IJHSR
ijhighschoolresearch.org
RESEARCH ARTICLE

Development of Chitosan-Coated Filter for the Isolation of Harmful Chemicals from Discarded Cigarette Butts

Chong Min

Seoul International School, 15, Seongnam-daero 1518 beon-gil, Seongnam-si, Gyeonggi-do, 13113, Republic of Korea; gosyber1@gmail.com
Mentor: Woo Rin Lee

ABSTRACT: Cigarette butts are hazardous to living organisms due to the presence of heavy metal ions that can cause damage to DNA and cells. Chitosan, a natural polymer derived from chitin, has excellent adsorption properties and is a potential solution to this problem. The study aims to develop a chitosan filter and evaluate its ability to remove harmful heavy metals from cigarette butts and investigate the toxicity of chemicals extracted from cigarette butts on human cultured cells. To conduct this study, we quantified the amount of iron (Fe), copper (Cu), mercury (Hg), zinc (Zn), and lead (Pb) in samples of CBEs (cigarette butt extracts). The cytotoxicities of CBE with various concentrations were tested on the human skin cell Detroit 551. Cytotoxicity was also measured with CBE using the chitosan-coated filter. It was made by immersing the Whatman paper filter into a synthesized chitosan solution and drying it. It was then compared with the toxicity of other samples without the filter. Using the chitosan-coated filter, concentrations from the cigarette butt extract decreased in particular heavy metals: Cu (92.1%), Zn (98.2%), and Pb (94.4%). Testing the cytotoxicity of CBEs, the results show a significant decrease in the live cell number starting from 5% CBE. The last experiment showed that 5% CBE with chitosan-coated filter paper, resulting in 1.21 x 10^6 cells/mL, was more effective in preventing cell deaths compared to non-filtered 5% CBE (0.32 x 10^6 cells/mL) and filtered 5% CBE (0.21 x 10^6 cells/mL). These results are significant as it shows how the chitosan-coated filter is a working method to isolate the heavy metals inside cigarette butt extracts and how it can have a positive impact on the environment with future research.

KEYWORDS: Environmental Engineering; Recycling and Waste Management; Chitosan Filter; Cytotoxicity.

Introduction

Approximately 4.5 trillion cigarettes are discarded each year worldwide, which makes it the most littered item in the world.[1] Cigarette butts are often disposed of on streets, sidewalks, and other public areas, and they eventually make their way into various parts of the environment, such as the ocean.[2]

Cigarette butts contain heavy metals such as cadmium, copper, and lead. As metal ions are correlated to DNA damage and cell cycle modulation, carcinogenesis, and apoptosis, the heavy metals in cigarette butts pose a risk to organisms.[3] The accumulation of heavy metals in water bodies, soil, and organisms continues to present toxicity risks to humans and other organisms.[4]

Chitosan is a natural biopolymer derived from deacetylating chitin, and it is a structural component in the shells of crustaceans such as shrimp. Chitosan has a positive charge and solubility in acidic to neutral solutions. It is commonly used in the biomedical industry as it can rapidly clot blood.[5] Also, chitosan has excellent adsorption properties due to the presence of high-functioning groups – amine and hydroxyl in its backbone, which act as active sites for metal ion adsorption.[6] This characteristic of chitosan makes it a potential solution to the problem that heavy metals inside cigarette butts inflict on our environment.[7] Chitosan is biodegradable and inexpensive, making it a realistically viable solution.[8]

Cigarette butt waste is a significant environmental issue. When discarded, heavy metals and toxic chemicals seep from the butts, posing a severe threat to human health and the broader ecosystem. To address this problem sustainably, this study investigates the use of chitosan as a filtration medium. The research objective is to create a chitosan-based filter that can be incorporated into cigarette butts to isolate and sequester hazardous substances. Additionally, the study aims to evaluate the toxicity of the chemicals extracted from cigarette butts on human cultured cells. This multifaceted approach can help mitigate the harmful impact of cigarette butt pollution and improve our understanding of the potential health risks associated with these common urban pollutants.

