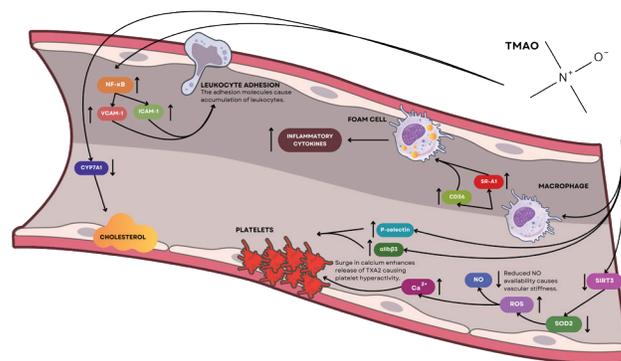
### Cholesterol Metabolism Disruption and Dyslipidemia:

Unchecked levels of cholesterol in the bloodstream elevate the risk of dyslipidemia and atherosclerosis.<sup>68</sup> TMAO is also known to suppress the activity of cholesterol 7- $\alpha$  hydroxylase (CYP7A1), an enzyme that aids in the breakdown of cholesterol into bile acids, which is important for cholesterol clearance from the liver.<sup>69</sup> Due to TMAO's suppression, the reduced activity of CYP7A1 leads to a decrease in bile acid production and impairment of the liver's ability to break down and excrete cholesterol.

### Pro-thrombotic Effects and Platelet Activation:

TMAO-induced oxidative stress triggers the release of calcium from intracellular stores through IP3 receptors, which increases the propensity of platelet activation to prothrombotic stimuli.<sup>70</sup> This surge in intracellular calcium enhances the release of thromboxane A2 (TXA2), a vasoconstrictor and platelet activator that further supports thrombus formation.<sup>71</sup>

Moreover, TMAO promotes the expression of adhesive molecules, such as P-selectin and integrins (specifically  $\alpha\text{IIb}\beta 3$ ) on the platelet surface.<sup>72</sup> These molecules cause platelets to adhere to the vascular endothelium and each other more readily, leading to increased clot stability.<sup>73</sup> P-selectin also interacts with P-selectin glycoprotein ligand-1 (PSGL-1) on leukocytes to enhance platelet-leukocyte aggregation. This aggregation is known to represent biomarkers of thrombo-inflammatory cardiovascular disease.<sup>74</sup>



**Figure 2:** Summary of the mechanisms by which TMAO impacts cardiovascular disease. This figure illustrates the multiple pathways through which elevated TMAO levels promote the development of cardiovascular disease and atherosclerosis via the mechanisms discussed in this section. *This is an original figure created using Canva and the NIH BioArt repository.*

## Sex-Based Differences in TMAO Metabolism and Cardiovascular Health

While the relation between TMAO and CVDs is well-established, more recent research investigating the intersection of sex-based differences in TMAO metabolism and cardiovascular risk has yielded conflicting results. This variance may be due to the influence of confounding factors such as age, gut microbiome diversity, and hormonal status. Some studies, such as those by Manor *et al.*, Chen *et al.*, and Stubbs *et al.*, suggest that males have greater circulating TMAO levels.<sup>32,75,76</sup> On the contrary, studies by Bennett *et al.*, Obeid *et al.*, and Falls *et al.* demonstrate that females may have higher levels.<sup>27,39,77</sup>

A primary factor in these differences is the effect of sex hormones on hepatic FMO3 expression. A 1997 study by Falls *et al.* investigated the regulation of flavin-containing monooxygenase 1 (FMO1), a flavin-containing monooxygenase isoform found in mice, and FMO3 in mice using sex steroids.<sup>77</sup> They found that the FMO1 and FMO3 expression is downregulated by the high levels of serum testosterone in male mice and that after castration, these mice demonstrated levels of FMO3 that were similar to those of females. However, once treated with testosterone after castration, the FMO3 levels in male mice returned to baseline. When unsprayed female mice were treated with testosterone, their FMO3 levels reached those of male mice, virtually undetectable. These findings suggest that testosterone suppresses the expression of FMO3. This study is supported by another one; Bennett *et al.* concluded that in a similar mouse model, females had much higher FMO3 expression when compared to their male counterparts.<sup>27</sup> This provides evidence for the positive relation between estrogen and FMO3 expression.

It is to be noted that estrogen levels in females vary with age, particularly due to menopause, which is a natural process that is characterized by the end of the monthly menstrual cycle due to ovarian follicular function. Menopause typically occurs in women between the ages of 45 to 55,<sup>78</sup> and causes a decline in estrogen. Post-menopausal women, therefore, have lower estrogen, and thereby lower FMO3 activity stimulation when compared to premenopausal women; this may lead to differences in TMAO levels, which is evident through a study

by Spencer *et al.*, which found that plasma TMAO levels are reduced after menopause.<sup>31</sup>

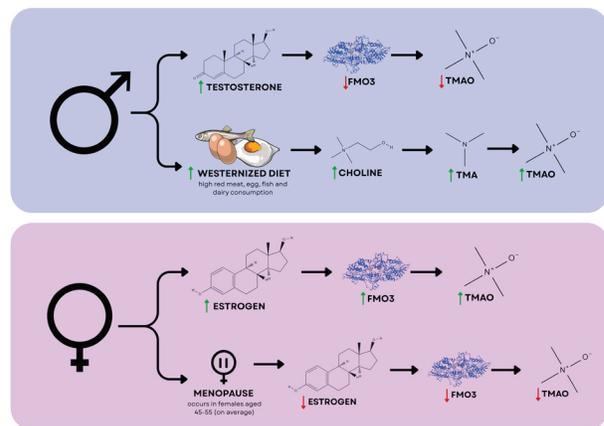
Manor *et al.* conducted a cohort study with 52% females and 48% males, with an average age of  $50 \pm 13$  years, and concluded that males showed significantly higher TMAO levels than females.<sup>32</sup> However, it can be speculated that the high proportion of elderly women, who may be postmenopausal, might explain the observed sex differences, as reduced estrogen levels can account for lower FMO3 activity and thereby a decrease in TMAO production. Another study in 2024 by Almer *et al.* had similar findings, with males showing significantly higher levels of serum TMAO concentrations than females.<sup>79</sup> The age group for this study was 11 to 26 years for both sexes. This can rule out our previous speculation that the results are being affected by postmenopausal hormonal changes. An alternative explanation for the observations in these studies is the dietary habits of males compared to females. Studies have previously found that men tend to have a higher consumption of red meat, fat, and a westernized diet than women;<sup>80, 81</sup> This may cause an increase in the TMA-precursors and thereby more synthesis of TMA, and by extension, TMAO.

Other studies have also shown evidence for higher levels of TMAO in females than in males. A study conducted by Veeravalli *et al.* in 2017 found that female mice had much greater TMAO levels than male mice. They proposed that this was due to the fact that when male mice reach 5-6 weeks of age, the expression of FMO3 is switched off, which does not occur in humans.<sup>82</sup> The study by Bennett *et al.*, which has been previously discussed, had also found that in the mouse model, there were much higher levels of plasma TMAO in females than in males, and higher levels of TMA in males than in females. This observation allowed them to conclude that the conversion of TMA to TMAO is more efficient in female mice. It also highlighted that the differences in TMAO levels were not replicated in human populations, causing them to believe that this may be due to the differences and variability in the diet consumption of humans. A visual summary of these key pathways through which sex differences affect plasma TMAO levels is provided in Figure 3.

However, not all studies abide by a sex-different hypothesis. Several studies did not detect significant sex-specific differences in TMAO levels; they did, however, find that the concentration of certain precursors is greater in males than in females. All three studies by Obeid *et al.*, Rohrmann *et al.*, and Mueller *et al.*, conducted on human models, found that betaine is significantly higher in males than in females; however, only Rohrmann *et al.* found that choline was higher in males than in females, where the other two did not correlate it significantly with sex.<sup>83-85</sup>

It is essential that we understand the variations in TMAO levels with sex that are observed before and after cardiac events to better inform treatment and rehabilitation plans in the future. Thus far, only one study has been conducted on TMAO levels following a cardiac event. This was a study by Baranyi *et al.* in 2022, which investigated the effect of sex-specific differences on TMAO concentrations before and after cardiac rehabilitation in patients who suffered an acute myocardial

infarction (AMI).<sup>86</sup> Their sample had an average age of 57.9 ( $\pm 12.0$ ) years and included 45 Caucasian males and 11 Caucasian females, all of whom had suffered an AMI. All patients underwent 4 weeks of cardiac rehabilitation. They found that female patients consistently had higher levels of TMAO blood concentration than males from the first day after AMI, and the difference was significant till the start of cardiac rehabilitation. By the end of the rehabilitation period, both males and females had had similar levels of TMAO concentration. This proves the efficacy of cardiac rehabilitation for patients who have suffered major adverse cardiovascular events (MACEs). They speculated that females may be at a greater thrombotic risk than men due to the higher TMAO concentrations and therefore higher levels of TMAO-induced platelet hyperactivity, as discussed in the preceding sections. This study, being the first of its kind, highlights the need for future studies that look at TMAO levels before and after MACEs, with a more generalizable sample and a multi-omic approach.



**Figure 3:** Summary of the pathways by which sex affects the TMAO levels. In both males and females, different routes can act to either elevate or reduce circulating TMAO levels, which may partially account for the inconsistent findings reported across previous studies. *This is an original figure created using Canva, MolView, and the SWISS MODEL database.*

## Discussion

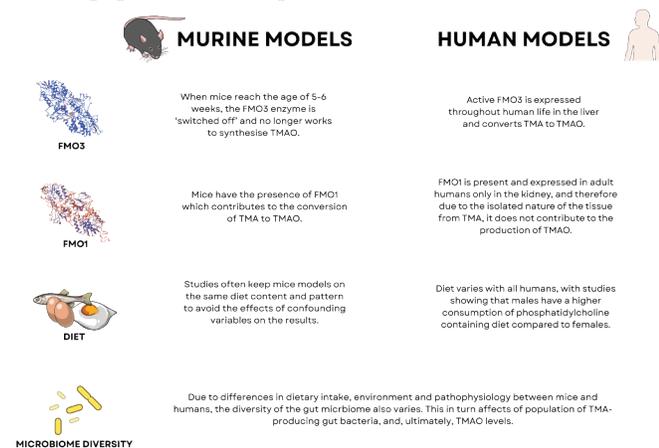
This analysis of decades of existing research has evaluated the complex role of TMAO in cardiovascular diseases and how it links to sex-based differences. While TMAO is a well-established biomarker for cardiovascular events,<sup>87</sup> its exact effect on the pathophysiological mechanisms remains to be fully explored by researchers due to its intersection with several factors, including sex-based differences.

Sex differences are essential in understanding the complete role of TMAO in cardiovascular physiology. It is possible that the variations in results that arise from different studies may exist due to several factors, such as focusing only on the postmenopausal or premenopausal phases, as estrogen levels in both are different and may act as an external variable in the studies.

When comparing mouse studies to humans, there are an array of factors that must be taken into account for better generalizability and applicability to humans, such as dietary intake, gut microbiome diversity, and even kidney function. The key matter of concern is the fact that FMO3 is, as previ-

ously discussed, switched off in male mice following 5–6 weeks of age, which makes the model much less reliable. Apart from these, a key factor is also the presence of hepatic FMO1. Unlike mice, adult humans do not have the presence of the FMO1 enzyme in the liver, and it is only expressed in the kidney and small intestine; therefore, it cannot play an active role in the production of TMAO, as studies have found no production of TMAO in cells isolated from these tissues.<sup>88, 89</sup> Furthermore, mouse studies typically involve all mice kept on the same diet to avoid confounding variables; however, this does not follow the diet differences between male and female humans, raising concerns regarding the validity of the comparisons drawn between the two models. This may also be a possible explanation for why mouse model studies tend to find females with higher levels of TMAO. Figure 4 provides a detailed summary of the differences between murine and human models that must be taken into account when evaluating the applicability of murine model studies to human populations.

Future research in the area must consider studies that account for sex and hormonal status in greater detail. These studies must separate sex differences from other factors to reach a conclusive result on variations and cause-and-effect in TMAO with sex, while also including both pre- and postmenopausal women to make clear the impact of hormonal changes on TMAO metabolism and CVD risk. Studies should also focus on the dietary aspect and the sex differences in TMAO levels following a MACE to identify critical threats to individuals undergoing recovery from a cardiovascular event and create better strategies to tackle these threats. Further research should address the conflicting data and evaluate TMAO-focused interventions in diverse populations to improve cardiovascular health.



**Figure 4:** Differences between murine and human models that limit the direct applicability of findings on sex-linked differences in TMAO levels from mouse studies to human populations. *This is an original figure created using Canva, MolView, the NIH BioArt repository, and the SWISS MODEL database.*

## Conclusion

This review has synthesized a vast body of literature on the sex differences in TMAO levels as CVD risk factors, outlining the complicated interactions between biological, dietary, sex, and age factors. While several studies suggest higher levels of TMAO in males relative to females, possibly due to dietary factors, others conclude that females have comparatively greater

levels of TMAO than males, which could be caused by the up-regulation of FMO3 by estrogen, especially in pre-menopausal women. In addition, research on TMAO levels post-cardiac events is scarce and should be the focus of future studies to allow effective treatment and rehabilitation.

## Acknowledgments

The author wishes to acknowledge the guidance of Abigail Evered from the University of Wisconsin-Madison for her mentorship in the production of this paper.

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# A Survey of Optimization and Compression Techniques for Natural Language Processing Models

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**ABSTRACT:** Natural Language Processing models have proven to be incredibly useful, boasting a tremendous number of practical applications spanning nearly all fields of study. The ability of NLP models to understand human language can be applied to a wide range of tasks, from generating medical reports to advanced translation. However, these abilities scale significantly with model size, so smaller models experience an exponential performance falloff when compared to large models. Large Language Models (LLMs) are at the forefront of NLP, and can have up to trillions of parameters. These programs are incredibly resource-intensive in their training and operation, and smaller organizations may be unable to properly utilize the full potential of current NLP technology. This paper aims to provide a survey of techniques that can make high-performance NLP models more accessible, whether it be by increasing the performance of weaker models or compressing larger, high-performance models to have more manageable requirements.

**KEYWORDS:** Robotics and Intelligent Machines, Machine Learning, Natural Language Processing, Large Language Model, Small Language Model, Model Compression, Model Optimization.

## ■ Introduction

Natural Language Processing is a field of AI centered around the 'natural' understanding and manipulation of human language by AI models. Natural Language Processing models (NLP models) are AI models that are built with this exact purpose in mind. They can generate and analyze text, translate text between supported languages much more accurately than non-AI machine translation, and even solve problems through logic.<sup>1</sup>

Some researchers believe that NLP models merely display the facade of intelligence through statistical prediction,<sup>2-5</sup> and others believe in the legitimacy of their reasoning abilities.<sup>1,6-8</sup> Despite this debate, this logical behavior is still incredibly useful in fields like medicine,<sup>9</sup> finance,<sup>10</sup> and computer science.<sup>11</sup>

The incredible potential of NLP models to be utilized in the world has one major obstacle, however. All of these characteristics are highly dependent on the size of the model, which is incredibly expensive to scale. The training, tuning, and operation of large-scale NLP models (Large Language Models/LLMs) takes an enormous amount of computational power, training data, and time. Additionally, most current research on optimization focuses heavily on larger models, as they are the current state-of-the-art (SOTA) technology in this field.<sup>12-15</sup>

The best solution to this problem, and the main focus of this paper, is the utilization of Small Language Models (SLMs). SLMs are smaller NLP models that have much lower resource requirements, but this comes at the cost of significantly lower performance in NLP tasks. Fortunately, there exists ample research around both the optimization of smaller NLPs' performance through techniques like prompt engineering and fine-tuning, and the compression of LLMs into SLMs with techniques like pruning and distillation. This paper aims to

provide a survey of such methods and describe how they work; Examples of particular techniques will be provided in order to stimulate more interest in the specific field.

This paper will first examine the exact definitions of the terms LLM and SLM, the differences between the two, and how SLMs are a much better choice for the average person's utilization. Then it will review performance-enhancing methods for SLMs and compression methods for LLMs. Finally, it will provide an overview of promising future prospects, as well as a statement on the necessity of such innovation in order to further the state of the world.

## ■ SLMs and LLMs: Background Information

### 2.1. : What are LLMs and SLMs?

To fully understand what LLMs and SLMs are. It is necessary to first understand transformer models. Transformer models are a type of model that has an attention mechanism as the main component. A simplified explanation of how an attention mechanism works is that it allows the model to focus more on information that is deemed important by the mechanism. A much more mathematical and detailed explanation can be found in this paper by A. Vaswani *et al.* This unique characteristic of transformer models makes them very useful in a variety of fields,<sup>16</sup> but to understand this paper, all that you need to know is that almost all modern SOTA Natural Language Processing models are based on the transformer architecture.

Natural Language Processing models are a group of models designed to manipulate and 'understand' human language in a more human-like way. These models are incredibly versatile due to this function and are used in a variety of applications such as

chatbots,<sup>17,18</sup> better translation of languages,<sup>19</sup> and analysis of text.<sup>20</sup> Large Language Models and Small Language Models are subsets of Natural Language Processing models, being the categorization of NLP models into larger and smaller models by their number of parameters.

LLMs are NLP models with incredibly large numbers of parameters. The current state-of-the-art is hundreds of billions to trillions of parameters. SLMs are NLP models with relatively low amounts of parameters, ranging from hundreds of millions to a few billion. The definitions aren't quite as concrete because of the rapid growth of the field, and now even models with as many as ten billion parameters can be considered SLMs as well, depending on sources.

### 2.2. : *Difference in Requirements:*

The main difference between LLMs and SLMs is, of course, their size, with the largest LLMs having easily thousands of times the parameters of an average SLM. This is why LLMs perform much better than SLMs in language processing. However, this comes with a major downside, which is the cost.

Training a state-of-the-art level LLM can require up to thousands of petaFLOPS ( $10^{15}$  floating point operations per second) of computational power. This is provided by hundreds to thousands of very expensive specialized GPUs, each costing in the range of tens of thousands of dollars. These GPUs can also need to run for up to several months in order to fully train such an LLM, and this incurs further tens to hundreds of thousands of dollars in electricity prices. On top of that, LLMs need terabytes of high-quality training data, which can be hard to procure and, of course, is very expensive.

Overall, LLMs need enormous amounts of resources to train, which is also reflected in requirements to optimize them using strategies like fine-tuning or reinforcement learning, where you are essentially just training the model more.

