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From Bottles to Brains: The Role of Microplastics in Enhancing Brain Cancer Cell Growth

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ABSTRACT: Numerous studies have revealed that microplastic particles in most food and beverages can harm humans. Microplastics are found to penetrate the blood-brain barrier that protects the human brain from harmful substances, causing severe damage and even cancer cell proliferation. This study determined the impact of microplastics on brain cancer cells. In addition, the study was continued in more detail about how the higher concentration of microplastics affects brain cancer cells. However, the microplastic floating above the cancer cells never interacted with each other. As a solution, microwaved microplastic (MMP) was used to make the particles smaller and less dense so that they would not float but interact with the cancer cells. As a result, it was found that 2% MMP may support brain cancer cell proliferation, but 20% MMP may induce cytotoxicity in brain cancer cells. Overall, these findings indicate that small amounts of microplastics can promote cancer cell growth, highlighting the need for people to be aware of the presence of microplastics in their daily lives.

KEYWORDS: Cellular and Molecular Biology; Cell Physiology; Micro-plastic; Brain cancer; Proliferation.

■ Introduction

Microplastics have been found everywhere on the planet. Tiny particles of this anthropogenic material have been found buried in Antarctic Sea ice, inside the guts of marine animals inhabiting the deepest ocean trenches, and in drinking water worldwide.[1]

A new study has found that bottled water can contain up to 100 times more tiny pieces of plastic than was previously estimated. According to a study by Columbia and Rutgers universities in the US, the average liter of bottled water contains almost a quarter of a million nano-plastic fragments.[2] The researchers analyzed five samples of three common bottled water brands. They found nano-plastic levels ranging from 110,000 to 400,000 per liter, with an average of around 240,000. Scientists say much of the plastic appears to be coming from the bottle itself and that it is unknown whether plastic ingestion poses a severe health risk.[3]

In addition, microplastics exist almost everywhere around us. According to a 2022 analysis, many microplastic was found in many farms and crops.[4] For example, 90 trillion to 700 trillion microplastics were found in one of the European farms. Also, as microplastic was found in many farms, a significant amount was found in many root plants such as carrots, radishes, and turnips.[5]

These days, all humans are struggling with the problem of microplastic. Almost every food we intake has at least a small amount of microplastics. Especially if the plastic cup or bowl holds hot food or drink, it can be more harmful to humans. Previous research showed a risk of microplastics and toxic substances by humans during the daily use of disposable plastic materials.

Previous research identified the relationship between microplastic and the brain. All humans have a barrier that filters almost everything, including the blood-brain barrier (BBB). Unfortunately, previous research showed that microplastic can go through that barrier and damage our brains in mouse experiments.[6]

In detail about mouse experiments, researchers treated young and old mice with water that contained fluorescently labeled pristine polystyrene microplastic.[6] Surprisingly, after consuming the water, mice had some cognitive changes, such as behavioral changes and alterations in immune markers in liver and brain tissue. Also, it was discovered that the reaction caused by microplastic changes differed depending on mouse age.

Previous research shows that every food or drink contains tons of microplastic.[7] Plastic can be deadlier than we think if we put hot substances into it. Unfortunately, a previous experiment on mice discovered that microplastic can even pass through the blood-brain barrier, which almost every living creature has, and damage our brains. Therefore, microplastics continuously penetrate deep inside our bodies, which may cause serious health problems.

We hypothesized that both conditions may increase brain cancer cell proliferation. For the two conditions we made, one involved putting microplastic directly into the cultured brain cancer cell, and the other involved microwaved microplastic. Therefore, the final goal of this study is to find the novel effect of microplastic on human brain cancer cells.

■ Methods

Cell culture:

Brain cancer cells, A172 and SHSY5Y, were used in this experiment. We stored the brain cancer cells in a 6-well culture plate with nutrient-full media RPMI1640. We also used trypsin-EDTA media to separate the cells from a culture plate. All

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the brain cancer cells were kept healthy and maintained in a CO_2 incubator at 37 ˚C.

Microplastic treatment:

The microplastic stock solution was prepared with 0.1 g of microplastic and 1 mL of PBS solution. Four conditions were used: 0%, 2%, 10%, and 20% of microplastic in the cultured plate with the brain cancer cell for 48 hours. Cell A172 was mainly used in this experiment.

Microwaved Microplastic treatment:

A microwaved microplastic stock solution was prepared with 0.1 g of microwaved microplastic and 1 mL of PBS solution. We microwaved the microplastic for 1 minute and 30 seconds by microwaving it 30 seconds at a time. There were four conditions: 0%, 2%, 10%, and 20% of microplastic in the cultured plate with the brain cancer cell for 48 hours. Cell A172 was mainly used in this experiment.

Live cell imaging with an inverted microscope:

The image was captured using a Nikon inverted microscope. Nikon image capture software and image sensors can also observe live photos.