Materials and Methods

Cigarette butt collection:

Cigarette butts were gathered from the urban streets of Seoul, South Korea. Protective gloves, specialized tongs, and dedicated collection bags were used for safety during collection. The collection process was executed in a manner that ensured minimal disruption to the surrounding environment, thereby enhancing data accuracy and mitigating the potential for bias.

Extracting the chemicals from the cigarette butt:

Ten cigarette butts were carefully selected and ground into a finer powder using a mortar and pestle to increase the surface area for extraction. Then, the finely ground cigarette butt powder was transferred into a glass container. Next, a solution of 50 mL (50% ethanol blended with distilled water) was added to the glass container containing the ground cigarette butt powder to facilitate chemical extraction. This solution was

DOI: 10.36838/v6i10.10

considered a 100% extracted solution. This solvent mixture was chosen to extract water- and ethanol-soluble heavy metals effectively. Then, the samples were incubated overnight to allow for chemical extraction. Then, the samples were filtered to remove any solid particles. The filtered solutions were stored in a clean container. The extracted samples were stored at 4 °C.

Analyzing the heavy metals from cigarette butt extracts:

The quantification of heavy metals of iron (Fe), copper (Cu), mercury (Hg), zinc (Zn), and lead (Pb) in the extracted samples was analyzed using Atomic Absorption Spectroscopy (AAS). Different wavelengths were used to quantify the absorbance of each heavy metal: Fe (248.3 nm), Cu (324.8 nm), Hg (253.7 nm), Zn (213.9 nm), and Pb (283.3 nm). Before quantifying the heavy metals from the extracted samples, the calibration standards were injected into AAS (model: 230ATS). After plotting the calibration curve for each heavy metal, the measured absorbance was converted to the concentration (μg/g).

Chitosan synthesis from chitin powder:

Ten grams of chitin powder were measured. Then, 1M of sodium hydroxide solution was added to the chitin powder for the deacetylation. The mixed solutions were incubated overnight at room temperature. After the deacetylation reaction, the mixture was neutralized by adding 1M of acetic acid. Then, the mixture was filtered using filter paper to separate the solid chitosan from the liquid. After the chitosan was washed with water, the solid chitosan was dried until a dry powder was obtained.

Creating chitosan-coated filters:

The chitosan solution (0.5% w/v) was prepared using diluted acetic acid solution (0.1% v/v). Then, the Whatman paper filter was immersed in the chitosan solution for 24 hours. After the filter was lifted from the solution, it was dried at room temperature to allow the chitosan to coat it as it dried evenly.

Analysis of cytotoxicity of cigarette butt extracts:

The Detroit551 human skin cell was purchased from Korea Cell Line Bank. The cytotoxicity of cigarette butt extract was measured using the LUNA-FL (Logos Biosystems) cell counter device. After the cells were stained with Acridine Orange and Propidium Iodide solution, the live cells were stained in green, and the dead cells were stained in red. After the staining, the cells were inserted into the plastic slide. Then, the slide was inserted into the LUNA-FL cell counter device. This fluorescence cell counting device automatically counts the live and dead cells.

Statistical analysis:

One-way ANOVA and Tukey's post hoc test were used to calculate the p-value using the Prism 8 program.

Result and Discussion

Table 1: Five heavy metals were quantified using Atomic Absorption Spectroscopy (AAS) from the cigarette butt extracts. Before and after filtered extracts using the chitosan-coated filter were analyzed. The chitosan-coated filter was ineffective in filtering Fe and Hg, but it effectively filtered Cu, Zn, and Pb, reducing each concentration by over 90%.