SLMs, on the other hand, are much more efficient in almost every way. While their performance is limited due to their smaller scale, the training requirements are equally reduced. SLMs can require thousands of times less computing power and can even be trained on the average laptop/desktop graphics card instead of specialized hardware. They also train much faster, even with this very reduced amount of computing power, and they take on the scale of weeks or days rather than months. They also require much less data to train, being on the scale of gigabytes rather than terabytes or even petabytes. All of this makes SLMs much easier to train and fine-tune. This leads to one of SLMs' main strengths over LLMs in terms of utility: they can be easily and quickly custom-trained to work well on one specific task, and cost thousands of times less.

## ■ Optimization Methods

### 3.1. : *Fine Tuning:*

Fine-tuning is a technique usable to optimize any kind of AI model. This technique involves taking a smaller, more specialized dataset and using it to further train a model to be better at a specific task. This technique was brought to the field of NLP by J. Howard & S. Ruder in 2018,<sup>21</sup> who took the concept from CV (computer vision) modeling and modified it to work

with NLP models. Fine-tuning optimizes NLPs by focusing them on a particular set of data. Specifically for reasoning, it is done with data sets containing reasoning-related question sets and their answers. It can also be done with a chain of thought, examples like the ones given in CoT prompting.

Fine-tuning is a well-established method of focusing a model on a particular task or subject, and it is also much more efficient to apply to SLMs. This is because SLMs have many fewer parameters and are usually trained with less available data. Fine-tuning allows SLMs to perform comparably to much larger models using significantly less data. Some examples of fine tuning methods that are better for SLMs are: fine tuning only bias terms,<sup>22</sup> which involves only fine-tuning a small subset of a model's parameters for increased efficiency; Distillation,<sup>23</sup> which is a technique in its own right, involving the transfer of training from larger models to small models; and even fine tuning for multiple different tasks,<sup>24</sup> which involves fine tuning using more than one specialized dataset to allow fine-tuned models to not be as restricted to a single task. Fine-tuning is an excellent, data-efficient technique to optimize a Small Language Model's performance in any field.

### 3.2. : *Prompt engineering:*

Prompt engineering is a very broad term that can be used to describe any technique to optimize a model by modifying the input sequence. Whether it be providing examples of inputs and outputs to acclimate a model to a particular question type,<sup>25,26</sup> instructing a model to approach a question in a particular way, such as breaking a problem into simpler steps,<sup>27</sup> or telling the model to break up the problem on its own,<sup>28</sup> providing examples of how to break down a question into multiple steps.<sup>29</sup> A couple of other particularly important sections of prompt engineering include zero-shot prompt engineering and automated prompt engineering. Zero-shot prompt engineering aims to guide the model to better utilize the information it has from training, and can be achieved through simply telling the model to take the problem step-by-step,<sup>30</sup> telling the model to take a deep breath,<sup>31</sup> and even appealing emotionally.<sup>32</sup> Automated prompt engineering involves using a model to optimize prompts for you, whether it be automatically generated CoT and few-shot learning examples,<sup>33,34</sup> automatically generating questions that models can understand well,<sup>35</sup> or distilling necessary context in a prompt by removing distracting information.<sup>36</sup> In this paper, we will only go over a few of the many, many ways to engineer prompts.

### *In-Context Learning:*

In-context learning is a prompt engineering technique that can be applied to almost any type of model and any type of task. It is done by simply giving the model examples of prompts and the desired answer. This can be done either manually with human-generated examples or with examples generated by another model. T. Brown *et al.* designed GPT-3 to test this technique in the realm of NLP,<sup>25</sup> and was able to beat the at-the-time SoTA model on the LAMBADA benchmark (Turing-NLG with 17B params at 68%) by about 10% accuracy with just 2.7B of its own parameters, advancing this

to an 18% increase in accuracy with their 175B parameter version. This does show that the benefits of ICL do not scale linearly with size, as an almost 70-fold increase in parameters only improved the performance of the model by roughly 8%. On the other hand, S. Min *et al.* investigated the question: “Why does in-context learning work?”<sup>26</sup> They discovered that, bizarrely, the labels assigned to the demonstrations don’t matter that much because when randomizing them, the performance was barely affected. They found that most of the benefits of ICL come from the example prompts and the example answers analyzed separately rather than paired, so as long as the example-answer sets are the same, you can pair them up in any fashion and have only a marginal reduction of benefits. In-Context Learning has an interesting effect when the size of the model is considered. Z. Shi *et al.* sought to find out how LLMs and SLMs process ICL examples differently,<sup>37</sup> and concluded that while they both have their skills, LLMs perform better due to their increased overall comprehension of the examples. However, SLMs were shown to notice smaller, more hidden trends in the examples that LLMs missed. This is a promising feature of them that could be used in conjunction with LLMs to improve overall performance.

#### *Instruction-Based Prompting:*

Instruction-based prompting is a prompt engineering technique based on giving a model a prompt that tells it to approach a problem in a specific way. One particular technique is plan and solve prompting,<sup>28</sup> which involves telling the model to make a step-by-step plan to solve the problem before actually following through on that plan. This strategy regularly beat standard zero-shot CoT (“think about it step by step”), and even barely outperformed manual few-shot CoT on the SingleEq, AddSub, and GSM8K benchmarks. Another technique is instructional prompt reframing.<sup>27</sup> This technique is centered around rewriting prompts to be better understood by the particular model(s) by breaking the problem down into steps, using more specific language, breaking up answer criteria into multiple instructions, turning ‘don’ts’ into ‘dos’ (NLP models struggle with negative commands), and adding restrictions to ensure a proper output format. In their experiment, they did this with the GPT models in mind and were able to improve GPT3’s performance on several datasets by an average of 17% and 8% over the raw prompt for zero-shot and few-shot trials, respectively.

#### *Chain of Thought Prompting:*

Chain of Thought Prompting, or CoT prompting, is an umbrella term referring to any prompt engineering technique aimed at getting the model to break down a task into many steps. This technique is broken down into two different kinds. Few-shot CoT prompting is the first kind of CoT prompting. Developed by J. Wei *et al.*,<sup>29</sup> this version of CoT prompting involves giving the model a few example questions paired with the answers and the chain of thought used to get from the context to the correct solution. Their experimental results found a particular improvement of the GSM8K problem set with models like GPT-3, Codex, and PaLM-540B, having an in-

crease of 31.3%, 43.4%, and 39% respectively. However, when tested on smaller models such as LaMDA 420M and GPT-3 350M, CoT prompting was found to have a negative effect on their performance, dropping their GSM8K accuracy rates by more than a factor of 4. This would suggest that CoT prompting has little to offer in the field of SLM optimization, but other studies have demonstrated potential. L. Ranaldi & A. Freitas use a method they call Instruction-Tuned- CoT, which involves using a teacher model to demonstrate chain-of-thought reasoning to SLMs.<sup>38</sup> They observed improvements of 5-10% on various benchmarks when using a teacher model of GPT-3.5 on the Llama-2 7B and Llama-2 13B models. While these improvements are not as significant as the ones that CoT has shown to have on LLMs, they show promise in the application of this idea to SLMs.

#### *Zero-Shot Prompt Engineering:*

Zero-shot Prompt Engineering is the field of prompt engineering surrounding zero-shot prompts, which are prompts that do not include any examples and require the models to answer solely with their training. One example of zero-shot prompt engineering is zero-shot CoT prompting. This technique, developed by

T. Kojima *et al.*,<sup>30</sup> involves prompting a model to take a problem step by step without any examples. They utilized the phrase “let’s think step by step” to induce this behavior, and their model showed a significant improvement on both the MultiArith and GSM8K datasets (17.7-78.7% and 10.4-40.7% respectively, from zero-shot to zero-shot CoT). Zero-shot prompting can also be achieved in some strange ways, such as in a study done by Yang *et al.*<sup>31</sup> In this study, they utilized LLMs as tools to generate optimized zero-shot prompts, and the best prompt phrase that their method generated was the phrase “Take a deep breath and work on this problem step-by-step.” This prompt achieved an 80.2% accuracy on the GSM8K dataset on a pre-trained PaLM 2-L model. This is a significant improvement compared to just the question, which measured a 34% accuracy on the same dataset and model. Another of these strange zero-shot prompt engineering techniques is emotional prompting. C. Li *et al.* propose that LLMs can be boosted through emotionally charged prompts.<sup>32</sup> Their techniques induce self-monitoring behavior by either asking the model if it is sure, or providing encouragement with phrases usually associated with boosting self-esteem i.e., “Remember that progress is made one step at a time. Stay determined and keep moving forward.” This study aimed to learn how LLMs process these kinds of human encouragement, and they tested their prompts on the TruthfulQA set, which is measured by both truthfulness and informativeness of a generated text. Their best result was using ChatGPT, and this was an improvement from 75% true, 53% informative, to 87% true and 67% informative.

#### *Automated Prompt Engineering:*

Automated prompt engineering is the field of prompt engineering that involves the utilization of a model in order to optimize prompts. For example, Yang *et al.* discuss the utilization of LLMs to optimize zero-shot prompts.<sup>31</sup> Their best

generated prompt was “Take a deep breath and work on this problem step-by-step,” which gave them an 80.2% accuracy on the GSM8K benchmark, a massive improvement from the baseline for no modifications, which was 34% accurate. Another couple of papers that demonstrate automatic prompt engineering are those by Z. Zhang *et al.* and A. Sevinc & A. Gumus.<sup>33,34</sup> These papers focus on the automatic generation of CoT prompts. Z. Zhang *et al.* achieved results comparable to manually generated CoT using examples generated by another LLM using a “let’s think step by step” (zero-shot CoT) prompt. A. Sevinc & A. Gumus use a strategy that uses rationales generated by a better model (GPT-4) to improve the performance of a weaker/smaller model.<sup>33</sup> They observed an increase of 20% accuracy when tested using GPT-4 on the StrategyQA benchmark. Further automatic prompt engineering techniques include “System 2 Attention” or S2A by J. Weston & S. Sukhbaatar, which is a technique that utilizes a model to remove any distracting information and optimize a prompt for itself.<sup>36</sup> They found that their method could successfully remove distracting details from the GSM-IC dataset, which has problems with irrelevant and distracting sentences included within it. Their method successfully got the accuracy of the prompted model to be just about the same as that of the oracle prompt (a prompt for the question without the distractions). A final method of automatic prompt engineering is presented by J. Weston & S. Sukhbaatar.<sup>36</sup> They propose another method of generating high-quality prompts for any task by utilizing a masked language model (MLM), which is a model that is trained by ‘masking’ or covering up words in a text, and having the model predict them. These models specialize in filling in spots in a given text. Their study suggests that this method could be a replacement for fine-tuning in certain situations. They tested it on the GLUE benchmark using the BERT and RoBERTa models, achieving a 19.1% and 6.2% improvement, respectively.

### 3.3. : Test-Time Compute:

Test-time compute is an optimization field that involves the reallocation of resources during inference. This allows models to respond much more accurately to difficult questions at the cost of inference time. This technique is very good at optimizing SLMs, due to the fact that it allows SLMs to take a long time to answer questions to a degree of accuracy higher than some LLMs. One example of this utilization is demonstrated by E. Akyürek in abstract reasoning.<sup>39</sup> They use the benchmark of the Abstraction and Reasoning Corpus (ARC), and tested Llama-3 8B and Llama-3.2 1B, and 2B. Their findings are promising, as the 1B and 3B models solved 6x and 3x as many tasks, respectively, when TTT (test-time-training) was enabled. While the 8B Llama model performed well, TTT proved to be effective at improving the 1B and 3B parameter models to a level competitive with the 8B model. The downside to test-time compute is the extended inference time, which negates one of the advantages of SLMs: fast inference time. M. Alfarra *et al.* aim to combat this longer inference time by promoting methods that take less time by giving them more encouragement through data.<sup>40</sup>

## ■ Compression Methods

### 4.1. : Knowledge Distillation:

Knowledge Distillation is a technique that transfers knowledge and skills from one model to another model.<sup>41-43</sup> There are three different techniques that are used in distillation: offline distillation, online distillation, and self-distillation. Offline distillation is the traditional distillation, which involves a large, pre-trained teacher model teaching a much smaller student model.<sup>44</sup> It is a good method to train SLMs that can run on much more constrained devices while retaining a good amount of the teacher model’s performance. Online distillation is a different approach, where both the teacher and student models are learning at the same time.<sup>45</sup> This is best when a pre-trained teacher model is not easily accessible. Self-distillation is a very drastically different technique where an already somewhat trained model trains itself and uses its deeper layers to improve its shallower layers.<sup>46</sup> This technique is best for optimizing a single model with little performance sacrificed when you have even more of a resource constraint.

One example of a knowledge distillation technique is Spot-adaptive Knowledge Distillation by J. Song *et al.*<sup>47</sup> Their work demonstrates a novel technique that adaptively chooses layers to learn from. Traditional methods typically use a manually selected set of layers to use in distillation, but this can be very sub-optimal in circumstances that are not ideal. They demonstrated their method in CV benchmarks and recorded small but consistent improvements across various model sizes and benchmarks. Another example of a knowledge distillation technique is shown in the paper “Heterogeneous Knowledge Distillation Using Conceptual Learning” by Y. Yu & N. Kim.<sup>48</sup> Their technique involves teaching the smaller model higher-level concepts in the field of knowledge, rather than just the information required to solve the target problems. This has the benefit of allowing the distilled model to better generalize to a broader group of related tasks. They tested this on the 20 Newsgroups dataset, and, similarly to the other study, showed a small but consistent advantage over traditional knowledge distillation techniques. While these adaptations of distillation show relatively minor improvements over traditional distillation, traditional distillation is incredibly efficient at compressing the model’s size, as shown by A. Rashid *et al.*, with a recorded up to 30x reduction in size while only losing 8-25% of the original model’s accuracy.<sup>49</sup>

The further development of distillation techniques is necessary because, while the improvements made over traditional distillation are small, distillation is still one of the best ways to provide close to LLM-level performance with a fraction of the required computational power.

### 4.2. : Pruning:

Pruning is a technique that involves carefully removing less important parts of models in order to reduce their operating requirements while not affecting performance too much. Pruning is separated into two main categories: structured pruning and unstructured pruning. Structured pruning is the process of removing large clusters of weights in a model, and unstructured pruning is the process of removing individual weights in

a model. These processes all aim to increase a measurement called sparsity, which is essentially the percentage of weights that are set to 0. This greatly reduces the required computing power as a large amount of calculations can be essentially skipped. The process typically targets weights that are close to zero already, so the model does not lose very much accuracy. One example of such is shown by R. Xu *et al.*, who developed a framework that utilizes the supervision of other models during the pruning process.<sup>50</sup> They achieve a 90% sparsity using structured pruning, and 97% sparsity using unstructured pruning.

They only lose 10.5-.7% and 7-.7% accuracy on various datasets such as QQP and SST-2 using structured and unstructured versions of their technique, respectively. Another example is in a paper by J.- H. Luo *et al.*, which demonstrates another framework for pruning that achieved only a 10% decrease in accuracy on the Indoor-67 dataset while achieving over 100-fold decrease in parameters.<sup>51</sup> While one of these studies is on computer vision deep neural networks rather than NLP models, they both demonstrate how pruning can decrease model sizes by orders of magnitude while still retaining much of their accuracy.

## ■ Discussion and Future Directions

### 5.1. : Prompt engineering

While prompt engineering techniques can provide little or even negative effects on SLMs, the core principles behind the field of prompt engineering can be implemented to improve SLM performance. The idea of Chain of Thought reasoning has been demonstrated to be teachable to smaller models,<sup>37</sup> and further developments in how prompts can be engineered to more effectively work on smaller models would make a massive impact on people's ability to utilize AI in small-scale settings.

### 5.2. : Fine Tuning:

Fine-tuning is a very well-used technique in SLMs, one of SLMs' main strengths being the ease of fine-tuning. This technique is generally beneficial, and important fields of improvement would be the versatility of fine-tuned models, the further increase of fine-tuning's effectiveness, and the decrease of computational requirements for fine-tuning. Fine-tuning is already a very effective and widely used technique to optimize SLMs for commercial usage, and further improvements are in progress as this is a more prevalent technique. Therefore, even more research into this field is likely not required, and efforts should be concentrated on newer, less studied techniques.

### 5.3. : Test Time Compute:

Test Time Compute is a technique in the field of NLP that has recently gained more widespread prevalence primarily due to OpenAI's o1 model line, and is overall a technique with much room for development. It is a field that needs much more research than the others due to its massive potential to make smaller models as good as larger models, and larger models even better. Of course, this technique is receiving the amount of attention it needs in the research space, but looking into applications and optimization for applying this technique

on smaller models is somewhat less popular, and developments in that specific field would massively increase the accessibility of high-performance AI models, which can combine the strengths of both LLMs and SLMs.

### 5.4. : Distillation:

Distillation is a relatively well-established technique, and therefore has extensive existing research. Additionally, its sole purpose is to optimize and compress, optimizing a large model by compressing a larger model's knowledge into it. For our purposes, that makes any research into distillation beneficial towards the accessibility of AI models. However, it has become clear that most improvements upon distillation techniques are near marginal, only improving performance by a couple of percentage points. Therefore, a large breakthrough in the field is required before we make significant progress.

### 5.5. : Pruning:

Pruning is another extremely effective technique, and is well established as well. It is very similar to distillation in relevance to our goal of accessibility and research status. It also has much existing research, but also only minor improvements are being made over the original technique. Similarly to distillation, a massive breakthrough needs to be made in this field, as current performance returns on research invested are low.

## ■ Methodology and Limitations

This survey was primarily conducted using Google Scholar with the key phrases of "NLP Compression", "SLM Optimization", and the names of the specific sections of the 'Techniques' section. The exploration of relevant citations was also utilized. This study was limited by a strict time limit, and due to this, it was heavily focused on well-established and cited papers. This reflects the main purpose of this paper, which is not to provide a comprehensive review, but to incite interest in this field as it is of great importance.

## ■ Conclusion

In this survey, we have gone over various methods that can be used to optimize small NLP models, as well as methods that can compress larger ones. This is done to focus on the practical utilization of NLP models in real-life applications. The development of these techniques is incredibly important for AI deployment in a small-scale setting. The increased accessibility of NLP models and AI in general for the general public is corresponding with increased public interest and investment in further development of AI. Therefore, it is just as important to work on smaller, more accessible models for public use as it is to work on massive, state-of-the-art models because this development of public AI is crucial to foster interest in the field of AI and eventually will lead to an increase in research and funding in the field as a whole.