Results and Discussion

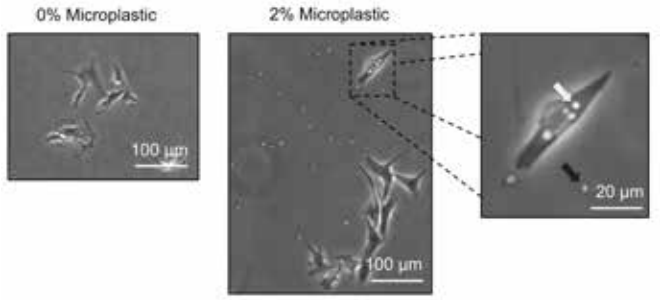

Figure 1: Microplastic particles attach to A172 brain cancer cells, suggesting potential for cellular damage. This figure illustrates the observation that, after 48 hours of incubation, microplastic particles attach to the A172 brain cancer cells. The attachment of microplastics, indicated by white arrows, suggests that microplastics can potentially cause cellular damage by interacting with the cell membrane. This observation supports our hypothesis that microplastics can influence brain cancer cell behavior by promoting conditions favorable for cancer cell proliferation.

We aim to find microplastic's direct impact on brain cancer cells. The other aim was to discover the interaction between microplastics and brain cancer cells. A172 cells were treated with 0% and 2% microplastics and were incubated for 48 hours in a CO_2 incubator. All the microscope images were captured using a Nikon inverted microscope (Figure 1). The result was that A172 cells with 0% microplastic had no microplastics observed, and the cells with 2% had some white dots around the cells. Two main types of microplastics were observed. One type was attached to the cells (indicated as a white arrow), but the other type was not attached to the cells (indicated as a black arrow). Therefore, the microplastic particles may attach to the human brain cells, cause severe damage, or induce cancer cell proliferation.

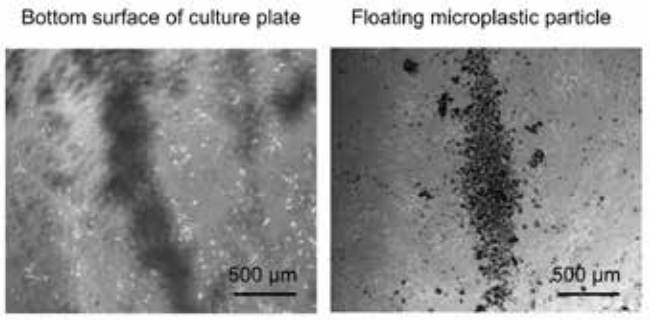

Figure 2: Higher microplastic concentrations float above A172 cells, reducing their interaction with the cells. In this experiment, A172 cells were exposed to increased microplastic concentrations (up to 10%) and incubated for 48 hours. We observed that, due to the lower density of microplastics compared to the culture media, excess microplastic particles floated on the surface of the culture media rather than interacting directly with the cells. This suggests that while lower concentrations of microplastics may facilitate interaction and influence cellular processes, higher concentrations lead to decreased cell contact, limiting the microplastic's direct impact on the cells.

We aim to find how the higher concentration of microplastics affects brain cancer cells. A172 cells were treated with increased concentration to 10% of microplastics and incubated for 48 hours in a CO_2 incubator. There were two main types: bottom surface focused image and floating microplastic particles (Figure 2). The result was that there were cancer cells on the bottom, and microplastics was floating on the surface. As the microplastic is less dense compared to the cell culture media, the microplastics floated. There was no attached microplastic. Therefore, higher concentrations of plastic particles showed a similar pattern as 2% microplastic particle treated samples, except the excess plastics floated on the culture media's surface. Next, we decided to test the effect of microwaved microplastics on the brain cancer cells to solve the floating problem.

The floating microplastics observed in Figure 2 likely significantly impacted the experimental results, notably by limiting the direct exposure of brain cancer cells to the plastic particles. Since the microplastics floated above the cell culture media, their interaction with the cells was reduced compared to the lower concentrations, where particles adhered more closely to the cells. This separation could have mitigated the cellular uptake of microplastics, thereby decreasing their potential to induce proliferation or cytotoxic effects. The lack of direct contact with the cells in higher concentrations suggests that the floating nature of these particles altered the experimental conditions, potentially leading to an underestimation of the impact that higher concentrations of microplastics might have if they were closer to the cells. This floating phenomenon emphasizes the need for methodological adjustments to ensure uniform exposure and accurately assess microplastics' dose-dependent effects on cancer cell behavior.