	Before filter μg/g)	After filter (μg/g)
Fe	210.1	191.2
Cu	130.2	10.3
Hg	145.5	100.4
Zn	73.1	1.3
Pb	21.5	1.2

The objective of this experiment was to determine the concentrations of five heavy metals (Fe, Cu, Hg, Zn, Pb) in cigarette butt extracts. We compared the concentrations of these heavy metals before and after filtering with a chitosan-coated filter to analyze the filter's effect on heavy metal filtration (Table 1). We used ten cigarette butts to extract the heavy metals, using a solution of 50% ethanol and 50% water in 50 mL quantity. After extraction, we analyzed the concentration of each heavy metal. Table 1 shows that the concentration of Fe decreased slightly by 9.0% after filtering, while that of Cu decreased by 92.1%. The concentration of Hg reduced somewhat by 31.0%, and that of Zn decreased by 98.2%. The concentration of Pb was also reduced by 94.4%. We concluded that the chitosan-coated filter was ineffective in filtering Fe and Hg. Still, it effectively filtered Cu, Zn, and Pb, reducing each concentration by over 90%.

The chitosan-coated filter selectively adsorbs certain heavy metals through a combination of ion exchange, chelation, electrostatic attraction, and physical adsorption mechanisms. The amino and hydroxyl groups on chitosan interact with metal ions, where amino groups facilitate ion exchange and form stable chelate complexes, particularly with divalent and trivalent metal ions like copper (Cu^{2+}), lead (Pb^{2+}), and chromium (Cr^{3+}). Chitosan's cationic nature under acidic to neutral pH conditions enhances electrostatic attraction with negatively charged metal species. At the same time, its porous structure supports physical adsorption via Van der Waals forces and hydrogen bonding. The adsorption efficiency is also pH-dependent, where varying pH levels can optimize the filter's selectivity and performance for specific metals by influencing the protonation of chitosan's functional groups. These combined mechanisms may make the chitosan-coated filter an effective tool for selectively removing heavy metals from contaminated water.

Chitosan, a biopolymer derived from chitin, has gained significant attention as an effective material for removing heavy metals from water due to its unique chemical properties. Its structure consists of amino ($-NH_2$) and hydroxyl (-OH) functional groups that can chelate or bind with heavy metal ions, facilitating their adsorption onto the chitosan surface. This binding occurs through mechanisms such as ion exchange,

electrostatic interactions, and complexation, which are highly dependent on factors like pH, metal ion concentration, and the degree of deacetylation of chitosan. These functional groups enable chitosan to act as an excellent filter, effectively capturing heavy metals such as lead, copper, and cadmium from contaminated water. The eco-friendly, biodegradable nature of chitosan further enhances its appeal as a sustainable solution for water purification.

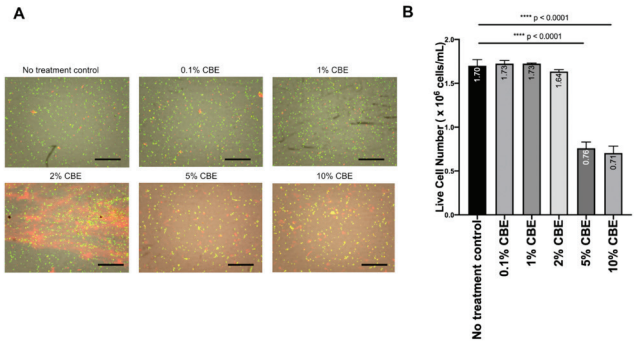

Figure 1: Cigarette butt extract (CBE) induces cell death of Detroit551 human skin cells. This result showed that up to 2% CBE, there was no significant decrease in the live cell number. However, from 5% CBE, there was a significant decrease in the live cell number. (A) Fluorescence image of Detroit551 cells incubated for seven days with six different concentrations of CBE: no treatment control, 0.1%, 1%, 2%, 5%, and 10%. (B) Bar graph of mean and standard deviation of live cell number. One-way ANOVA and Tukey's post hoc test were used to calculate the p-value.