Increasing accessibility of these models would also boost efficiency and improve the quality of life for countless small businesses and organizations.

## ■ Acknowledgments

I would like to thank Professor Siddarth Krishnan and Dr. Plinio Zanini for their mentoring during the process of writing this paper. Thanks to them, my own view of research as a whole has been completely changed.

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Nathan Lam is a Junior at Stuyvesant High School in New York, New York, at the time of writing. He is passionate about all things engineering, and he aspires to integrate Artificial Intelligence and Machine Learning into any project where it may improve it.

# An Insulin Delivery Patch Pump with 3D Printed Microneedles

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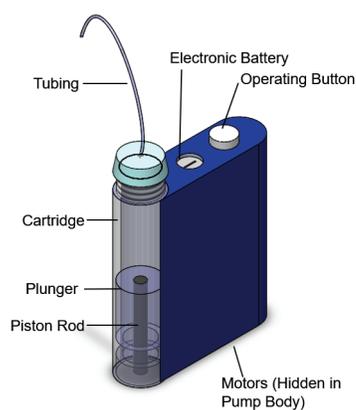
Mentor: Zahra Faraji Rad

**ABSTRACT:** Current insulin pumps, which are serving 200 million diabetics worldwide who require insulin therapy, face critical limitations, including high costs (~\$4000), bulky designs, pain associated with hypodermic needles, and occlusion-prone tubing. This project develops a low-cost, painless, and wireless insulin delivery pump by integrating fluids, electronics, and microneedle modules. The fluids module uses a piezoelectric actuator that achieves target flow rates of 0.02 - 3.0  $\mu\text{L}/\text{min}$  at 10 - 20 Hz frequencies, demonstrating a linear relationship between frequency and flow rate. The electronics module utilizes Bluetooth components from commercial earbuds, generating 0.4V signals (measured via an oscilloscope), with plans to integrate amplifiers for 40 $\times$  signal boosting to meet 15V system criteria within \$20 cost cap. The microneedle module features two designs: hollow and open microchannel. Computer simulation results showed that the open microchannel design was more resistant to lateral forces. Two hollow microneedles achieved 90% penetration depth under 0.5 N in synthetic skin, meeting insertion force criteria (< 3 N) within the 3.5  $\times$  3.5 mm<sup>2</sup> size constraint. This approximately \$30 prototype addresses key limitations through wireless connectivity, painless microneedle delivery, and affordable piezoelectric actuation, potentially improving the quality of life for over 200 million diabetics.

**KEYWORDS:** Biomedical Engineering, Biomedical Devices, Insulin Pump, Piezoelectricity, Fluids, Electronics.

## Introduction

Diabetes is a serious chronic condition where the patient cannot produce enough insulin to regulate their blood sugar. At least 200 million people across the globe require insulin therapy.<sup>1</sup> Basic treatment is through multiple daily injections (MDI). Insulin pumps are the current state-of-the-art treatment method, as they are less painful and more precise than MDIs.<sup>2</sup> Existing insulin pumps consist of a reservoir, a sensor/starter, an actuator, transportation pathways, and a catheter or needle (Figure 1).<sup>3</sup>



**Figure 1:** Components of a typical insulin pump.

Yet, existing pumps come with their own problems, including high costs, inconveniently long tubing, fixed dosages, and a large size. These disadvantages not only inhibit freedom of movement and cause insulin waste but can also lead to life-threatening complications, as the tubing can build up pressure and lead to clogging and hyperglycemia.<sup>4</sup> Moreover,

even the best insulin pumps on the market are insufficient in flow rates, discreteness, and costs. Table 1 shows a comparison of typical insulin pumps available on the market. Expensive versions, such as Medtronic and Tandem, often feature integratable Continuous Glucose Monitor (CGM) software and extended infusion sets, as seen in Medtronic's case. Medtronic holds a 41.4% market share in the insulin pump market, while Tandem has a 9.2% share, and Insulet's Omnipod has a 16.2% share.<sup>5</sup>

**Table 1:** Comparison chart of typical insulin pumps on the market and size comparison of a Band-Aid and the fluids module of this project.

	Omnipod DASH	Medtronic Minimed 630G	Tandem Tslim	V-Go	This Project
<b>Cost (w/o insurance)</b>	\$600	\$3,500	\$4000	\$635	<b>\$30</b>
<b>Dimension (mm)</b>	53 x 52 x 10	39 x 98 x 24	51 x 80 x 15	61 x 33 x 13	<b>40 x 10 x 3</b>
<b>Actuating Method</b>	Step Motor	Step Motor	Step Motor	Compressed spring	<b>Piezoelectric</b>
<b>Tubeless</b>	Yes	No	No	Yes	<b>Yes</b>
<b>Adjustable rates</b>	Yes	Yes	Yes	No	<b>Yes</b>
<b>Painless Injection</b>	No	No	No	No	<b>Yes</b>

In addition, it is important to note that basal treatment of continuous subcutaneous insulin infusion (CSII) works by delivering rapid-acting insulin doses at the set basal rate, unlike that of multiple MDIs, in which long-acting insulin is injected in one go.<sup>6</sup> Therefore, the key to designing an insulin pump is to control basal and bolus rates accurately.

The inverse piezoelectric effect can effectively achieve this goal, as piezoelectric materials deform elastically under electric fields, enabling each stroke to generate a small but precise displacement.<sup>7</sup> The displacement leads to a pressure change in the pump chamber, and therefore drives the fluid forward. A piezo actuator (such as a piezo buzzer) is thin, typically 0.2 mm in thickness, and inexpensive (around \$0.50 apiece), adding to its appeal for insulin micropumps. To engineer a precise micropump, the piezo buzzer's displacement, and hence flow rate, must have a monotonic dependence on the driving signal's amplitude. Previous research showed that the actuation voltage and displacement are approximately proportional within a certain range.<sup>8</sup> Piezoelectric systems are not yet in commercial insulin pumps due to hysteresis leading to actuator fatigue, high sensitivity to air bubbles that can lead to inaccurate dosage, and the general high regulatory burden that requires intensive testing (as piezoelectric pumps are still relatively new).<sup>9</sup> The existing piezoelectric micropumps are too large (80 × 50 × 22 mm) to be a patch pump.<sup>10</sup> Additionally, the complex dual chamber structure is unsuitable for low infusion rates, such as for Type 2 diabetes.<sup>11</sup>

Tubing increases the risk of occlusion, which is the blocking of insulin.<sup>12</sup> Pumps like Medtronic Minimed and Tandem Tslim still have tubing, which limits mobility. Tubing and wires both inhibit freedom of movement. Smart healthcare systems, including wireless monitoring, demonstrate improvements in overall quality of life, particularly in blood sugar management.<sup>13</sup> Therefore, a Bluetooth connection would eliminate the need for wires and would start the pump wirelessly. Just as a mobile phone would send out a signal to earbuds for the speaker to actuate, it also sends out signals to the piezo buzzer at the target frequency and voltage.

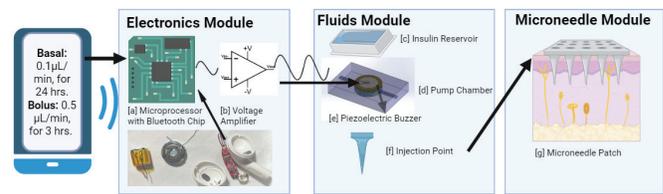
Current needles cause pain, while 10% of diabetes patients have needle phobia. Current insulin pumps' infusion sets use hypodermic needles. Typical hypodermic needles are blunt and trigger pain receptors; an alternative option is microneedles. They are extremely sharp and small, so they bypass the stratum corneum barrier of skin without pain.<sup>14</sup> The length of the microneedles is ~ 2.5mm at maximum.<sup>15</sup> The criterion for painless injection is an administrative force of < 3 N.<sup>16</sup> Furthermore, hollow microneedles work well with an auxiliary device.<sup>17</sup> Having open microfluidic channels is new and promising, and easier to manufacture.<sup>18</sup>

Therefore, to engineer a better wireless, discreet, painless, and precise micropump, the goals of this study are to (1) apply the piezoelectric effect to engineering a discreet and precise fluids pump chamber, (2) incorporate wireless electronics that actuate the piezo buzzer on command, and (3) engineer a minimally invasive, painless microneedle patch to deliver liquid.

## ■ Methods

Figure 2 shows the overall process and components of the proposed insulin delivery system. First, a mobile phone with data of the target flow rate is sent to the electronics module (Figure 2 [a]). The microprocessor receives the information and sends sinusoidal signals. The signal is amplified by an amplifier to drive the piezo buzzer (Figure 2 [e]), which bends

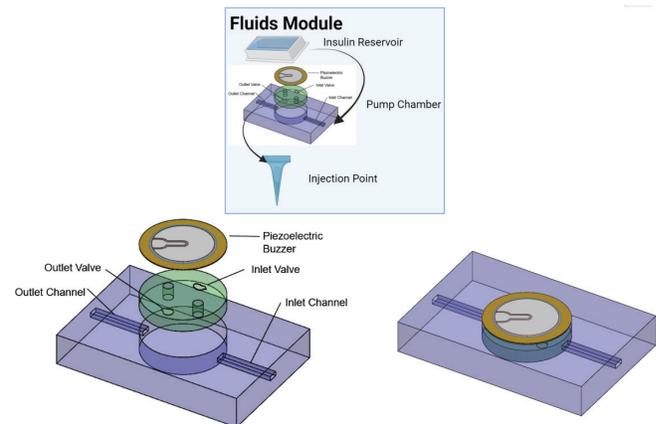
up and down, leading to a change in the volume and pressure of the pump chamber (Figure 2 [d]), therefore activating the fluids module. The fluid is sucked from the reservoir (Figure 2 [c]) and delivered to the end of microneedle patches (Figure 2 [f]), finally exiting to enter the patient's body (Figure 2 [g]).



**Figure 2:** Workflow diagram of insulin pump design, from actuation to insulin entering the patient's body. Created with BioRender.com.

### *Pump Chamber:*

The insulin will travel from the thin reservoir through the actuating chamber with two check valves, layered microfluidic channels, and an outlet (usually a needle) for insulin infusion (Figure 3). The Total Daily Dose (TDD) calculation is usually 0.4 - 1 units per kilogram of bodyweight for Type 1 Diabetics<sup>19</sup> and 0.1 - 0.2 units per kilogram of bodyweight for Type 2 Diabetics.<sup>20</sup> The standard split between basal and bolus rates is 50/50. Therefore, the bolus flow rates will be 0.2 - 3.0  $\mu\text{L}/\text{min}$ , based on the floor and ceiling numbers of 4 - 62 units per 3.5 hours for U100 insulin.<sup>21</sup> Therefore, the criterion is a flow rate of 0.02 - 3.0  $\mu\text{L}/\text{min}$ .



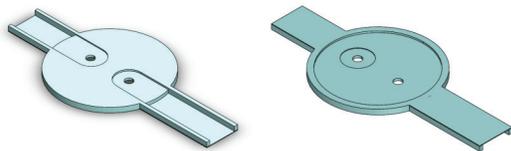
**Figure 3:** Pump chamber diagram.

(Note: These images are only models to demonstrate the structure of the pump chamber and are not designed to scale; the actual pump chamber is much thinner.)

The actuation amplitude, frequency, and waveform were controlled by the function generator, and the signal was delivered to the piezoelectric buzzer. The flow rate was then observed by tracking the motion of moving specks in the simulated pump chamber. The flow rate was calibrated by placing an object with a known size under the microscope and converting the actual measurements to pixels on screen; therefore, the distance travelled by the fluid was calculated.

After receiving electrical signals, the piezo buzzer (Figure 2 [e]) deforms vertically, leading to a change in the volume and pressure of the chamber (Figure 2 [d]), which causes insulin to enter and exit the chamber through one-way check valves. The pump will reciprocate continuously. In engineering the pump

chamber, it is essential to minimize the volume of the pump to reduce errors; therefore, it is designed in layers. This design will be effective, relatively easy to manufacture, and inexpensive (Figure 4).



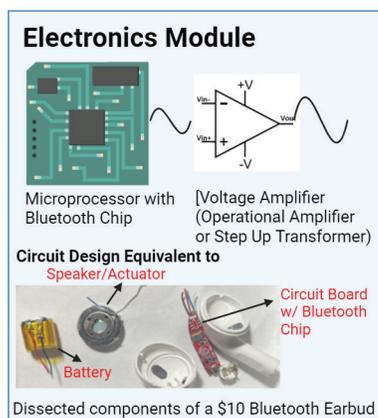
**Figure 4:** Final pump chamber body and channels design, front and back.

Water was used as the testing liquid because it has approximately the same viscosity as lispro rapid-acting insulin ( $1.0 \text{ mP} \cdot \text{s}$  vs.  $1.1 \text{ mP} \cdot \text{s}$ ).<sup>21</sup>

The line of motion of water through the pump chamber outlet channel was tracked over time under a microscope to determine the overall flow rate. The micropump's pump chamber, valves, and fluidic channels were made by layers of vinyl cut by a Cricut Joy® Paper Cutter™ (a popular tool among DIYers, which creates layers as thin as  $50 \mu\text{m}$ , finer than most 3D printers can print).

#### Wireless Electronics:

To establish a connection with mobile phones, a low-cost ( $\sim \$10$ ) wireless Bluetooth earbud was directly dissected (Figure 5). Only the actuator would need to be replaced with a typical speaker to a piezo buzzer. So the speaker was cut off, and a piezo buzzer was soldered on. The amplitude and waveform of the signals were measured with an oscilloscope and further adjusted with an amplifier. The criteria were a secure connection with a stable amplitude of  $15\text{--}20 \text{ V}$  (as this was the actuation voltage used to achieve the target flow rate range) and adjustable frequency.

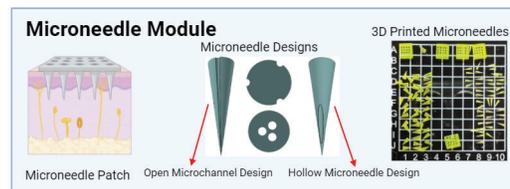


**Figure 5:** Electronics design further breakdown.

#### Microneedle Patches:

The microneedle had two designs: hollow and open microchannels (Figure 6) were fabricated by a high-precision 3D printer at Parvus Microstructures LLC (Minnesota, United States) with  $\sim 20 \mu\text{m}$  resolution. SOLIDWORKS stress analysis was used as a preliminary test to check the validity of the design and collect data on the various tensile and compressive forces. Empirical testing was later performed by measuring the

insertion forces, using weights and synthetic skin for different design types and needle spacing. The criteria were to ensure minimal pain with mechanical stability with an insertion force  $< 3 \text{ N}$ , and needle tip deformation  $< 250 \mu\text{m}$ . The constraints were a size of  $< 3 \times 3 \text{ mm}$ .

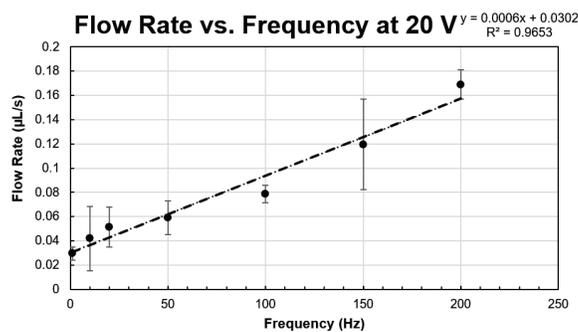


**Figure 6 :** The microneedle module breakdown.

## Results and Discussion

### Pump Chamber:

The data indicate an overall linear trend between the frequencies and flow rate (Figure 7). However, the error possibility at  $150 \text{ Hz}$  is high and might disrupt this conclusion. Currently, frequencies of  $10 \text{ Hz}$  and  $20 \text{ Hz}$  satisfy both criteria for a flow rate of  $0.02\text{--}3.0 \mu\text{L}/\text{min}$  and a frequency below  $20 \text{ Hz}$ . The final pump chamber design was manufactured using 3 layers of poly-coated paper and had lift check valves to control the direction of flow. This design was mostly successful because it effectively decreased the dynamic load (which is a key factor that limits the performance of piezoelectric pumps) by embedding check valves.<sup>22</sup> However, there were small gaps on the side of the pump chamber formed by uneven adhesive, which likely led to the leakage at the sides when pumping fluid out. Hysteresis and the instability of the input voltage signal might have also contributed to the leakage at  $< 17 \text{ Hz}$ .<sup>23</sup>



**Figure 7:** Flow rate vs. actuation frequency in simulated pump chamber, with error bars. There is an overall linear trend between the flow rate and actuation frequency when the voltage is set.

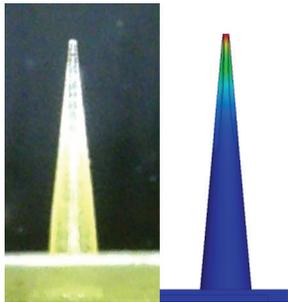
### Bluetooth Electronics:

The piezoelectric buzzer was soldered onto the circuit board of the Bluetooth earbud. The Bluetooth earbud was paired and connected with a mobile phone, which was playing a sinusoidal signal using a function generator app. The ends of the Bluetooth earbuds were connected to an oscilloscope, which measured the signal that the Bluetooth earbuds received. This validated the signals the mobile phone was sending out to the Bluetooth earbud, and there was no signal distortion. The amplitude of the initial sinusoidal signal was  $0.4 \text{ V}$  peak-to-peak, while the target amplitude was  $15\text{--}20 \text{ V}$ ; therefore, the signal needed to be amplified. A step-up transformer (which was the original selected amplifier) was soldered between the piezo

buzzer and microcontroller, but the signal could not be measured because the tension of the clips caused the connection between the transformer and microcontroller to break. This was a limitation in hitting the target voltage, and the amplifying circuit component should be changed. Therefore, future research will focus on switching the Digikey ATB322515-0110 transformer component (3.20mm x 2.50mm x 1.55mm) to a general-purpose operational amplifier, LMV358. However, the concept of dissecting a Bluetooth earbud to act as a controller for the insulin pump system has been validated. The signal before amplification was 20 Hz, with a peak-to-peak voltage of 0.4 V.

### Microneedle Patches:

The hollow microneedles were printed out of PMMA and were manufactured accurately as per design (Figure 8). The open microchannel microneedles have had manufacturing issues and have been unable to be inserted. The hollow microneedles have been able to withstand force without large tip deformation while being inserted over 90% into the synthetic skin when each was given a 0.25 N injection force (Figure 9).