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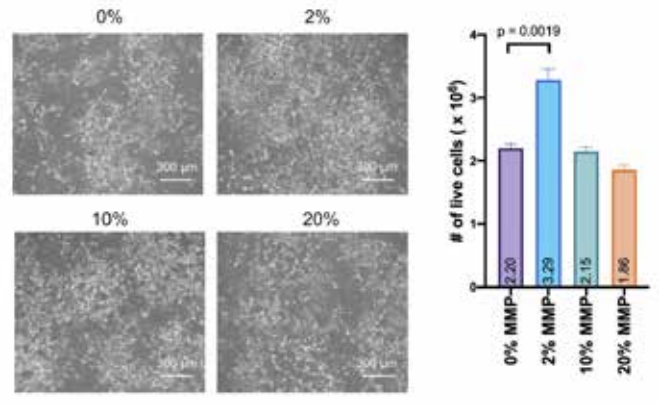

Figure 3: Microwaved microplastic (MMP) enhances A172 brain cancer cell proliferation at low concentrations, while higher concentrations induce cytotoxicity. This figure demonstrates the effect of varying concentrations of microwaved microplastics (MMP) on A172 brain cancer cells. At 2% MMP, there was a significant increase in cell proliferation (approximately 149.5% compared to the control), supporting our hypothesis that low levels of MMP can promote cancer cell growth. However, at 10% and 20% MMP concentrations, a decrease in live cell count was observed, indicating cytotoxic effects at higher concentrations. These findings suggest a concentration-dependent relationship between MMP exposure and brain cancer cell viability.

Next, we aim to find the effect of microwaved microplastic (MMP) on the proliferation of A172 brain cancer cells. Four concentrations of MMPs were tested (0%, 2%, 10%, and 20%). The Nikon inverted microscope captured all the microscope images. Back to the main idea, the MMP was for 48 hours with A172 cells (Figure 3). As a result, compared to the 0% negative control sample, 2% MMP increased the number of live cells from 2.20 x 10^6 to 3.29 x 10^6, indicating that the cell number had increased to 149.5%. However, both live cells of 10% MMP decreased to 98%, and 20% MMP decreased to 85% compared to 0% MMP samples. Therefore, 2% MMP may support brain cancer cell proliferation, but 20% MMP may induce cytotoxicity in brain cancer cells.

Several strategies could be employed to mitigate the harmful effects of microplastics, especially given their role in enhancing brain cancer cell proliferation, as demonstrated in this study. First, reducing disposable plastics can minimize exposure, particularly in food packaging and bottled water. Switching to alternatives like glass or metal containers may prevent the release of microplastic particles into consumables. Public awareness campaigns focused on educating the public about the risks of microwaving food in plastic containers, which can further reduce microplastic leaching. Governments and industries should also collaborate to develop more biodegradable and non-toxic plastic alternatives. Additionally, filtration technology advancements at water treatment facilities and within household appliances can capture microplastics before they enter the water supply. Finally, further research should be encouraged to develop methods for breaking down microplastics at the molecular level, mitigating their persistence in the environment and reducing long-term health risks.

Conclusion

In Figure 1, our experiment aimed at the impact of microplastics on brain cancer cells. The result was that the microplastic particles may attach to the human brain cells and cause severe damage or induce cancer cell proliferation. Figure 2 shows how the higher concentration of microplastics affects the brain cancer cells, and the higher concentrations of plastic particles showed a similar pattern to that of 2% microplastic particle treated samples, except that the excess plastics floated on the culture media's surface. Next, we decided to test the effect of microwaved microplastics on the brain cancer cells to solve the floating problem. In Figure 3, the goal was to find the impact of microwaved microplastics on the proliferation of A172 brain cancer cells. It was found that 2% MMP may support brain cancer cell proliferation, but 20% MMP may induce cytotoxicity in brain cancer cells.

While this study provides valuable insights into the effects of microplastics on brain cancer cell proliferation, it is essential to acknowledge its limitations. The experiments were conducted solely *in vitro* using a single cell line, A172, limiting the findings' generalizability. Brain cancer is highly heterogeneous, and different cell types may respond variably to microplastic exposure. Therefore, to fully understand the implications of microplastic-induced cancer proliferation, further studies should include multiple cell lines representing diverse cancer subtypes and non-cancerous cell models to assess broader cytotoxic effects. Additionally, *in vivo* studies using animal models would be essential to simulate real-life conditions and evaluate the biological interactions of microplastics in a living organism, particularly their ability to cross biological barriers and accumulate in tissues. Such studies would provide a more comprehensive understanding of the health risks associated with microplastic exposure and its potential to contribute to cancer progression.

We identified that microwaved microplastics may induce human brain cancer proliferation. There are two possible explanations of how microwaved microplastics induces human brain cancer proliferation: chemical leaching and physical leaching. First, chemical leaching explains how microwaving plastics can lead to the leaching of various chemicals, some of which may be carcinogenic or promote cancer cell growth. For instance, bisphenol A (BPA) and phthalates, often found in plastics, can act as endocrine disruptors and have been linked to various health issues, including cancer. In contrast, an explanation states that microplastics might directly interact with cells and tissues, causing physical damage or stress responses that could increase proliferation. In addition, microplastics' size, shape, and surface characteristics can influence their biological interactions, potentially leading to enhanced inflammation or cellular stress, which can contribute to cancer progression.

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Han Jun Lee is a student at the Cardigan Mountain School who likes science, especially chemistry, biology, and experiments. He loves to try special experiments about chemistry and cells.

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