This experiment aimed to measure the toxicity of cigarette butt extract (CBE) on the human skin cell Detroit 551, testing with six different concentrations of CBE: no treatment control, 0.1%, 1%, 2%, 5%, and 10%. We hypothesized that increasing the concentration of CBE would decrease the number of live cells in human skin cells.

Detroit 551 human skin cells were incubated for seven days with the six concentrations of CBE mentioned above. The live cell number was then quantified using the automatic live cell counter LUNA-FL device. This device detects the live cells (green) and dead cells (red) using the fluorescence color of the cells. Images from the LUNA-FL device were used to observe the effect of increasing concentrations of CBE on the live cells. For each concentration of CBE tested, green fluorescence represented live cells, while red fluorescence represented dead cells. It was observed that from no treatment control CBE to 1% CBE, there was generally no red signal background (Figure 1A). However, this red signal background was more visible in the 5% CBE and 10% CBE, representing more dead cells.

The live cell number (x 10^6 cells/mL) for each concentration sample of CBE was quantified, and the results were presented in Figure 1B. The no-treatment control sample, 0.1% CBE sample, and 1% CBE sample showed no significant difference in the live cell number. The 2% CBE sample slightly decreased the live cell number, possibly explaining its slightly red signal background. The 5% CBE sample significantly decreased the live cell number compared to the no-treatment control and 2% CBE samples. The 10% CBE sample significantly decreased the live cell number compared to the no-treatment control sample, but there was only a slight decrease in the live cell number compared to the 5% CBE sample. The results of this experiment revealed that up to 2%, there was no significant decrease in the live cell number. However, from 5%, there was a significant decrease in the live cell number.

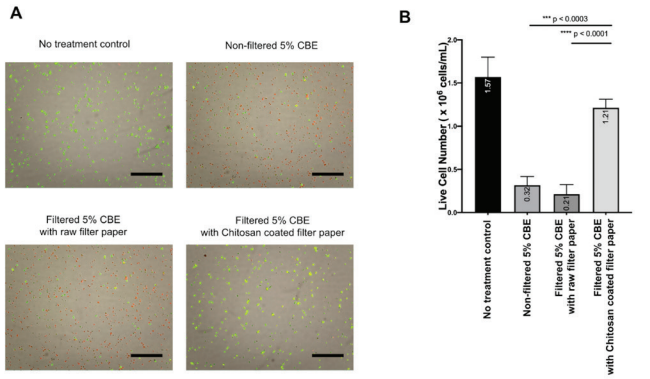

Figure 2: Chitosan-coated filter paper protects the Detroit 551 skin cells. The results show how the chitosan-coated filter paper effectively filters heavy metals that would reduce the live skin cell number. (A) Fluorescence image of Detroit551 cells incubated for ten days with four different conditions of CBE: no treatment control, non-filtered 5% CBE, filtered 5% CBE with raw filter paper, and Filtered 5% CBE with chitosan-coated filter paper. (B) Bar graph of mean and standard deviation of live cell number. One-way ANOVA and Tukey's post hoc test were used to calculate the p-value.

Next, this experiment aimed to collect data on the effectiveness of the chitosan-coated filter in reducing the harm caused by the cigarette butt extract on the human skin cell Detroit 551. We hypothesized that the chitosan-coated filter would be more effective than using raw filter paper or not using the chitosan-coated filter paper. Detroit 551 human skin cells were incubated for ten days, with one control sample using no treatment and the other three using 5% CBE. The concentration of 5% was used as there was not much change in the live cell number below 5% and roughly the same above it in the previous experiment (Figure 2A). The first 5% CBE was unfiltered, the second was filtered using raw filter paper, and the third was filtered using chitosan-coated filter paper. Then, the live cell number was quantified using the automatic live cell counter LUNA-FL device. Figure 2A shows images from the LUNA-FL device for no treatment control, non-filtered 5% CBE, filtered 5% CBE with raw filter paper, and filtered 5% CBE with chitosan-coated filter paper. The live cell number of the non-filtered 5% CBE sample is shown to have a significantly decreased number from the no-treatment control (Figure 2B). The filtered 5% CBE with raw filter paper shows similar results, meaning that raw filter paper does not have much effect in lowering the number of cell deaths. However, the filtered 5% CBE with chitosan-coated filter paper shows significantly fewer cell deaths, with most of the live cell number being preserved. These results are reflected in Figure 2B, which numerically shows how filtered 5% CBE with chitosan-coated filter paper was more effective in preventing cell deaths with 1.21 x 10^6 cells/mL compared to non-filtered 5% CBE with 0.32 x 10^6 cells/mL and filtered 5% CBE with 0.21 x 10^6 cells/mL. The results of this experiment show how the chitosan-coated filter paper effectively filters heavy metals