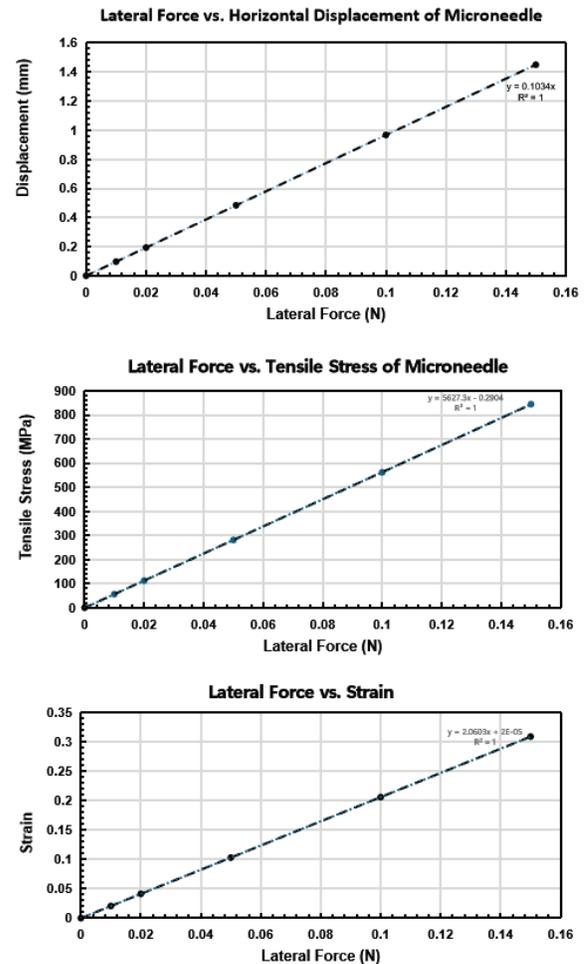


**Figure 8:** Printed hollow microneedle vs. in simulation.



**Figure 9:** Top view showing hollow microneedles being injected into the skin model.

However, when empirically testing the microneedles, there may have been uncontrolled pressure during handling with tweezers. This could be improved upon by using a precise Universal Tensile Machine, which has been the standard for microneedle testing.<sup>24</sup>



**Figure 10:** Lateral force vs. horizontal displacement, stress, and strain for open microchannel microneedle. The graphs show a linear relationship between lateral force and the other variables.

The simulation data show that both designs' tip displacement, strain, and stress grew linearly with the applied force (Figure 10). The simulation used a standard mesh in SOLIDWORKS, and forces of 0.01 N, 0.02 N, 0.05 N, 0.10 N, and 0.15 N were applied at the tip of the design, with the base plate fixed. The hollow microneedle previously performed better than the open microchannel microneedle in simulation, but this was due to an error in the positioning of the insertion force. Even then, the vertical tip displacements were very similar ( $7.16 \times 10^{-3}$  mm for hollow microneedle vs.  $8.82 \times 10^{-3}$  mm for open microchannel microneedle when 0.125 N vertical force was applied). This was unexpected because it was predicted that the open microchannel microneedle would perform better, as it has more mass and therefore was expected to be more mechanically stable.

Later, the positioning of the forces was adjusted to be identical, and lateral (instead of vertical) forces were applied at the tip, as buckling forces tend to cause more mechanical stress on thin microneedles, especially since the aspect ratio is 1:5 for the designs.

Under the same lateral forces (0.01 N) and material (Polycarbonate), the open microchannel design had a smaller displacement (0.097 mm) than the hollow microneedle (0.113 mm). The same experiment was repeated with two materi-

als-polycarbonate and stainless steel. Polycarbonate is more biocompatible and easier to manufacture than stainless steel, but stainless steel is more rigid than polycarbonate and has a higher elastic modulus. If  $\delta$  represents the horizontal displacement by the horizontal force, and  $E$  represents the material's elastic modulus, then the product of them should be a constant value for the same design, as the force applied is also a constant value. Thus,  $k = \delta E/F$ , where  $k$  is a constant with a unit of  $m^{-1}$ . The higher the  $k$  is, the more prone the material is to bending. The value  $k$  can also be used to calculate the displacement  $\delta$  when the material and applied force are known. It was calculated that:

$$k_{\text{Hollow Microneedle}} = 2.6 \times 10^7 \text{ m}^{-1}$$

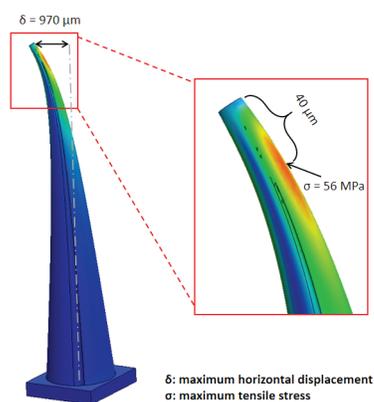
$$k_{\text{Open Microchannel Microneedle}} = 2.2 \times 10^7 \text{ m}^{-1}$$

Therefore, it can be concluded that the open microchannel microneedle is more rigid. However, these designs show similar  $k$  values, so the difference may be within experimental error.

Injecting at a 45-degree angle with the same lateral force resulted in the same horizontal displacement (both 0.97 mm) and in a smaller maximum stress (original was 563 MPa, 45-degree was 562 MPa).

Therefore, lateral forces should be minimized during the injection of the microneedle patch. This includes injecting at an angle or accidental rubbing, as keeping the movement strictly vertical would be ideal for preserving the mechanical shape of the microneedles, minimizing the injection force, and reducing pain.

At 0.1 N lateral force, both microneedle designs displace a significant amount ( $\sim 1$  mm), about 1/5 of the total length of the microneedle. More importantly, at a 0.04 mm distance to the tip, the open microchannel microneedle experiences peak stress ( $56150000 \text{ N/m}^2$ ), which is close to the maximum tensile strength of the Polycarbonate material ( $62700000 \text{ N/m}^2$ ); therefore, the microneedle is close to failure (Figure 11). The safety factor of this design was 1.12 (maximum tensile strength/stress the microneedle experiences). Therefore, the lateral force should be reduced as much as possible, and perhaps in the future, an attachment or fixture to the microneedle patch should be designed to keep the injection motion strictly vertical.



**Figure 11:** Open microchannel microneedles' displacement and stress under lateral force at the tip under 0.1N lateral force. The microneedle experiences a bending of 0.04 mm from the tip and a horizontal displacement of 0.97 mm.

## ■ Conclusion

The purpose of this project was to develop an insulin treatment system for diabetes patients by developing the components of a piezoelectric pump chamber, wireless Bluetooth electronics, and a painless microneedle patch.

The micropump chamber was able to push liquid forward at the target flow rate (0.02 - 3.0  $\mu\text{L}/\text{min}$ ). The Bluetooth earbud functioned as a complete microcontroller system and successfully actuated the piezoelectric buzzer, similar to System-on-Chip designs.<sup>25</sup> However, it did not hit the target voltage (15 -20V) to successfully actuate the pump yet because numerous attempts to solder on a step-up transformer failed because of the small size of the electronics, and might not have been effective due to core losses and low frequency inefficiency. Simulation experimentation of the two microneedle designs showed that the open microchannel microneedle is more resistant to lateral forces, and vertical motion should be maintained through injection. The hollow microneedles were inserted over 90% into the synthetic skin when each was given a 0.25 N injection force.

**Table 2:** Summary of key metrics & results.

	Flow rate	Wireless actuation	Microneedles
Results	Pump chamber target flow rate (0.02-3.0 $\mu\text{L}/\text{min}$ ).	Bluetooth earbud signal was 0.4V.	Hollow microneedles were inserted over 90% into the synthetic skin when each was given a 0.25 N injection force.
Limitations	High maintenance of the pump chamber and high fatigue of the valves.	Signal too weak and did not hit the target voltage, substitute with an operational amplifier or other components in the future.	Have not been tested as a microneedle patch.

These three individual components work smoothly, so it is predicted that when integrated, the micropump system has the potential to effectively assist diabetes patients in their treatment. This system has increased discreteness (3 mm thick) and convenience due to the lack of wiring and tubing. The painless microneedles may also lead to an improved experience. The low-cost manufacturing of piezoelectric micropumps has the potential for more affordable and accessible healthcare, saving the healthcare costs of over 11 million Americans. The material cost of the micropump is less than \$30, while current pumps cost \$700-\$3000 without insurance. This micropump has the potential to lower the risk of hyperglycemia and prevent hypoglycemia.

Future research will likely focus on integrating the micropump's three components by amplifying the actuation signal and improving the microneedle patch by adding fixtures. Experiment repeats should also be done to solidify findings.

## ■ Acknowledgments

The author thanks Dr.Faraji Rad of University of the Sunshine Coast for being a wonderful mentor, Parvus Microstructures LLC for generously 3D printing microneedles free of charge, and MIT THINK for generously providing funding that supported the continuation of this project.

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# Adults 65 and Older Navigating the Digital World

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**ABSTRACT:** As society becomes more digital than ever, older adults face increasing difficulties interacting with advancing technology due to physical, cognitive, and psychological barriers. This study investigates the connection between technology design, reliance on support, and confidence among adults aged 65 and older. A survey of 123 participants identified key usability obstacles, including small text, complex interfaces, and password management. While most older adults in the study report feeling confident in using devices, most still experience significant difficulties with technology. This suggests that confidence does not always translate to ease of use. The findings underscore the importance of inclusive design and structured support systems for older users, as they may be crucial in boosting confidence and promoting digital independence. This study fills a gap in existing research by simultaneously examining design features, support structures, and user confidence. There are several actionable areas for technology developers and policymakers looking to extend their devices to older generations.

**KEYWORDS:** Behavioral and Social Sciences, Sociology and Social Psychology, Older Adults and Technology, Digital confidence, User Interface Accessibility for the Elderly.

## ■ Introduction

As technology continues to advance at a rapid pace, older generations face increasing challenges in keeping up with digital tools and platforms. Older adults often struggle with technology due to physical limitations, cognitive barriers, and unfamiliarity with digital interfaces. This technological gap risks isolating older individuals from essential services, digital communication, and even social engagement, reinforcing generational divides and increasing dependency on younger family members or caregivers. Despite these challenges, many older adults express a willingness to engage with technology if provided with the necessary support and accessible designs. The barriers they face—including physical impairments like hand tremors or visual difficulties, cognitive decline affecting memory and processing speed, and psychological factors such as fear of making mistakes—can make technology use daunting and frustrating. To explore these issues further, this research aims to investigate the role support systems play in helping older generations navigate technology.

This research was conducted through a survey and qualitative analysis to identify the most significant challenges preventing older adults from effectively using digital tools and how support systems, such as family members, caregivers, and community programs, can bridge this gap. By understanding these dynamics, we can develop more inclusive technological solutions that empower older adults to remain connected, independent, and active participants in an increasingly digital world. According to Michelle Faverio, a research associate at Pew Research Center, 96% of people aged 18 to 29 own a smartphone, compared with 61% of those 65 and older, yet this statistic may have changed in the past few years.<sup>1</sup> Furthermore, according to Monica Anderson, the director of Internet and Technology research at Pew Research Center, “just 26% of internet users ages 65 and over say they feel very confident when

using computers, smartphones or other electronic devices.”<sup>2</sup> Malihe Yazdani Darki, at the Kashan University of Medical Sciences, stated that this could be partially due to physical limitations, including visual impairments, back pain, and hand tremors, making technology difficult for the older generation.<sup>3</sup>

### *Barriers to Technology Usage:*

Conducting further research in this area, Mujtaba Awan, in the Department of Software Engineering at the University of Gloucestershire, finds that technology such as smartphones may also be difficult for the older generation to manage, as they may have a hard time with small fonts, screen size, font type, buttons, and color contrast.<sup>4</sup> Exploring this from a different perspective, Charness and Boot, researchers in cognitive aging at Florida State University, highlight that reduced fine motor skills in aging adults make swiping or tapping on a touchscreen more challenging, increasing the risk of mistakes and, consequently, discouragement, especially since many devices have low-contrast displays or small text.<sup>5</sup> According to Dr. Sara Czaja, a behavioral scientist and Professor of Gerontology in Medicine, older adults’ ability to operate electronics such as smartphones and tablets is significantly affected by age-related impairments in motor control and dexterity, which can cause them to feel frustrated and reluctant to use technology.<sup>6</sup> Additionally, Tracy Mitzner *et al.*, a senior research scientist at Georgia Institute of Technology, discovered that aging can oftentimes bring a decline in working memory and processing speed, which can make it difficult to learn and retain new technological skills.<sup>7</sup> Compared to younger users, who often have stronger cognitive flexibility and more prior experience with technology, older individuals need more time and repeated exposure to learning new digital technologies. As an example of Mitzner *et al.*’s findings, Daniel Morrow *et al.*, a professor of Educational Psychology at the University of

Illinois Urbana-Champaign, find that when applications for tracking medical-related information required multiple steps and quick recall, older adults demonstrated significantly higher error rates and frustration levels than younger users.<sup>8</sup> Many people avoid digital tools entirely because they are afraid of making mistakes, accidentally deleting important documents, or falling victim to internet scams. Heart and Kalderon, researchers at Ben-Gurion University of the Negev, found that older adults who lacked experience with technology had increased insecurities when using devices.<sup>9</sup> Furthering this point, Eleftheria Vaportzis *et al.*, a senior lecturer at the University of Bradford, highlight that feelings of incompetence and exclusion can be reinforced when technology is viewed by older adults as being primarily made for younger users.<sup>10</sup> Overall, the older generations' adoption and use of technology are severely hampered by the aforementioned obstacles that they may encounter. Digital interactions become more difficult because of medical conditions like impaired motor control, slower processing speed, memory loss, and psychological issues, including social rejection and fear. This highlights the need for more inclusive technology design and educational programs to close the generational technology divide.

#### ***Reliance on Support:***

Research indicates that this digital divide is expanding due to older adults being unable to overcome technological issues independently. According to Kathleen Schreurs *et al.*, a research and statistics expert at Western University, adults frequently depend on younger family members for digital assistance, which can affect their self-esteem and sense of autonomy.<sup>11</sup> Building on this research, Charlene Quinn, an Associate Professor at the University of Maryland, finds that many older adults also rely on caregivers for technological support with mobile health management and online communication.<sup>12</sup> This leads to older generations relying on others, as "nearly 50% of older adults 65 and older and 40% of those ages 50-64 feel they need someone to assist them in learning and using a new technology device," as stated by Deborah Vollmer Dahlke, an associate Professor at Texas A&M University.<sup>13</sup> This reliance is often due to a lack of digital literacy, as older adults may not have had the same exposure to evolving technologies as younger generations. Healthcare and financial management are among the most common areas where older adults require digital assistance. Nancy Gell *et al.*, an assistant professor at the University of Vermont, states that older adults with physical impairments are less likely to use technology independently, often requiring assistance for tasks such as online banking and accessing health information.<sup>14</sup> Guo Yin *et al.*, at the Sanquan College of Xinxiang Medical University, explore this idea and conclude that this reliance on others not only limits their independence but also creates security risks, as they may share sensitive information with family members or caregivers rather than managing it independently.<sup>15</sup> The combined findings of Gell *et al.* and Yin *et al.* indicate that the lack of confidence in using technology also contributes to older adults' reluctance to explore digital tools independently. Many older adults depend on family members, caregivers, or professional assistance

to navigate digital technology, which can reinforce the generational digital divide that results from younger generations viewing older adults as inept with technology. Additionally, technology interfaces are often designed with younger users in mind, making them less intuitive for older generations. This ongoing reliance on others for digital navigation underscores the importance of targeted training programs and simplified user interfaces, which have been found to improve confidence, increase their likelihood of independent technology use, and reduce their reliance on others. However, according to Yvonne Barnard at the University of Leeds, programs that incorporate peer mentorship—where older adults teach each other digital skills—are more effective than traditional classroom settings.<sup>16</sup> Without these interventions, older adults may continue to depend on family members and caregivers, further limiting their independence in an increasingly digital world. Overall, a major obstacle to older persons' technological independence is their reliance on others for digital help. This dependence is exacerbated by low digital literacy, confidence problems, and technology designed for younger users, which limits their independence and puts them in danger of security breaches. By implementing focused training programs and peer mentorship activities, older persons can become more digitally inclusive and less reliant on family members and caregivers by learning how to use technology with confidence.

#### ***User Interface:***

Many older adults also struggle with technology because of design choices that do not consider their needs. According to Sergio Sayago *et al.*, an Assistant Professor in Human-Computer Interaction at the University of Lleida, many digital devices prioritize aesthetics and innovation over accessibility, which can make them hard for older adults to use.<sup>17</sup> Professors of Psychology at the Georgia Institute of Technology, Rogers and Fisk, highlight some examples, such as small text, complex menus, and touchscreen sensitivity issues, which make technology challenging to use, in turn leading to frustration and a lack of desire to use.<sup>18</sup> Jia Zhou *et al.*, a researcher in human-computer interaction and co-chair of the International Conference on Human Aspects of IT for the Aged Population (ITAP), states that older adults are significantly less likely to use smartphones and tablets when the interface requires precise motor control or involves a lot of scrolling and tapping.<sup>19</sup> According to Dan Hawthorn at the Unitec Institute of Technology, many seniors benefit from larger font sizes, high-contrast displays, and simplified navigation.<sup>20</sup> When technology incorporates adjustable text size and voice commands, older adults are more likely to engage with digital devices and have a better user experience. According to Alisha Pradhan, an assistant professor at the New Jersey Institute of Technology, voice-activated assistants, such as Amazon Alexa and Google Assistant, can help older adults interact with technology more easily, particularly those with vision impairments or limitations with mobility.<sup>21</sup> A survey of technology developers, conducted by Dr. João Leitão *et al.*, an associate Professor of Economics with Habilitation at the University of Beira Interior, found that only 20% of professionals considered older adults a primary audience

when designing new digital tools.<sup>22</sup> Considering the current state of technology development referenced above, Siân Lindley *et al.* at Microsoft Research share that because of this, older adults often feel alienated from technology and struggle to integrate it into their daily lives.<sup>23</sup> The implementation of design principles, such as intuitive layouts, clear instructions, and multimodal interaction options, could help improve accessibility for older generations. By prioritizing user-friendly features, incorporating accessibility tools, and considering seniors in the design process, technology companies can create more inclusive products. Older adults face significant challenges in adopting technology due to physical limitations, cognitive decline, reliance on others, and poor design choices that fail to accommodate their needs. These barriers make digital engagement frustrating and discourage seniors from using technology independently. Many older adults risk losing access to essential services and communication tools without targeted interventions, as they increasingly shift online. To improve accessibility, technology companies must prioritize senior-friendly designs, such as larger text, voice commands, and intuitive interfaces. Additionally, structured training programs and peer mentorship can help older adults develop digital confidence and reduce reliance on family members for assistance. By addressing these issues, society can ensure that older adults remain engaged, independent, and empowered in an increasingly digital world, allowing them to benefit from technological advancements rather than being excluded. The culmination of research on technology usage amongst the older generation addresses what percentage of older adults are online, what technology they use, how they may benefit from technology, and more. While there have been studies that have explored older adults' confidence with technology, specific aspects that make it more user-friendly, and the individuals that older adults rely on for technology problems, no published studies have tried to connect all three of these factors in the same study. The lack of research around these points to the gap that this study aims to fill. With this understanding, this paper's research question is: To what extent do technology features and support systems play a role in confidence levels with digital devices among adults aged 65 and older? The researcher hypothesizes that the users who had the most ease with technology features and who relied on support systems less frequently would report higher confidence levels with technology. Participants who have more difficulty with features or regularly seek technology support would feel less confident with technology and therefore use it less frequently.