DOI: 10.36838/v6i10.10

that would reduce the live cell number. This allows for it to preserve most live cells successfully.

Conclusion

Table 1 aimed to analyze the effectiveness of the chitosan-coated filter paper in filtering heavy metals. The results showed that the chitosan-coated filter paper was significantly effective in filtering the heavy metals Cu, Zn, and Pb. It reduced each concentration by over 90% before and after filtering. To determine the effectiveness of the chitosan-coated filter paper in filtering heavy metals and reducing the number of human cell deaths, we conducted an experiment shown in Figure 1. We measured the toxicity of cigarette butt extract (CBE) on human skin cells (Detroit 551).

Figures 1A and 1B of this experiment indicate that up to 2% CBE, there was no significant decrease in live cell count. From 5% CBE, there was a significant decrease in the live cell count. This data was important in the experiment, as shown in Figure 2. This experiment aimed to analyze the effectiveness of the chitosan-coated filter paper in reducing the harm caused by cigarette butt extract on Detroit 551.

The data from Figure 1 led us to experiment with samples excluding the no-treatment control sample having 5% CBE, the concentration where a significant decrease is observed and maintained. Figures 2A and 2B show the results of this experiment. The filtered 5% CBE with chitosan-coated filter paper was significantly more effective in preventing cell deaths, with a live cell count of 1.21×10^6 cells/mL. This was compared to non-filtered 5% CBE with 0.32×10^6 cells/mL and filtered 5% CBE with 0.21×10^6 cells/mL. In summary, the chitosan-coated filter paper effectively filters heavy metals that can reduce the live cell count. It preserves most live human cells, making it a viable solution for filtering cigarette butt extract and reducing the potential harm caused by it on human skin cells.

The overall adsorption is enhanced by the electrostatic attraction between the positively charged protonated amino groups of chitosan and negatively charged metal ion species, particularly under acidic conditions where chitosan is more protonated. With its amorphous regions, chitosan's semi-crystalline nature provides a porous structure that increases the surface area available for adsorption, facilitating the physical capture of metal ions through Van der Waals forces and diffusion into the polymer matrix. This combination of chelation, electrostatic attraction, hydrogen bonding, and physical adsorption allows chitosan to sequester heavy metals selectively and effectively from contaminated environments.

One limitation of this research is that experimentation was only conducted on the human cell Detroit 551. This is a significant drawback as the lack of experimentation on other animal cells means that the chitosan-coated filter's environmental impact is not fully reflected in the experiments. Various animal cells could be used to determine whether the chitosan-coated filter effectively reduces cell deaths in most animals or humans.

Another research limitation was that the first experiment only analyzed the concentrations of five heavy metals (Fe, Cu, Hg, Zn, Pb) in CBE. However, other heavy metals in the CBE could also be significant or potentially more significant in causing cell death. This limitation is paired with the research limitation in which no experiment is used to explain why the chitosan-coated filter effectively preserves the live cell number. As no experiment reveals which heavy metal in CBE has the most impact on damaging human cells, it is unclear how the filter works.