## ■ Methods

A survey was used to gather evidence about how older adults interact with technology. Participants, aged 65 and above, were selected from retirement homes using paper surveys as well as WhatsApp group chats using digital surveys (see Appendix A) to ensure a broad representation across different age groups and technology skills. Participants were recruited through two methods. First, administrators at independent-living retirement communities granted permission for the researcher to invite residents aged 65 and above to participate; residents

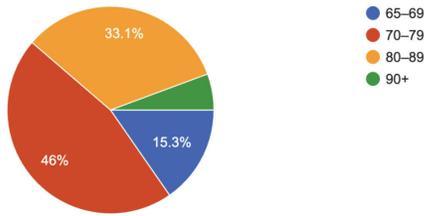
who volunteered were then given the paper survey to complete. Second, older adults in WhatsApp community groups were sent a posted message, and those who chose to participate completed the digital version of the survey. Some participants chose to fill out the survey independently, while others received assistance with reading or writing if needed. Data collection methods include surveys to gather data on the amount of screen time, technology use, technological help, and health aspects. The researcher asked participants these specific questions, as it allowed for data to be collected to identify a potential correlation between age and confidence level specific to usability features or types of technology. The specific survey questions were selected to measure three main areas: technology usage patterns, confidence levels, and the usability barriers most frequently documented in prior research. All questions appeared on the written or digital survey for participants to answer independently; none were verbally asked. Additionally, it aims to identify the specific challenges they face when using technology, explore the assistance they rely on, to develop strategies that can enhance their ability to adapt to technological advancements. The researcher decided to specifically identify through the survey some specific features, such as small text, touchscreen sensitivity, or confusing interfaces, to confirm the findings of other researchers. However, other features, such as battery life or popups, were not expected to have as high a result but were included to determine if other factors contribute to a lack of confidence among older adults using technology. By addressing these barriers, the research aspires to improve the overall quality of life for older adults by developing technology that is easier for them to use. The researcher included children, grandchildren, technology professionals, Information Technology (IT) service providers, as well as assistants, nurses, and aides, as these are thought to be the most often sought out support when it comes to technology help for older adults. There were 123 respondents to the survey, sent to people aged 65 and older, asking how often they use technology, their confidence with it, what they struggle with, and more. In this method, 5.8% of the respondents were 90 and older, 32.2% were aged 80 to 89, 46.3% of the respondents were aged 70-79, and 15.7% of the respondents were aged 65 to 69 years old. The resulting age gap allowed the researcher to gather information from diverse ages among the older generations. A varied age group was used to determine differences between all selected age groups amongst the older generation. Participants were not given an incentive and instead participated out of the desire to increase research on technology to better accommodate the older generation. All study procedures were reviewed and approved by the Institutional Review Board (IRB), ensuring ethical oversight of research.

## ■ Results and Discussion

Paper surveys were handed out to residents at a South Florida Nursing Home, and they were given assistance by the researcher if they needed clarification on what the survey asked or needed help writing down answers. Surveys were also forwarded through WhatsApp groups digitally. This ensures

a diverse age range, technological skill, and mental awareness level.

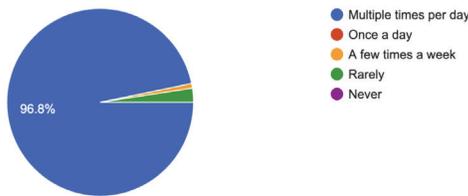
What is your age group?  
124 responses



**Figure 1:** Age distribution of participants aged 65 and older. This figure displays the breakdown of participant ages, showing a diverse sample spanning from 65 to over 90 years, which allows for an understanding of technology use and challenges across a broad age range of older adults.

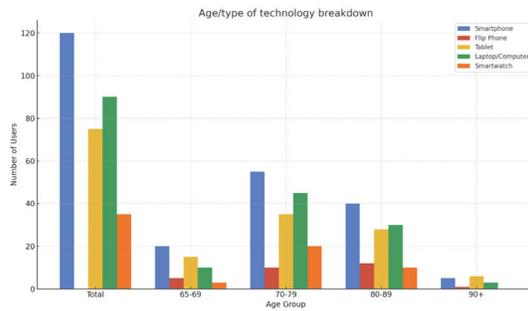
Figure 1 shows the distribution of ages among the 123 survey respondents. The majority of participants (46.3%) were between the ages of 70-79, followed by 32.2% aged 80-89, 15.7% aged 65-69, and only 5.8% aged 90 and older. The range reinforces that the study’s findings are not isolated to a specific age subset but span much of the older adult population. This strengthens the study’s ability to generalize findings across the older adult population and examine how older adults interact with technology and the confidence they feel in it.

How often do you use a smartphone, computer, or tablet?  
124 responses



**Figure 2:** Frequency of electronic device use among older adults. This figure shows that the majority of older adults use digital devices such as smartphones, tablets, or computers multiple times per day, highlighting widespread daily engagement with technology.

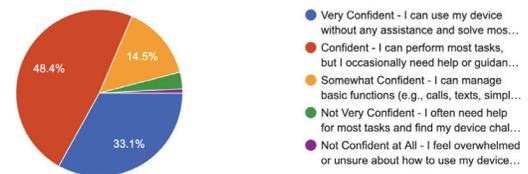
Figure 2 illustrates how often participants use devices such as smartphones, tablets, or computers. The majority of the respondents (96.7%) use a smartphone, computer, or tablet multiple times per day. 2.4% of the respondents use an electronic device rarely, and 0.8% of the respondents use an electronic device a few times a week. This demonstrates that regular digital activity is very common among older generations, with only a very small group not using the most common digital devices. This shows that frequent digital engagement is widespread among older adults, contradicting stereotypes of digital disconnection. Despite this high usage, later figures reveal that many still experience difficulty navigating technology. This supports a core finding of the study: frequent use does not eliminate barriers such as poor technological design, a lack of confidence navigating technology, or over-reliance on support.



**Figure 3:** Types of digital devices owned by older adults. Smartphones are the most commonly used device, followed by laptops/computers, and then tablets, indicating the critical importance of mobile device usability across devices for older adults’ digital experiences.

Figure 3 identifies which digital devices respondents currently own. Out of 123 responses, the total section indicates that nearly all participants (95.1%) use a smartphone. Most of the respondents have a laptop/computer (73.9%) or a tablet (63.4%). Many fewer have a smartwatch (27.6%), and barely any own a flip phone (0.8%). Based on this, the researcher can conclude that the majority own a smartphone, and within the 60s, 70s, and 80s age groups, more than 95% of the participants had a smartphone. Also, in each of these age ranges, 60% or more had a tablet and/or a laptop/computer. The 90s group also mirrors this; however, it is more difficult to be certain because this age range had the smallest number of participants. This demonstrates that smartphones are the dominant device across the older generation, with tablets and laptops also widely used. Because smartphones are the most common digital device, their design plays a crucial role in shaping older adults’ digital experiences. Poor design decisions in mobile apps could therefore have a very negative effect on this demographic and confidence in these devices.

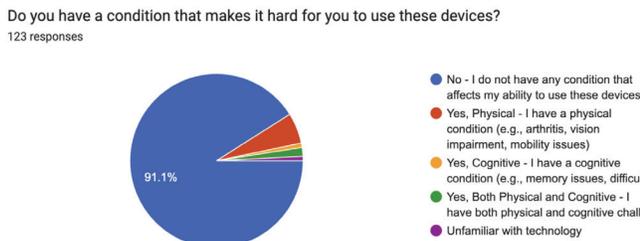
How confident do you feel in using your device?  
124 responses



**Figure 4:** Self-reported confidence levels in using digital devices. Most participants consider themselves confident or very confident in using digital devices, although this confidence contrasts with reported difficulties, revealing a gap between perceived ability and actual usability difficulties.

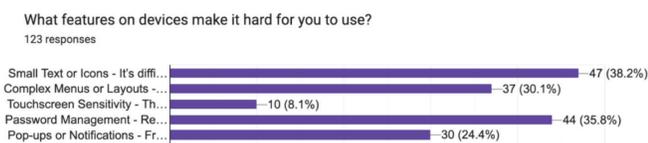
Figure 4 highlights the self-assessed confidence levels of the participants when using digital devices. Most participants feel confident using their devices, with 48.8% saying that they are “Confident” and 33.3% saying that they are “Very Confident.” A smaller group of 13.8% said that they are “Somewhat Confident,” meaning they can handle basic tasks online but may struggle with more advanced ones. Few participants fall into the “Not Very Confident” or “Not Confident at All” categories, indicating that only a small percentage frequently need assistance. While most respondents can navigate their

devices well, some still require occasional help. This perceived confidence contrasts with widespread usability difficulties shown in Figure 6, where most participants reported struggling with basic features. This disconnect between confidence and actual ease of use is one of the central findings of this study, showing the need for technology with better design that may be easier to use.



**Figure 5:** Health-related conditions affecting participants' technology use. The majority of participants report no physical or cognitive impairments impacting their device use, suggesting that usability and design issues, rather than health limitations, are the main barriers for older adults.

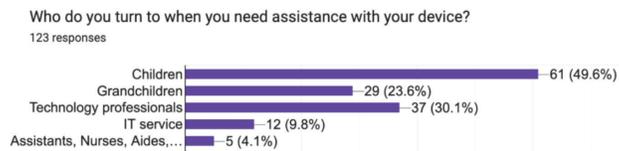
Figure 5 breaks down which participants report health-related conditions that hinder technology use. While 91.8% report no such conditions, a small subset cites physical challenges (e.g., arthritis, vision loss), cognitive decline (e.g., memory loss), or both. Some participants have both physical and cognitive challenges, making devices more difficult to use. While most participants have no accessibility barriers, a small group may need additional support or accommodations due to cognitive or physical issues. This supports the study's finding that age-related health conditions are not the main barrier to technology use among older adults. Instead, the challenges older adults face tend to stem largely from poorly designed interfaces and a lack of tailored support.



**Figure 6:** Health-related conditions affecting participants' technology use. The majority of participants report no physical or cognitive impairments impacting their device use, suggesting that usability and design issues, rather than health limitations, are the main barriers for older adults.

Figure 6 highlights the digital features that respondents find most difficult to use. 20.5% of respondents report no challenges; however, among the remaining 79.5%, there were a variety of features that made technology difficult to use. The results found that the most common device features that participants find challenging include small text or icons, password management, and complex menus or layouts, as well as pop-ups and technical jargon. Some of these findings, such as difficulties with small text, complex menus, and touchscreen sensitivity, mirror the findings of Rogers and Fisk.<sup>17</sup> An unanticipated finding was the difficulty of managing passwords (35.2%), which is not a device-specific difficulty because it relies on the user to be able to remember or recall the password. Small text, password management, and complex layouts are

the leading difficulties with technology, highlighting design as a core usability barrier. These difficulties reveal that older adults have difficulty navigating technology because many digital interfaces fail to accommodate their needs. This figure directly supports the study's central call for a more inclusive, age-friendly design.



**Figure 7:** Sources of technology support for older adults. Nearly half of the surveyed seniors rely on their children for technical assistance, with a majority depending on family members overall, indicating strong social reliance that may affect their autonomy and privacy.

Figure 7 highlights the people that older adults turn to for tech support. Nearly half of the respondents (49.2%) turn to their children for assistance with their devices, making them the most common source of help with electronics. The combined 72.2% of children and grandchildren shows the role of family members in technology assistance for older adults. This is similar to the findings of Schreurs *et al.*, who found that older adults often turn to younger relatives for assistance.<sup>11</sup> Technology professionals (30.3%) are also significant sources of support, and a smaller percentage (9.8%) rely on IT services, while only 4.1% seek help from assistants, nurses, or aides. This reflects a heavy reliance on family support rather than formal services, underscoring the need for intuitive design and better training options. The heavy reliance on family reinforces the study's finding that without a more thoughtful design and structured support, many older adults struggle to engage confidently with digital technology.

### Analysis:

The researcher hypothesized that those who reported difficulty with technology features would indicate lower levels of technological confidence. As demonstrated in Figures 4 and 6, although most users report confidence, many report difficulty with device features such as small text and password management. This was different from the researcher's findings since, according to Figures 4 and 6, the majority of respondents (82.1%) feel a sense of confidence using their devices; however, at the same time, 79.5% reported difficulty with features. These features include small print (37.7%), password management (35.2%), and complex menus (30.3%). This shows that confidence does not necessarily equate to ease of use, and underscores the need for a more user-friendly design. This could include voice-activated assistants, as shown by Pradhan *et al.*; however, this was not specifically investigated in my research, as it is a growing technology that fewer individuals currently have access to. Revisiting Leitão *et al.*'s findings, which showed consideration of older adults as a minority (20%), these findings indicate a significant need for the older generation's voices when designing new technology. While development is part of the issue, further consideration needs to be given

to the personal support that older adults may need. Figure 7 illustrates this reliance clearly, with children and grandchildren being the main sources of help. These results echoed those of Schreurs *et al.*, finding that people rely more on relatives than any other group for assistance. Nearly half (49.2%) of parents turn to their kids for help, with technology workers (30.3%) and grandkids (23%), who also play important roles. Few turn to aids (4.1%) or IT services (9.8%) for assistance, indicating a preference for unofficial support. While many of the participants rely heavily on family members, there is still a greater preference for help from actual people rather than automated systems, as indicated by multiple responses to question 10 of the survey (Appendix A).

#### **Future Directions:**

The findings of this study can be used by technology companies and app developers to create more user-friendly technologies for older adults. This can be implemented in companies such as Apple, Samsung, Microsoft, and Google to develop smartphones, tablets, or even software updates that address the key challenges faced by the older generation identified in this study. Phones such as Lively's Jitterbug offer larger buttons and emergency features that could be useful for older adults; however, they still fall short with slow performance, required PINs, no biometric logins, and limited apps.<sup>24</sup> Since around 30% of study participants struggled with password management, my findings suggest that software-based solutions—not just physical design—are essential. To address this, technologies such as Lively's Jitterbug phone can incorporate facial or fingerprint recognition, reducing the need for memorized passwords. Additionally, the major technology companies mentioned above could make facial and fingerprint recognition setups easier to follow, reducing the reliance on PIN passwords for older adults. Greater ease of use would likely lead to a decreased reliance on other people for help, which could boost confidence when using technology. Companies can also use this feedback to understand that, despite existing options for increasing font size, older adults may need assistance and education for setup. The findings indicate that many older adults still struggle with preventable issues, which points to an existing need to guide older adults to better understand the capabilities that the technology they are purchasing may already include. Technology companies may consider design technology that incorporates step-by-step instructional guides that walk users through downloading and navigating apps during the initial setup of the device. These guides could include interactive tutorials, voice-assisted instructions, and demonstrations tailored to different skill levels, to ensure that older adults can use their devices confidently and without external assistance. Further research into the cause of a lack of confidence, especially regarding falling for scams, making mistakes, and deleting important files, may be helpful to this area of research. Technology companies can incorporate built-in fraud alerts that warn about clicking on certain links or visiting potentially fraudulent websites, to protect users from online threats and boost their confidence when using technology, as discussed by Brands and van Wilsem.<sup>25</sup> By implementing these

recommended improvements, technology companies can help make the online world more inclusive. Improving accessibility will allow seniors to reap the benefits of the digital age and businesses to benefit from increased usability across more generations, given that it can be designed with their needs in mind. Additionally, looking ahead, future cohorts of older adults may interact with technology differently as digital tools become more deeply integrated into daily life. Because of this, usability challenges and support needs observed in today's older adults may shift significantly as future generations age. Continued research will be necessary to understand how evolving levels of digital exposure shape confidence, reliance on support, and technology engagement in later life.

#### **Limitations:**

The main limitation present in this study arose when recruiting participants to answer the survey. Some older adults may have a hard time answering a digital Google Form. To combat this limitation, the researcher printed out the surveys and handed them out at a local nursing home. The researcher helped some of the residents in the nursing home to fill in the questions or to clarify the questions if needed. Additionally, the sample size is limited and, therefore, may not capture generational trends in countries outside of the United States and among people from different socioeconomic backgrounds. Individuals' self-reported perceptions of their technological abilities may be biased and not standardized. It is important to note that the generalizability of these findings is limited by the narrow demographic information collected. Since the study focused on gathering age data, it does not account for other factors, such as education, socioeconomic status, or prior technology experience, which may influence confidence levels.

#### **Conclusion**

This study aimed to identify if confidence among older technology users was impacted by various technology features and the support that they received from others. The survey findings show major obstacles older adults encounter while utilizing technology, such as physical restrictions, cognitive decline, dependence on others for assistance, and subpar design decisions that do not meet their unique needs. Even though many of the participants reported feeling some level of confidence with technology, issues like small font, complicated menus, and password management still make it difficult for them to do so. Implementing more user-friendly design concepts and offering structured educational programs are crucial to ensuring older individuals stay connected and independent in an increasingly digital culture. Technology developers may help create a more inclusive digital environment and enable older generations to use technology with increased confidence and effectiveness by tackling these issues.

#### **Acknowledgments**

I would like to express my deepest gratitude to the school's Advanced Placement Research class for providing me with this invaluable research opportunity. This class has been instru-

mental in facilitating my exploration and understanding of the digital challenges faced by older adults. I would also like to thank my AP Research teacher, Taly Furer, for her guidance, feedback, and continuous support throughout the development of this project.

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## ■ Author

Rebecca Elitzur is a devoted student researcher with a strong interest in the intersection of aging, technology, and mental health. She intends to pursue a degree in psychology and public health to create inclusive solutions that support digital equity, mental health, and healthier communities for all.

## ■ Appendix A

### Research on Technology Usage

Hi, I am an AP Research student, researching technology usage in society. The purpose of my research is to determine what ways technology can be changed to assist the older generation.

\* Indicates required question

1. By selecting "yes", you agree to anonymously participate in the survey and have the information used in the research project. \*

Mark only one oval.

- Yes  
 No

2. What is your name? (Your name will not be included in the study; it is only for administrative purposes) \*

\_\_\_\_\_

3. What is your age group? \*

Mark only one oval.

- 65-69  
 70-79  
 80-89  
 90+

4. How often do you use a smartphone, computer, or tablet? \*

Mark only one oval.

- Multiple times per day  
 Once a day  
 A few times a week  
 Rarely  
 Never

5. Do you have an electronic device? \*

Check all that apply.

- Smartphone  
 Flip Phone  
 Tablet  
 Laptop/Computer  
 Smartwatch  
 Other: \_\_\_\_\_

6. How confident do you feel in using your device? \*

Mark only one oval.

- Very Confident - I can use my device without any assistance and solve most problems on my own.  
 Confident - I can perform most tasks, but I occasionally need help or guidance for more complex issues.  
 Somewhat Confident - I can manage basic functions (e.g., calls, texts, simple apps) but struggle with advanced features.  
 Not Very Confident - I often need help for most tasks and find my device challenging to use.  
 Not Confident at All - I feel overwhelmed or unsure about how to use my device and require frequent assistance.

7. Do you have a condition that makes it hard for you to use these devices? \*

Mark only one oval.

- No - I do not have any condition that affects my ability to use these devices.  
 Yes, Physical - I have a physical condition (e.g., arthritis, vision impairment, mobility issues)  
 Yes, Cognitive - I have a cognitive condition (e.g., memory issues, difficulty understanding instructions)  
 Yes, Both Physical and Cognitive - I have both physical and cognitive challenges  
 Other: \_\_\_\_\_

8. What features on devices make it hard for you to use? \*

Check all that apply.

- Small Text or Icons - It's difficult to read or see due to small text or icons.  
 Complex Menus or Layouts - The navigation or organization of features is confusing or overwhelming.  
 Touchscreen Sensitivity - The touchscreen is too sensitive or not responsive enough.  
 Password Management - Remembering and managing passwords is challenging.  
 Pop-ups or Notifications - Frequent pop-ups or notifications are distracting or confusing.  
 Limited Physical Accessibility - Buttons or other controls are hard to use due to physical limitations (e.g., arthritis).  
 Short Battery Life - Devices require frequent charging, making them inconvenient.  
 Technical Jargon - Instructions or settings use terms that are hard to understand.  
 None - I do not experience any difficulties with device features.  
 Other: \_\_\_\_\_

9. Who do you turn to when you need assistance with your device? \*

Check all that apply.

- Children  
 Grandchildren  
 Technology professionals  
 IT service  
 Assistants, Nurses, Aides, etc  
 Other: \_\_\_\_\_

10. What's your biggest struggle with technology and how do you think it can be fixed? \*

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

# Targeted Drug Delivery with Nano-Antibiotics to Mitigate Antibiotic-Related Adverse Effects and Mortality

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**ABSTRACT:** Antibiotics have revolutionized modern medicine over the last century, saving millions of lives and extending the average human lifespan. Twenty percent of people who receive antibiotics in the hospital experience at least one side effect. These can range from mild gastrointestinal irritation, skin, musculoskeletal, cardiac, and neurologic side effects to severe life-threatening events such as anaphylactic shock, Stevens-Johnson Syndrome, or *Clostridium difficile* infections. Antibiotic resistance is another global issue that is responsible for millions of deaths. Antibiotic resistance occurs when bacteria develop resistance to antibiotics bioengineered to kill them. Among bacteria that have developed ways to evade antibiotics' effectiveness are Methicillin-Resistant *Staphylococcus aureus*, *Escherichia coli*, *Mycobacterium tuberculosis*, and *Pseudomonas Aeruginosa*. Nano-antibiotics are being investigated to utilize antibiotics efficiently and reduce antibiotic resistance. Nano-antibiotics are either nanoparticles that naturally have antibacterial properties or traditional antibiotics that are packaged inside specialized nanoparticles. The unique properties of nanoparticles facilitate targeted, controlled drug delivery, thereby enhancing the bioavailability of the drugs. Nanotechnology has shown promising results in the treatment and prevention of *Clostridium difficile* infections. This research paper reviews antibiotic resistance and the improvement of antibiotic delivery using nanotechnology to reduce antibiotic-related adverse effects and mortality.

**KEYWORDS:** Materials Science, Nanomaterials, Nano-antibiotics, Targeted Delivery.

## ■ Introduction

Antibiotic resistance occurs when bacteria develop the ability to combat the drugs engineered to kill them. According to the CDC's 2019 Antibiotic Resistance Threat Report, more than 2.8 million antibiotic-resistant infections occur each year in the United States, and more than 35,000 people die as a result.<sup>1</sup>

According to a report by the World Health Organization, drug-resistant diseases could force up to 24 million people into poverty by 2030. Additionally, drug-resistant diseases could cause 10 million fatalities annually by 2050. If no mitigation or prevention strategies are available, antimicrobial resistance (AMR) can become a leading cause of death, even surpassing cancer by the year 2050. In addition to increased mortality, antimicrobial resistance increases the chance of secondary infection, making many medical procedures riskier. Antibiotic resistance is predicted to cost an additional \$1 to \$ 3.4 trillion in healthcare costs by 2050.<sup>2</sup>

*Clostridium difficile* is a bacterial infection that occurs when antibiotics are used to treat other infections. Antibiotic use causes a temporary disruption of normal gut flora, which allows *C. difficile* endospores to germinate and proliferate, resulting in a gut infection.<sup>3</sup> According to the Mayo Clinic, the symptoms range from mild to severe diarrhea, and it can be life-threatening in extreme cases.<sup>4</sup> If this infection is added to the CDC data, the total number of infections in the US per year exceeds 3 million infections per year, and the number of deaths exceeds 48,000 per year, making *C. difficile* the bacterium with the highest fatality rate due to antimicrobial resistance.<sup>1</sup>

A landmark study published in *The Lancet* forecasts that antimicrobial resistance (AMR) could be associated with the death of 169 million people from 2025 to 2050 if no preventive measures are taken. From now to 2050, yearly deaths from antibiotic-resistant infection are expected to rise to over a million, with 1.91 million deaths being estimated in 2050. The regions most likely to be affected are South Asia, Southeast Asia, and sub-Saharan Africa. These areas experience limited access to medical resources and lack proper regulation regarding the use of antibiotics. However, new and innovative drugs against gram-negative bacteria (labeled by the World Health Organization as the biggest threat to human health) could avert an estimated 11.1 million deaths. In addition to improved medications to treat antimicrobial-resistant bacteria, other strategies, such as infection prevention, vaccination, decreasing inappropriate use of antibiotics in farming and humans, could save an estimated 93 million deaths by 2050.<sup>5</sup>

Due to the detrimental outcomes of antimicrobial resistance on humanity, scientists are working on finding innovative prevention and treatment strategies to reduce the fatalities associated with antibiotic resistance. This will improve health outcomes in developing countries and provide valuable insight into the prevention of the antimicrobial crisis in the future.

Nano-antibiotics are currently being explored to reduce antibiotic resistance. Nano-antibiotics are conventional antibiotics that are encapsulated in specifically developed nanoparticles called nanocarriers. Many nanoparticles have intrinsic antibacterial activity. Utilizing their unique chemical properties, nanocarriers can deliver antibiotics to specific bacterial target sites via various mechanisms.<sup>6</sup> Many research studies have

demonstrated promising results of nano-antibiotics in the prevention and treatment of drug-resistant diseases. The implementation of effective prevention strategies and the discovery of efficient nano-antibiotics have the potential to reduce mortality associated with infectious diseases.

However, determining the specific material for encapsulation, its bioavailability, biocompatibility, degradability, specificity for individual bacteria, and long-term safety are some challenges that new nanotechnology will have to overcome. The objective of this literature review is to provide valuable insight into the potential use of nanotechnology in developing nano-antibiotics to reduce antibiotic resistance, antibiotic-associated adverse effects, and mortality, as well as prevention strategies for *C. difficile*.

## ■ Methodology

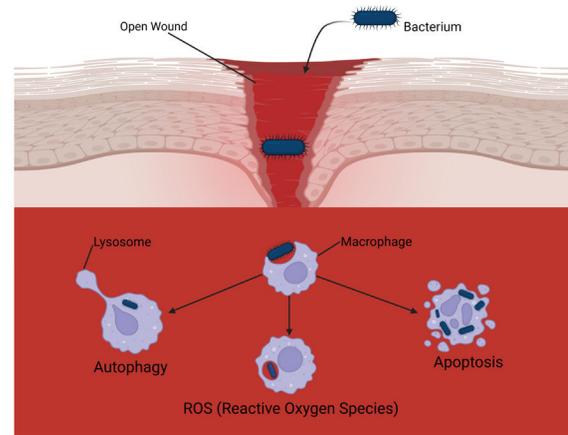
The primary objective of this study was to investigate whether nano-antibiotics can be utilized to mitigate antimicrobial resistance and antibiotic-associated adverse effects. This research paper is a literature review of multiple peer-reviewed research articles and studies. The qualitative method of analysis was employed in this research paper to explore and investigate innovative prevention strategies for antibiotic resistance using nanoparticles. To complete this method of analysis, numerous articles were reviewed. Articles were selected based on their publication in peer-reviewed journals or reputable medical organizations, their relevance to antimicrobial resistance, the current state of nano-antibiotic research in combating antimicrobial resistance, antibiotic encapsulation by nanoparticles, *C. difficile* prevention, and the clarity of their methodologies. Based on study design, sample size, and experimental controls, each study was evaluated for any conflicting results. Studies lacking methodological detail or relevance to the research focus were excluded. Potential research biases and contradicting information were removed in part or in their entirety. Further research was conducted on the research gap: prevention of *C. difficile*, including the bacterium's pathophysiology, clinical features, diagnosis, and prevention strategies. No physical research or materials were used in this research paper, except for credible online resources such as the World Health Organization (WHO), Mayo Clinic, National Institutes of Health (NIH), and the University of Minnesota's Center for Infectious Disease Research and Policy (CIDRAP) websites.

## ■ Results

### *Internal Defense Against Bacteria:*

As shown in Figure 1, the body's first line of defense is the skin; however, through an open wound, bacteria can easily infiltrate the defense. Once inside the body, the bacteria seek to reproduce quickly and spread; however, the body's second line of defense, the macrophages, is quick to respond to the new bodily threat. The macrophages have three main methods of destroying bacteria: autophagy, Reactive Oxygen Species (ROS), and apoptosis. Autophagy occurs when the bacterium is trapped inside a membrane called an autophagosome, which then joins a lysosome. The lysosome releases hydrolytic

enzymes, which then digest the bacterium. ROS are created when a macrophage produces an enzyme that releases hydrogen peroxide, which kills harmful bacteria. Apoptosis occurs when a macrophage engulfs bacteria and self-destructs to limit bacterial replication and prevent further spread.<sup>7</sup>



**Figure 1:** Innate defense mechanisms against bacteria. This figure shows different processes used by macrophages to kill bacteria, such as autophagy, ROS & apoptosis. Created by Pawar using Biorender.

### *Antibiotic Resistance:*

Antibiotic resistance occurs when bacteria develop the ability to resist destruction from antibiotics. Bacteria develop resistance by genetic mutations or by acquiring resistant genes from other bacteria. Excessive antibacterial exposure or the use of lower doses of prescribed antibiotics helps resistant bacteria survive and multiply.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a resistant strain of *S. aureus* that causes infection when open wounds or injuries are exposed to the bacteria. MRSA often arises in East Asian and South American countries due to excessive and unregulated antibiotic usage in those countries.<sup>8</sup> Approximately 1% of the general population carries MRSA. Untreated infections result in gangrene or deep abscesses in the skin, sometimes resulting in sepsis or death.<sup>9</sup> MRSA is a specific strain of *Staphylococcus aureus* that has developed resistance to many commonly used antibiotics. This bacterium acquires a *mecA* gene, which encodes an altered penicillin-binding protein (PBP2a) with reduced affinity for antibiotics such as methicillin. This allows bacteria to survive and grow even in the presence of antibiotics.<sup>10</sup>

Tuberculosis is a bacterial infection that occurs when the bacterium *Mycobacterium tuberculosis* enters the body through the nasal passages when one breathes in the droplets released by an infected person. The bacterium is then phagocytosed and destroyed by either T cells or lysosomal enzymes.<sup>11</sup> Mycobacteria develop drug resistance through mutations in different genes that help the bacteria survive anti-tuberculosis drugs. Phenotypic resistance is common in *M. tuberculosis* and results from changes in cell structure, which limit antibiotic access to their targets. Other resistance mechanisms seen in *M. tuberculosis* include efflux pumps and target pressure. Efflux pumps force the drug outside the bacterial cell, so it does not get killed. Target pressure occurs where *M. tuberculosis* develops mutations in genes that code for the antibiotic target site.<sup>12</sup>

*Escherichia coli* is an infectious bacterium that enters the human body after consumption of contaminated food or liquid, and it results in mild symptoms such as nausea, vomiting, watery diarrhea, or some toxin-producing strains can cause serious illness, such as kidney damage. This bacterium can spread from human to human under unhygienic conditions and through the fecal-oral transmission route.<sup>13</sup> Drug resistance mechanisms in *E. coli* include horizontal gene transfer, DNA integration, and porin modification. With horizontal gene transfer, *E. coli* can acquire resistant genes from other bacteria. DNA integration happens when the bacterium inserts a plasmid into its genetic code. Porin modification occurs when *E. coli* alters the pores in its cell membrane to control the flow of substances. This prevents the antibiotics from entering the bacterium, allowing it to survive and reproduce inside a host.<sup>14</sup>

*Pseudomonas* is a genus of bacteria known for causing severe infections. The most common species is *P. aeruginosa*, which is spread through contaminated water, soil, food, and medical devices such as ventilators and urinary catheters. According to a report by the Cleveland Clinic, it is one of the most common bacterial infections, making up to 25% of bacterial infections. *P. aeruginosa* is resistant to most antibiotics, as it can rapidly evolve to develop resistance in a specific strain. The drug resistance mechanisms in *Pseudomonas* include intrinsic resistance, mutations, and biofilms. Intrinsic resistance mechanisms include low outer-membrane permeability and drug efflux pumps. Additionally, *P. aeruginosa* can mutate, making it immune to some of the most effective antibiotics, such as penicillin and carbapenems. *P. aeruginosa* in the biofilms, commonly found in medical devices inside the body, leads to reduced antibiotic susceptibility and persistent infections.<sup>15</sup>

Antibiotics use leads to the elimination of susceptible bacteria. However, some bacteria survive through a variety of mechanisms of drug resistance, allowing them to survive, reproduce, and pass the mutations to their next generation. Over time, natural selection can cause resistant bacteria to become a dominant strain. Thus, the bacteria develop antibacterial resistance due to their existing genetic variations and evolution through natural selection. Understanding these drug resistance mechanisms is crucial in addressing the global challenge of antibiotic resistance.

### ***Clostridium Difficile:***

*C. difficile* is a gram-positive, spore-forming bacillus. In the environment, it survives in the spore form. It is spread via contact with the contaminated objects or the hands of healthcare workers. Once these spores are in the intestines, they convert into their functional, toxin-forming form. Antibiotic use is one of the major risk factors for *C. difficile* infection. Antibiotics can adversely affect the healthy gut flora, leading to an excess growth of *C. difficile*. Healthy infants and adult patients in hospitals or nursing homes may carry *C. difficile* in their gut flora, but it is often kept in check by other gut bacteria. Symptoms arise when there is overgrowth of *C. difficile*, and certain strains produce toxins. This can lead to mild symptoms like watery diarrhea and stomach cramping, while in severe cases, this infection can be life-threatening.<sup>16</sup>

Currently, antibiotics such as Vancomycin, Metronidazole, and Fidaxomicin are used to treat this infection. In some cases, these antibiotics are ineffective. Like other drug-resistant bacteria, *C. difficile* develops resistance by mechanisms such as genetic mutations, alterations in metabolic pathways, and biofilm formation.<sup>17,18</sup> Understanding drug resistance mechanisms of *C. difficile* and implementing those strategies to develop novel antibiotics, such as nano-antibiotics, would prove to be more beneficial in the prevention and treatment of this disease.<sup>19</sup>

### ***Nano-carriers (Nano-antibiotics):***

Nanotechnology is a branch of science that focuses on the development and usage of nanoparticles, or particles with a size between 1 nm and 100 nm. The nanoparticles are extensively utilized in biomedical sciences and engineering applications, primarily due to their high surface area, ease of surface modification and functionalization, and highly tunable structural and chemical properties. A proposed method to combat antibiotic resistance is the use of nano-carrier encapsulated antibiotics (also known as nano-antibiotics). Encapsulation of commonly used antibiotics with nanoparticles can serve several purposes. Nanoparticles can be bioengineered to deliver antibiotics to their target sites more efficiently. In addition, nanoparticles can protect the antibiotics from degradation by enzymes, which increases their half-life and bioavailability. If this can be properly executed, the effective dose of currently used antibiotics can be lowered. Additionally, metal nanoparticles such as gold, silver, copper, and iron are currently being used for their antibacterial properties.<sup>20</sup> As they act through multiple non-specific mechanisms, such as the generation of reactive oxygen species or membrane disruption, they have a broad spectrum of antibacterial activity. Incorporating nanoparticles into current infection management strategies can help reduce side effects associated with many antibiotics.<sup>21,33</sup>

As the concept of nano-antibiotics is still in its developmental stage, it is unclear if these nano-antibiotics would have any undesired short-term or long-term adverse effects. To avoid any adverse effects of nano-antibiotics, it is crucial to utilize a nano-carrier that has increased bioavailability and biodegradability, and is non-toxic to mammalian cells.<sup>21,33</sup>

**Table 1:** Comparative analysis of various nanoparticles. The table displays various types of nanoparticles, their composition (organic/inorganic), shapes, and antibacterial activity.