In future research, we plan to expand our study to include the adsorption of additional heavy metals, specifically cadmium (Cd) and chromium (Cr), to provide a more comprehensive assessment of the chitosan-coated filter's capabilities. We will also broaden our cytotoxicity tests to include a variety of animal cell lines, such as liver, kidney, and neuronal cells, to evaluate the filter's safety and biocompatibility more thoroughly across different biological systems. Additionally, we intend to investigate the long-term stability and reusability of the filters under various environmental conditions and conduct pilot-scale field tests in real-world contaminated water bodies. Finally, we will perform a comprehensive environmental impact assessment to ensure the filter's large-scale deployment is effective and environmentally sustainable.

This research may have a significant impact on the environment as the Chitosan-coated filter presents a method to isolate the heavy metals inside cigarette butt extracts. This research can help reduce the leakage of heavy metals from cigarette butt extracts that make their way into the environment. Additionally, although the danger and toxicity of cigarette butt extract to human skill cells were previously underestimated, this research can make people realize the true potential for harm of this issue. As cigarette butt extracts proved harmful to human skin cells, this research also suggests that cigarette butt extracts could also be toxic to animal cells, which is important to test as many cigarettes make their way into animal ecosystems.

Acknowledgments

I want to thank Prof. Lee, my research advisor, and my parents for their support and guidance throughout this research.

References

1. World Health Organization. WHO raises alarm on tobacco industry environmental impact. www.who.int. https://www.who.int/news/item/31-05-2022-who-raises-alarm-on-tobacco-industry-environmental-impact.
2. Tchounwou, P. B.; Yedjou, C. G.; Patlolla, A. K.; Sutton, D. J. Heavy Metal Toxicity and the Environment. *Experientia Supplementum* **2014**, *101* (1), 133–164. https://doi.org/10.1007/978-3-7643-8340-4_6.
3. Qamar, W.; Abdelgalil, A. A.; Aljarboa, S.; Alhuzani, M.; Altamimi, M. A. Cigarette Waste: Assessment of Hazard to the Environment and Health in Riyadh City. *Saudi Journal of Biological Sciences* **2019**, *27* (5). https://doi.org/10.1016/j.sjbs.2019.12.002.
4. Vongdala, N.; Tran, H.-D.; Xuan, T.; Teschke, R.; Khanh, T. Heavy Metal Accumulation in Water, Soil, and Plants of Municipal Solid Waste Landfill in Vientiane, Laos. International *Journal of Environmental Research and Public Health* **2018**, *16* (1), 22. https://doi.org/10.3390/ijerph16010022.
5. Sudhakar, Y. N.; Selvakumar, M.; Bhat, D. K. Chapter 2 - Methods of Preparation of Biopolymer Electrolytes. ScienceDirect. https://www.sciencedirect.com/science/article/abs/pii/B9780128134474000029?via%3Dihub (accessed 2023-11-15).

6. Rouhani Shirvan, A.; Shakeri, M.; Bashari, A. Recent Advances in Application of Chitosan and Its Derivatives in Functional Finishing of Textiles. *The Impact and Prospects of Green Chemistry for Textile Technology* **2019**, 107–133. https://doi.org/10.1016/b978-0-08-102491-1.00005-8.
7. Upadhyay, U.; Sreedhar, I.; Singh, S. A.; Patel, C. M.; Anitha, K. L. Recent Advances in Heavy Metal Removal by Chitosan Based Adsorbents. *Carbohydrate Polymers* **2021**, 251, 117000. https://doi.org/10.1016/j.carbpol.2020.117000.
8. Cheung, R. C. F.; Ng, T. B.; Wong, J. H.; Chan, W. Y. Chitosan: An Update on Potential Biomedical and Pharmaceutical Applications. *Marine Drugs* **2015**, *13* (8), 5156–5186. https://doi.org/10.3390/md13085156

Author

Chong Min is a junior high school student at Seoul International School. As he engaged in and led local cleanups, Chong became interested in environmental research and how pollution may detrimentally affect people and nature.

DOI: 10.36838/v6i10.10