Material	Organic/Inorganic	Shape	Bacteria
Silver nanoparticles	Inorganic	Spherical, Cubic, Nanorod	<i>Mycobacterium Tuberculosis</i> , <i>P. Aeruginosa</i> , <i>E. Coli</i>
Gold nanoparticles	Inorganic	Spherical, Cubic, Nanorod	<i>Yersinia Pestis</i> , <i>Salmonella spp.</i>
Zinc Oxide nanoparticles	Inorganic	Spherical, Cubic, Nanorod	<i>Mycobacterium Tuberculosis</i> , <i>MRSA</i> , <i>E. coli</i>
Copper nanoparticles	Inorganic	Spherical, Cubic, Nanorod	<i>L. Pneumophila</i>
Polymeric nanoparticles	Organic	Spherical, Cubic, Nanorod	<i>Methicillin-Resistant Staphylococcus Aureus</i> , <i>Mycobacterium Tuberculosis</i>
Liposome-based nanoparticle	Organic	Spherical, Multilamellar vesicles (MLV), Unilamellar vesicles (ULV)	<i>M. Avium Complex</i>
Solid lipid nanoparticles	Organic	Spherical, Polymeric core-shell	<i>Methicillin-Resistant Staphylococcus Aureus</i>
Carbon nanoparticles	Inorganic	Cylindrical nanotubes	<i>Mycobacterium Tuberculosis</i>
Chitosan nanoparticles	Organic	Cylindrical, spherical, irregular	<i>Bruceella spp.</i> , <i>Salmonella spp.</i>

### Nanoparticles Classification:

As shown in Table 1, nanoparticles are a large and diverse group of compounds that vary in size, structure, and properties. They have different sizes and shapes: nanorods, nanospheres, nanotubes, nanowires, quantum dots, nano-shells, mesoporous silica, and nanotriangles, amongst many others. Nanomaterials have an extensive range of structures, such as fibers, fullerenes, hollow interiors, and reticular networks. The particles themselves have a wide variety of properties and molecular makeup. These nanoparticles differ in their properties, which include strength, elasticity, electrical conductivity, thermal conductivity, magnetism, and density. Metal nanoparticles include silver, gold, zinc oxide, and copper nanoparticles. Organic nanoparticles include polymeric, liposome-based, solid lipid-based, and chitosan.

Silver nanoparticles are one of the nanoparticles that have antibacterial properties. They exhibit the antibacterial effect by attaching to the bacterial cell surface, destroying proteins, and disrupting bacterial biofilms. Silver is a pure material because it has only one type of atom. Silver nanoparticles have been shown to have antibacterial effects on *M. tuberculosis*, *P. aeruginosa*, and *E. coli*.<sup>21</sup>

Gold nanoparticles can cause bacterial death by disrupting the bacterial respiratory chain, inhibiting the production of ATPase in bacteria, and decreasing the cell membrane potential. Gold is a pure substance and can halt the spread of *Yersinia pestis* and *Salmonella spp.*<sup>22</sup>

Zinc Oxide nanoparticles can be used to eliminate *M. tuberculosis*, *MRSA*, and *E. coli* by destroying and preventing bacterial biofilm formation, increasing the membrane permeability, elasticity, and degradation.<sup>23</sup>

Copper is a pure, inorganic metal and takes on almost all nanoparticle shapes. Copper nanoparticles have shown antibacterial activity against various bacteria such as *Staphylococcus aureus*, *Salmonella enterica*, *Campylobacter jejuni*, *Escherichia coli*,

*Listeria monocytogenes*, and *L. pneumophila*. Copper nanoparticles exhibit antibacterial activity against *E. coli* by generating reactive oxygen species, lipid peroxidation, protein oxidation, and DNA degradation.<sup>24</sup>

Polymeric nanoparticles are organic nanoparticles that have a polymer matrix and a polymer shell.<sup>25</sup> These polymers are best used as a nanocarrier for antibiotics due to their variety of beneficial properties, such as biodegradability, versatility, and low toxicity. According to a study by Pandey *et al.*, nebulized poly lactic-co-glycolic acid (PLGA) nanoparticles delivering anti-tuberculosis drugs in guinea pigs showed higher bioavailability than oral doses. This study demonstrated that using nanotechnology, it is possible to reduce the dosing frequency of the anti-tuberculosis drugs.<sup>26</sup>

Liposome-based nanoparticles are among the most widely used and effective nanocarriers because of their versatility. They can encapsulate most antibiotics, including hydrophilic and hydrophobic substances. A study by Mehta *et al.* showed that liposome encapsulation of a drug to treat *Mycobacterium Avium Complex* (MAC) infections in a mouse model reduced toxicity and improved therapeutic efficacy compared to the free drug.<sup>27</sup>

Solid lipid nanoparticles (SLNs) are colloidal carrier systems that have a solid biodegradable lipid matrix. They are organic, and their most common shape is spherical. They offer many advantages, such as improved stability of incorporated compounds and controlled release of drugs. Due to their biocompatibility, non-toxicity, and simple production procedures, SLNs are commonly used in biomedical applications. As nanocarriers, SLNs demonstrated enhanced antibacterial activity of drugs against resistant strains like *MRSA* and *P. aeruginosa*.<sup>28</sup>

Carbon nanoparticles, because of their chemical properties, are easily released into bacterial membranes. Due to their exceptional transportation skills, they are suitable to function as nanocarriers. Examples of carbon nanoparticles are quantum dots and 2D materials such as graphene and nanotubes. They are pure substances, meaning they consist of only carbon atoms. These nanoparticles can be used to treat *Mycobacterium tuberculosis* infection.<sup>29</sup>

### Effectiveness of Nano-antibiotics in Treating *S. aureus*:

A study published in 2023 by Le *et al.* demonstrated that nanocarriers can be used to increase the effectiveness of antibiotics compared to conventional antibiotics. The bacteria tested in this study were *Staphylococcus aureus*. The researchers tested two commonly used antibiotics to treat *S. aureus*: ciprofloxacin and levofloxacin. They encapsulated these antibiotics with polymer-based nanoparticles called poly lactic-co-glycolic acid (PLGA). In addition, the researchers modified the antibiotic surfaces by altering the antibodies complementary to the bacterium. Due to these modifications, the researchers demonstrated that the antibiotics were more efficient in identifying and eradicating bacteria. They observed bacterial growth using microscopes and fluorescent dyes that made the bacterial cells easier to visualize. After testing conventional antibiotics, targeted nano-antibiotics, and non-targeted nano-antibiotics, the researchers found that targeted nano-an-

tibiotics functioned better at killing *S. aureus* than conventional antibiotics. Non-targeted nano-antibiotics had slightly less efficacy compared to conventional antibiotics. However, the targeted nano-antibiotics were the most effective and efficient in killing *S. aureus*. Non-targeted nano-antibiotics and conventional antibiotics decreased the number of *S. aureus* colonies by around 10 times. Astonishingly, targeted nano-antibiotics decreased the number of colonies nearly 100-fold.<sup>30</sup>

#### **Effectiveness of Lipid Nanoparticles & Chitosan as Nanocarriers:**

Bacterial biofilms are a group of microorganisms that are ingrained into a substance called Extracellular Polymeric Substance (EPS), which is made of lipids, proteins, polysaccharides, and nucleic acids. Bacterial biofilms can stick to cell surfaces and lead to antimicrobial resistance because few antibiotics can penetrate through the biofilm. The bacterial biofilms are developed over time in five stages that include: attachment, colonization, development, maturation, and dispersal. There are several approaches currently being researched to inhibit the growth of bacterial biofilms.

A study published in 2024 by Ahsan *et al.* discussed the use of lipid nanocarriers in the treatment of bacterial biofilms. A combination of antibiotics with antimicrobial adjuvants such as EPS degrading enzymes or quorum-sensing inhibitors (QSI) can be encapsulated by lipid nanoparticles. EPS degrading enzymes can be used to eradicate biofilms, and QSI are the agents that block bacterial communication, halting the development of biofilm. Lipid nanocarriers are promising nanocarriers for this strategy due to their greater bioavailability, drug protection from enzyme degradation, and targeted drug delivery. This strategy can improve the effectiveness of antibiotics and decrease antimicrobial resistance.<sup>31</sup>

Chitosan is a naturally occurring amino polysaccharide that has extraordinary biocompatibility and biodegradability. For decades, Chitosan has been used in drug delivery systems and in different applications in various medical fields such as orthopedics, ophthalmology, and surgery. Chitosan nanocomposites contain chitosan polymers that are less than 100 nm. Thus, Chitosan nanocomposites are considered a promising and safe nanocarrier for biomedical applications.<sup>32</sup>

#### **■ Discussion**

As antibiotics have been used for decades, their mechanism of action, pharmacokinetics, and pharmacodynamics are well-studied. The FDA approves, on average, one to two antibiotics every year. However, resistant strains to these antibiotics appear more rapidly. Therefore, it is of vital importance to develop novel alternatives to traditional antibiotics. The nanotechnology industry has become a significant part of this endeavor by utilizing nanoparticles with antibiotic properties or by encapsulating antibiotics with nanoparticles. Despite the demonstrated antibacterial activity of some nanoparticles, such as silver nanoparticles, the precise molecular mechanism by which these nanoparticles exhibit the antibacterial effect is partially understood.

Currently, nanomaterials are used in targeted drug delivery to cancer patients. In these treatments, nanoparticles loaded with chemotherapy drugs are coated with ligands that bind to specific receptors on cancer cells. Cancer cells identify these ligands as their own and internalize the nanoparticles, enabling chemotherapy drugs to be delivered directly to cancer cells while sparing healthy tissue. This targeted approach improves effectiveness and reduces side effects compared to traditional cancer treatments. Similar strategies could be adapted for more precise antibiotic delivery. Currently used nanoparticles lack specificity for the bacteria. Therefore, developing nanocarriers with specific targeting molecules for pathogenic bacteria is very important. Since nanoparticles cannot distinguish between probiotic and pathogenic bacteria, dysbacteriosis may occur in the human body.

Utilizing targeted antibiotic delivery using nanoparticles as carriers would limit the antibiotic's exposure to non-targeted cells, thus decreasing their adverse effects and improving patient outcomes. Some nanoparticles, such as silver nanoparticles, are cytotoxic, which limits their *in vivo* applications. Managing this cytotoxic effect is essential to ensure the safety of nanoparticle-based treatments. Therefore, selecting or designing nanocarrier systems that maintain strong antibacterial properties while minimizing harm to human cells is a critical aspect of developing effective and safe nano-antibiotics. Currently, the best nanocarrier nanoparticle coatings are Solid Lipid Nanoparticles (SLNs), which are organic compounds with limited cytotoxicity and have a similar antibacterial effect to other inorganic nanoparticles.

Nano-antibiotics would have traditional antibiotics encapsulated with nanoparticles. The use of conventional antibiotics in the nanocarrier's core would deliver antibiotics to their target, allowing them to accomplish their goal. These nano-antibiotics combine the unique advantages of nanomaterials, such as enhanced bioactivity and reduced antimicrobial resistance, with the well-established mechanisms of traditional antibiotics, making them a promising next-generation antimicrobial treatment option.<sup>33</sup> In summary, advancing nano-antibiotics will require targeted research into their precise antibacterial mechanisms, pathogen-specific selectivity, and long-term biosafety.

#### **■ Conclusion**

Antibiotic resistance remains a major global threat to humanity, as antibiotics once considered dependable continue to be ineffective. Moreover, inappropriate use of antibiotics can lead to severe side effects and secondary infections, such as *C. difficile*, emphasizing the urgent need for safer and more efficient drug delivery systems.

Nano-antibiotics offer a promising solution to address this critical issue by encapsulating the conventional antibiotics within biodegradable, non-toxic nanocarriers. This approach can enhance targeted drug delivery, increase drug bioavailability, and minimize systemic toxicity. Additionally, nano-antibiotics demonstrate significant potential in treating the biofilm-associated resistance by achieving controlled and sustained drug release at the site of infection. Future research

should focus on understanding the long-term safety, pharmacokinetics, and potential cytotoxicity of these nanomaterials.

Ultimately, integration of nanotechnology into antimicrobial therapy could positively impact the existing model of infectious disease treatment. Through continued interdisciplinary research, nanotechnology truly has the potential to aid scientists in the fight against antibiotic resistance, enhance antibiotic efficacy, and contribute to the development of next-generation antibacterial therapies.

## ■ Acknowledgments

I would like to thank Professor Kristina Lilova of Arizona State University and Professor Virgel Torremocha of the University of Southeastern Philippines for their mentorship during this writing process.

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# Comparative Gerontology: A Binational Study of Senior Well-Being

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**ABSTRACT:** The quality of life among older adults is influenced by a dynamic interplay of physical health, emotional well-being, social engagement, financial stability, and environmental context. While existing research often centers on single-country analyses, there remains a significant gap in comparative studies across regions with distinct cultural, social, and healthcare systems. This study addresses that gap through a method approach of systematic surveys and interviews, offering a cross-cultural comparison between senior populations in India and the United States. Responses were categorized into four overarching themes: (1) Social Engagement & Activities, (2) Family Roles & Environment, (3) Self-Care & Independence, and (4) Healthcare Access & Community Resources. Advanced statistical comparative methodologies were employed to examine thematic patterns, identify cross-cultural parallels, and highlight region-specific distinctions. Findings reveal that self-rated happiness and health levels are statistically comparable, averaging approximately 8 on a 10-point scale. However, deeper analysis uncovered notable differences in lifestyle patterns and support structures, shaped by factors unique to each region. This research contributes to the field of gerontology and cross-cultural health by providing direct comparative insights into aging experiences across diverse contexts. The implications are particularly relevant for policymakers, caregivers, healthcare practitioners, and technological innovators.

**KEYWORDS:** Behavioral and Social Sciences, Physiological Psychology, Gerontology, Sociology, Quality of Life, Integral Support System.

## ■ Introduction

Older adults are a growing population in society whose quality of life is deeply dependent on social engagement, environment, and healthcare. In fact, by 2030, 1 in 6 people in the world will be aged 60 years or over, and by 2050, the world's population of people aged 60 years or older will double.<sup>1</sup> The quality of life for older adults is shaped by an interplay of physical, emotional, social, financial, and environmental factors. Physical health factors include chronic conditions, physical mobility, pain management, nutrition, and access to healthcare. Mental and emotional well-being depends on cognitive health, depression/anxiety, sense of autonomy/ dignity, and sense of purpose. Social factors, such as family/friends, community engagements, opportunities for expression, and intergenerational relationships, can have a significant impact. Financial independence is a critical factor that includes income/savings, affordable housing, and access to benefits like pensions, social security, etc. A living environment that includes safety, accessibility, cleanliness, and comfort is equally important. This information is an anecdotal basis for which each factor for each theme was based.

While these factors collectively shape the experience of the older adults, they do not exist in isolation. Rather, they are embedded within broader societal and systemic frameworks that vary significantly across cultures and nations. How a society prioritizes elder care, allocates resources, and designs its healthcare and social support systems can dramatically influence how these individual factors manifest and interact. Yet, despite widespread recognition of the systemic nature of ag-

ing-related challenges, much of the existing research tends to focus on individual countries, leaving a gap in the lack of comparison between two different countries with different healthcare systems, cultural contexts, and social frameworks. For example, studies done in India document the quality of life of older adults between rural and urban areas.<sup>2</sup> Furthermore, in the US, a study has been done to determine the lifestyle difference between older adults with and without disabilities.<sup>3</sup> Furthermore, the existing cross-cultural research is either outdated or not addressing quality of life; respectively, this is seen in a study about the relationship between emotional support and subjective well-being of married older people published in 1995,<sup>4</sup> as well as a cross-cultural study that analyzes health behaviors tied to aging without specifically addressing its impact on elderly quality of life.<sup>5</sup> This leaves a notable gap in comparative studies across regions with differing cultural, social, and healthcare frameworks.

This research attempts to address this gap and presents a novel cross-cultural comparison pertaining to the quality of life in older people. It builds on the hypothesis that differing values, infrastructures, and policy models can illuminate alternative pathways and innovative solutions. It undertakes a comparative exploration of the lifestyle of older adults in two distinct contexts: the United States and India. By examining how each of these populations navigates the complex terrain of aging, this research aims to draw conclusions from the interplay of influencing factors and uncover strategies that may be adapted or reimaged to improve the quality of life for elders across diverse settings.

## ■ Methods

To investigate the complex factors influencing the quality of life among older adults (those that this particular study defines as being 60+ in age), this research adopted a mixed-methods design that integrates both quantitative and qualitative approaches.

First, the focus is on the design and administration of a structured survey aimed at capturing the multifaceted dimensions of older adults' quality of life. The survey was carefully constructed to address five key domains: physical health, emotional well-being, social engagement, financial stability, and environmental conditions. With a thematic approach in mind, a survey was created and distributed in English and Marathi (an Indian language from the state of Maharashtra), depending on the respective location. While the core structure of the questionnaire remained consistent across both the United States and India, minor contextual adaptations were made to ensure cultural relevance and clarity for local participants. The survey consisted of 43 multiple-choice questions, enabling the collection of quantitative data across several categories, including demographic profiles, health routines, social interactions, living environments, self-reported happiness, and perceived health status. To ensure accessibility and appropriate inclusivity, the survey was administered in person, allowing participants above the age of 60 to engage directly with facilitators and clarify any uncertainties.

To complement the quantitative findings, in-depth interviews were conducted with a subset of survey participants. These interviews provided rich qualitative insights, enabling respondents to share personal narratives and reflect on life experiences within a thematic framework. Qualitative data from these interviews allowed for a clear interpretation of the survey results. Interview questions included themes of quality of life, professional career, hobbies, exercise routine, favorite time of day, proximity to children, social engagements, and healthcare plans. Note that the actual interviews in most cases deviated from these, depending on the conversation flow to include more specific responses related to a topic invoked from the questions. The interview transcripts were systematically analyzed using a thematic analysis, allowing for the identification of recurring patterns, emotional undercurrents, and culturally specific nuances.

Throughout the study, ethical standards were rigorously upheld. An Institutional Review Board (IRB) approval was obtained for this study. Furthermore, all participants were engaged under the principles of voluntary participation, informed consent, and strict confidentiality. These safeguards ensured that the research process honored the dignity, autonomy, and privacy of every individual involved.

## ■ Results

The study was conducted at the Gilbert Senior Center in Arizona, USA, and at Athashri Senior Living, a private residence in Pune, India. The focus was on urban locations for both countries to refrain from selection bias. Results include 48 responses from the USA and 72 responses from India.

About 10 total interviews were conducted as part of the study. All respondents filled out all the questions.

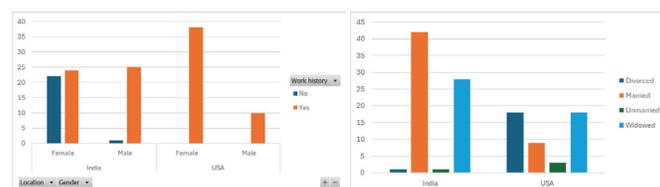
### *Demographic observations:*

Figure 1 shows the age and gender distributions. In India, 72% of older people are 70 or older, while in India this proportion was 81%. Additionally, the gender distribution among older people indicates that in India's sample, 64% are female, and in the US sample, 79% are female. This demonstrates that most respondents are above the age of 70 for both countries, with a larger ratio of females over males. This imbalance represents a broader demographic trend showing more female longevity in older age groups.



**Figure 1:** Age and Gender of the survey participants. The survey participants are predominantly female populations above the age of 70.

Figure 2 illustrates the marital and employment status of survey participants. Among female respondents in India, 50% did not engage in formal employment but identified as homemakers. In contrast, 100% of female participants in the United States reported being employed. The marital status distribution shows a statistically significant difference between the two countries: the majority of Indian women were either married or widowed, whereas in the U.S., most were divorced or widowed. The observed statistical difference in marital status between survey participants in India and the United States potentially reveals distinct cultural and generational patterns shaped by societal norms, historical context, and perceptions of relationships.



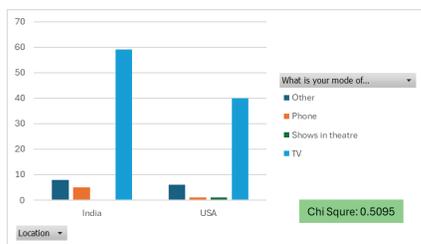
**Figure 2:** Marital and employment status of the survey participants. Older Indian women are often homemakers and married/widowed, while older women of the US are more often fully employed and divorced/widowed.

### *Analysis Approach:*

Out of the 43 survey questions, 2 items required participants to provide quantitative self-ratings (on a scale of 1–10) for their levels of happiness and health. These numerical responses were analyzed using descriptive statistical methods, focusing on mean comparisons and variance assessments to summarize key trends. The Student t-test was used to compare numerical data across the two countries.

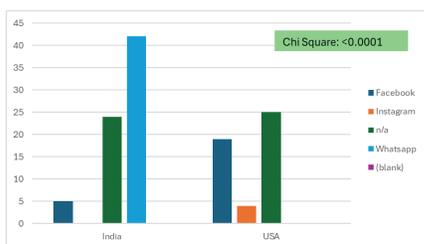
The remaining 41 questions were multiple-choice, generating categorical data. These were systematically organized into four overarching thematic categories: 1) Social Engagement





**Figure 6:** Mode of entertainment. Television emerged as the most common type of entertainment in both India and the US, reflecting similar entertainment preferences across cohorts.

**2) Choice of social interaction methods:** As shown in Figure 7, the survey reported no difference in the social media connectivity between the two cohorts. However, the choices of social media platforms were significantly different. While India favored WhatsApp as a media platform that favors direct and private conversations, the US favored Facebook, which is a broad and public platform. This was supported in findings from interviews, as many participants talked about their connectivity on WhatsApp in India, whereas in the US, WhatsApp was virtually unknown among the elderly population.



**Figure 7:** Preference for social media platform. While overall social media connectivity was similar across cohorts, Indians preferred WhatsApp for private conversations, whereas US participants favored Facebook for broader social interaction.

**3) Impact of pandemic on social engagement:** The survey also investigated the effects of the COVID-19 pandemic on participants' patterns of social interaction. Figure 8 shows the responses across different impact vectors. Analysis of responses from both cohorts revealed minimal change in the frequency or modality of in-person and online communication post-pandemic. Furthermore, participants from India demonstrated a comparatively higher propensity to report a full resumption of pre-pandemic lifestyle practices.

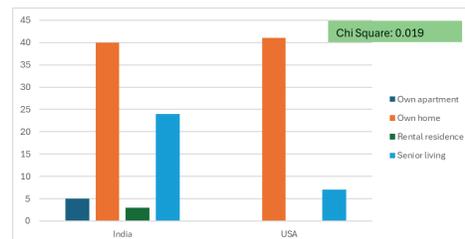


**Figure 8:** Impact of COVID-19 on lifestyle. Participants from both India and the US revealed minimal change in the frequency of in-person and online communication post-pandemic.

**Family Structure and Roles:** This category examines the composition of the family unit and explores the roles older adults occupy within familial interactions and responsibilities.

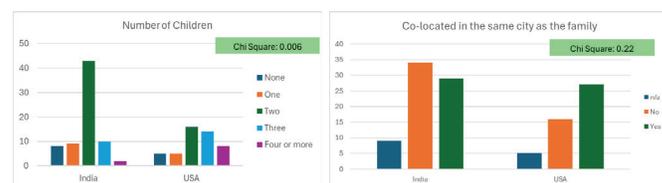
**1. Residential arrangement:** Residential arrangements differed considerably for the survey participants, as captured in Figure 9. In the U.S., 85.7% of older adults resided in privately

owned dwellings, with 12.2% in institutional care facilities. In India, private ownership rates were at 56.9%, with 40.3% residing in senior living facilities. Note that there is a selection bias in the study. One part of the survey in India was done at a senior living facility, which is reflected in the composition fractions. Pre-pandemic living arrangements were relatively stable in both countries, and the majority remained in the same residence during the COVID-19 pandemic (>90% in both cohorts).



**Figure 9:** Primary residence details. Indian seniors more often resided in senior living facilities than seniors in the US; more seniors from the US lived in private homes than Indian seniors.

**2. Family structure and proximity:** Family structure and proximity revealed distinct patterns across the Indian and U.S. senior cohorts. Figure 10 shows the family sizes and current proximity to the children. The average number of children reported by Indian participants was 1.8, notably lower than the U.S. average of 2.5. This divergence reflects historical public health initiatives in India, such as the "Hum Do Hamare Do" ("We are Two, will have Two Children") campaign launched in 1967, which played a seminal role in promoting family planning and population stabilization (Government of India, 1967).<sup>9</sup> One Indian interviewee explicitly referenced this campaign when discussing the normative shift toward two-child families. Despite differences in family size, co-location within the same city was comparable across both cohorts. Yet Indian respondents tend to have more children away from their current home. This is likely due to the young population leaving the city or country to pursue studies in STEM and business fields, as noted in several interviews.

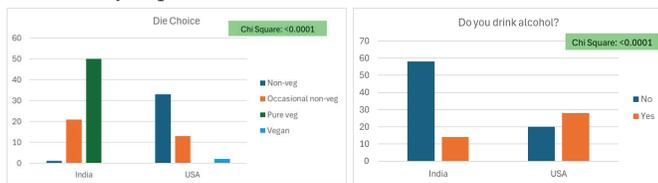


**Figure 10:** Family structure. Older adults in the US reported larger families on average compared to Indian seniors. Though older adults' proximity to children was similar across both cohorts, more Indian participants have children living away from them.

**Self-care and independence:** This category highlights how older adults engage in activities related to caring for their mental and physical health.

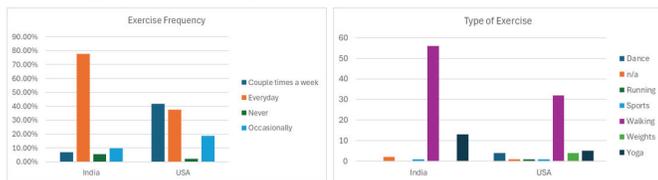
**1) Diet:** Figure 11 shows that dietary preferences reveal notable contrasts between the two cohorts. Approximately 70% of Indian respondents identified as vegetarians, while a similar proportion of U.S. respondents reported a non-vegetarian diet. Alcohol consumption patterns further underscore these cultural distinctions. Among Indian older adults, 80% reported

abstaining from alcohol, whereas 60% of U.S. older adults indicated regular or occasional consumption. >90% of respondents from both countries reported not smoking in both countries. Numerous interviewees in both India and the US stated that lifestyle choices among older adults are significantly shaped by their sociocultural conditioning and religious values, combined with implications for health behavior, social engagement, and community expectations.



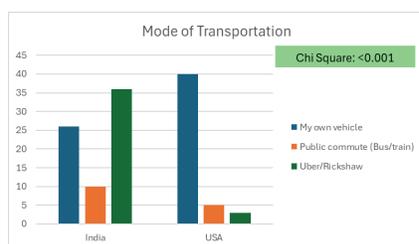
**Figure 11:** Diet choices. Indian participants were mostly vegetarian and abstained from alcohol, while US participants favored non-vegetarian diets with higher alcohol use.

**2) Exercise:** There was a marked difference in exercise frequency between the two cohorts. As shown in Figure 12, approximately 75% of respondents in India reported engaging in daily physical activity. In contrast, fewer than 40% of U.S. participants followed a daily routine, with many preferring to exercise a few times per week. Despite this variation in frequency, the type of exercise chosen was notably consistent across both groups, with walking emerging as the preferred form of physical activity.



**Figure 12:** Exercise choices. Daily exercise was far more common among Indian seniors than US seniors, though walking was the preferred activity in both groups.

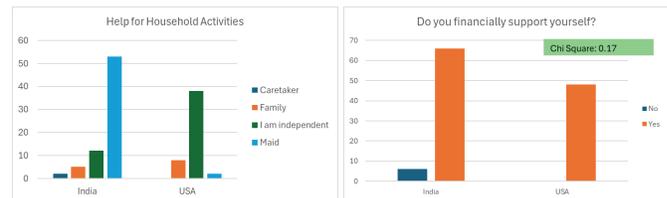
**3) Mode of commute:** Commuting practices reflected infra-structural disparities, with U.S. older adults utilizing personal vehicles and Indian older adults primarily relying on private feeder commute services like Uber™ and rickshaw, which are more economical modes of transportation. Figure 13 shows the preferred modes of commute between the two groups.



**Figure 13:** Mode of commute. US participants relied more on personal vehicles for commute, while Indian seniors favored services like Uber™ and rickshaws.

**4) Independent living:** Financial independence was also consistent across the two groups, with most older adults in both India and the U.S. reporting that they were financially self-sufficient. However, notable differences emerged in the domain of household support. In India, 58.3% of older adults

reported employing domestic assistance for daily household tasks, whereas U.S. older adults were more likely to perform these tasks independently. This contrast reflects broader socio-cultural norms and labor market structures influencing elder care and domestic labor. For example, in interviews with seniors in India, elders had readily available domestic assistance in their household, whereas in the US, there were rarely any caretakers or maids present at their will. The comparison charts for both these elements are shown in Figure 14.



**Figure 14:** Factors affecting independent living. While financial independence was common among both cohorts, Indian participants relied on domestic help more than US seniors, who managed household tasks more independently.

**Healthcare Access and Resources:** This category highlights access to health care and resources.

**1) Health insurance and expenses:** Access to health insurance plays a vital role in the well-being of older adults. Survey results revealed that over 99% of U.S. respondents had access to health insurance, compared to approximately 57% of Indian participants (see Figure 15). The lower adoption rate in India stems from a blend of cultural and economic factors. Insights from interviews highlighted several reasons why many Indians opt out of purchasing health insurance:

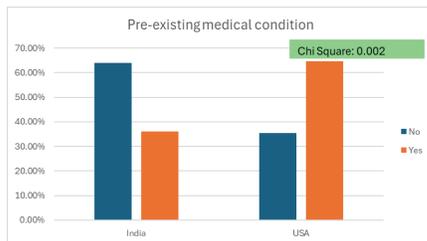
- Perceived high premiums make insurance seem unaffordable.
- Limited awareness of the process and benefits, especially since health insurance is not mandatory.
- A cultural preference for out-of-pocket payments often relies on personal savings or family support.
- A low-cost government-sponsored health care system in India.

Among those who do have insurance, the survey explored whether their plans adequately cover medical expenses. In the U.S., 65% of respondents reported that their insurance covered most or all of their healthcare costs. In contrast, only 45% of Indian participants felt similarly. This disparity addresses the differences in coverage levels and plan choices available or selected in each country. As a result, more Indian participants are paying out of pocket for health-related expenses compared to the US.



**Figure 15:** Health insurance and expenses. Nearly all US seniors had health insurance, versus only a little over half in India. Financial coverage was more comprehensive in the US, leaving many seniors to rely on out-of-pocket payments.

**2) Hospitalization:** The survey explored respondents' awareness of pre-existing medical conditions. Approximately 65% of participants from India reported being unaware of any such conditions, whereas a similar proportion of U.S. respondents indicated that they did have pre-existing conditions (see Figure 16). Given the self-reported nature of the survey, it is difficult to verify the accuracy of these claims. Around 50% of respondents in both countries have experienced hospitalization within the past five years, with over 80% of these cases unrelated to COVID-19. In both cohorts, more than 70% of hospital stays lasted less than one week. When asked about family involvement during hospitalization, about 75% of Indian respondents reported receiving visits from family members, compared to roughly 50% of older adults in the U.S.



**Figure 16:** Awareness of pre-existing medical condition. Indian participants were less aware of pre-existing medical conditions compared to US participants. During hospitalization, family visitation was more common in India than in the US.

## Discussion

The cross-cultural comparative study presented in this research paper demonstrates that despite distinct cultural, social, and healthcare contexts, older adults in India and the United States share similar overall self-perceptions of happiness and health, both averaging around 8 on a 10-point scale. This parity suggests that subjective well-being in older adulthood may be more strongly tied to intrinsic coping mechanisms, community ties, and perceived purpose than to the absolute availability of resources. However, beneath this surface similarity lie notable lifestyle differences with implications for health behaviors, social engagement, and care strategies.

Before moving on to the implications of these results, it is important to address potential limitations that could have been encountered. The biggest difference is that of the sample size. For the US, the sample size was  $n=48$ , while the sample size for India was  $n=72$ . This could skew comparative statistics, and this could make certain findings less reliable, due to the sample size not staying constant. The sampling bias also needs to be considered. For example, people were only selected from an upper-middle-class community in urban areas, and the data might not actually be completely representative of the broader elderly population. Across large and diverse countries like India and the US, there are bound to be limitations through sampling bias, resulting in a lesser generalizability of my results. Another limitation is self-reporting inaccuracies. For example, participants could have overestimated positive behaviors like exercise frequency or underestimated negative behaviors like alcohol use. Additionally, there were significantly more Indian older adults in the study who were in senior homes than American older adults, which could influence results on multiple fronts.

Socially, both cohorts maintain high engagement in leisure activities, favoring intellectually stimulating, creative, and physically active hobbies. Yet the nature of their social connections diverges in subtle but meaningful ways, as Indian older adults lean toward private, direct communication (WhatsApp). In comparison, U.S. older adults favor broader, more public networks (Facebook). This contrast may reflect differing cultural norms regarding privacy, self-expression, and intergenerational exchange. Additionally, pandemic-related disruptions were less enduring in India, where respondents more often reported returning to pre-pandemic social patterns, potentially indicating greater resilience or community integration. Family and living arrangements reveal sharper contrasts. U.S. older adults are more likely to live independently in private dwellings. In comparison, a larger proportion of Indian older adults reside in senior living facilities, a shift from traditional multi-generational cohabitation that may reflect evolving urbanization and lifestyle patterns. Interestingly, despite smaller family sizes in India, a likely legacy of public health campaigns, geographic proximity to children remains similar across countries, suggesting a persistent value placed on familial closeness in later life.

Self-care practices show cultural imprints: vegetarianism is predominant among Indian older adults, while non-vegetarian diets and higher alcohol consumption are common in the U.S. Indian older adults exercise daily at notably higher rates. However, walking is the preferred activity for both groups. Domestic assistance is also more prevalent in India, reflecting both cultural acceptance of household help and the affordability of labor markets, whereas U.S. older adults maintain greater independence in household management. Healthcare access and utilization mark a clear systemic divide. Nearly universal health insurance coverage in the U.S. contrasts sharply with partial adoption in India. Barriers in India include perceived affordability, lack of awareness, and cultural preference for out-of-pocket payments.



**Figure 17:** Spider charts showing the comparison of different factors across the four themes. There are similar patterns in family structure and social engagement between older adults' lifestyles in India and the US. However, there are differences between independence and healthcare access factors between the two cohorts.

Spider charts in Figure 17 show a high-level picture of how the different factors differ between the two countries across the four themes studied. While family structure and social engagement themes are comparable for the two countries, self-care/independence and healthcare themes have significant differences.

## ■ Conclusion

The findings in this research paper address the stated gap by comparing the lifestyles of older adults in India and the US, highlighting critical systems in each country that can be improved based on cultural background. Deep analysis underscores that successful aging is a multifactorial phenomenon, shaped as much by cultural values, social structures, and personal agency as by formal healthcare systems. For policymakers, the data highlights the need for context-specific interventions: in India, expanding affordable, comprehensible health insurance products and integrating preventive health education could address current coverage gaps; in the U.S., community-based aging models and structured opportunities for interpersonal connection could boost social engagement among independently living older adults. Caregivers and healthcare providers in both settings can benefit from culturally informed approaches that respect dietary norms, exercise preferences, and communication styles, thereby aligning interventions with older adults' lived experiences. Moreover, technology developers can create inclusive elder-care tech, such as simple health apps, telemedicine, and social connectivity tools, to support aging in place in both India and the U.S.

By learning from each other's strengths through family-based support in India and community/institutional support in the U.S., both countries can innovate policies and services that ensure older adults not only live longer but also live with a high quality of life.

## ■ Acknowledgments

The author would like to thank Dr. Rama Joshi Paranjape (Geriatrics physician, Pune) and Dr. Sampada Patwardhan (Medical Microbiologist at Deenanath Mangeshkar Hospital, Pune) for arranging the process of older adults taking the survey and having the author conduct interviews in India. The author would also like to thank Amanda Shepard (senior staff member at Gilbert Senior Center) for assisting with surveying the U.S.

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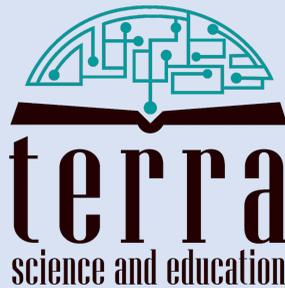
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