Rapid synthesis of copper oxide nanoparticles dispersed with carbon nanotubes, bound to a melamine sponge as an electrically conductive bactericidal filter for the inactivation of *Escherichia coli* and total coliforms via electroporation

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ABSTRACT

This paper presents a simple and cost-effective method for the production of electrically conductive sponges for water filtration. The materials for the filter can be synthesized in a developing country, because no specialized materials or equipment were used. I synthesized copper oxide nanoparticles in a rapid and affordable manner. There was very little waste product throughout the process, resulting in a highly efficient synthesis. Carbon nanotubes are hydrophobic, so a surfactant was needed in order to disperse them in distilled water. Sodium lauryl sulfate, bentonite clay, PEG and water were tested in dispersing carbon nanotubes, but sodium lauryl sulfate was chosen for is affordability and greater dispersion abilities. Sodium lauryl sulfate solutions of copper oxide nanoparticles and carbon nanotubes were created, and a standard melamine sponge was repeatedly dipped and dried in order to ensure the nanomaterials bind to the sponge's surface. I 3D printed a PLA enclosure that allowed for the placement of 2 sponges for the filtration of water. Production of the filter can be scaled to further reduce costs. In addition, solar cells can be used to power the system in place of AA batteries, which will allow for the filter to be self-sufficient. Due to the pore size of the sponges, biofouling is not an issue; however, further research must be carried out on the robustness and durability of the filter.

KEYWORDS

Short multi-walled carbon nanotubes, sodium lauryl sulfate, enhanced electric field, water disinfection

INTRODUCTION

Roughly 2.1 billion people obtain their drinking water from a fecally contaminated source (CAWST 2017). *Escherichia coli* are common water-borne bacteria with an infective dose as small as 100 organisms per liter that cause severe illnesses, such as urinary tract infections, meningitis and diarrhea. In developing countries, diarrhea can lead to severe dehydration and potentially death (WHO 2017). Contamination occurs when fecal matter from humans or animals comes in contact with a drinking water source (WHO 2017). Although there are thousands of water related deaths per year, this can be reduced by ensuring water is properly filtered or disinfected. Current water filters implemented in developing countries such as membrane, biosand, and ceramic filters are too expensive for most households to afford.

Membrane filters are the most portable and durable, but they are very costly. Ceramic filters are heavy and fragile; and biosand filters are extremely heavy and material intensive. The most common water filter in developing countries are biosand filters, which are capable of filtering large amounts of water and can be manufactured on site with common materials such as sand, gravel and cement (CAWST 2011). They remove between 87.9-98.5% of bacteria, but it takes approximately 30 days for it to reach these values due to the dependence of a biolayer, which is a layer on surface the filter with beneficial microorganism that kill harmful pathogens through predation (CAWST 2011). In addition, biosand filters are not the most reliable, so the percentage of bacteria removed will fluctuate depending on who built the filter, where the water is being obtained, the temperature of the environment, and the age of the biolayer (Schijven et al. 2013).

The least expensive membrane filters which cost around $20, not including shipping, are still relatively expensive for a household in a developing country to afford where on average someone dealing with poverty is estimated to earn $1.90 per day (Jolliffe and Prydz 2016). These lower end membrane filters only filter out large particles and pathogens, so smaller bacteria and viruses can easily penetrate through the membranes, leading to potential infections (AWWA 2008). The most effective filters are ceramic pot filters, but they are extremely fragile and difficult to manufacture locally since they incorporate a silver lining; therefore, ceramic filters are commonly shipped to their perspective locations, which greatly increase the overall cost of the filter. Thus, there is a need for an affordable, compact and efficient filter that can reliably remove waterborne pathogens.

The silver lining in ceramic pot filters has antibacterial properties which aid in disinfecting water. The process in which metals inactivate bacteria is called the oligodynamic effect. Essentially, positive metal ions bind to negatively charged pathogens and dissolve the cell membrane until the internal organs are exposed, leaving the pathogen to die (Azam et al. 2012, Shrestha et al. 2009). Other pure metals and metal oxides from copper, silver, gold and brass also possess antibacterial properties. The oligodynamic effect is highest for copper particles, and for gram-negative bacteria such as *E. coli* (Shrestha et al. 2009). Therefore, incorporating metal oxides into filters is an effective way to inhibit bacterial growth.

The oligodynamic effect's transfer of ions from a metal to the cell walls of bacteria is similar to the disinfection processes that occurs in graphene nanosheets. Graphene is a single layer of sp2 bonded carbon atoms in a honeycomb lattice structure (Geim and Novoselov 2007) with a large surface area (Lightcap et al. 2010). Graphene sheets are rolled into cylindrical shapes to produce carbon nanotubes. Oxygen radicals in water adhere to graphene nanosheets and carbon nanotubes, and cause oxidative stress upon contact with bacteria, resulting in protein and DNA damage (Cabiscol et al. 2000, Gurunathan et al. 2012). If sufficient oxidative stress is achieved it will lead to the inactivation of the bacteria. The use of graphene and carbon nanotubes for water treatment is improving and advancing but do their high cost it has restricted their implementation.

An efficient method of disinfecting water is through electroporation. An electric field is induced on a positively charged substrate, causing gram negative bacteria to become attracted to it. In the process, nanopores form on the bacteria's membrane, and the negatively charged DNA is extracted (Huo et al. 2017). If there is a sufficiently strong electric field the nanopores will enlarge, or the electric field will cause a rupture in the cell's membrane, exposing the bacteria's organelles, causing it to immediately die (Huo et al. 2017, Liu et al. 2013). Copper oxide nanoparticles and carbon nanotubes have a high surface area which can be used to attract gram negative bacteria (Huo et al. 2017).

The aim of the study is to determine how different nanomaterials impact the electroporation rate at different voltages. I will compare carbon nanotubes and copper oxide nanoparticles to identify the most efficient and effective nanomaterial that can achieve 2-log disinfection at the lowest applied voltage. I hypothesize that the carbon nanotube coated sponge with the copper oxide nanoparticles will possess the highest antibacterial properties due to a combination of high electro-conductivity from the carbon nanotubes and the antibacterial properties from the copper ions.

MATERIALS AND METHODS

Materials

Carbon nanotubes were purchased from US Research Nanomaterials Inc. Copper (II) chloride dihydrate, polyethylene glycol (PEG) 400 and sodium hydroxide were obtained from College of Chemistry at UC Berkeley. Ethyl alcohol was obtained from Dr. Mendez in the College of Natural Resources at UC Berkeley. Sodium lauryl sulfate was obtained from Amazon. A Coliscan Easygel kit was purchased from Weber Scientific.

Dispersing carbon nanotubes

Carbon nanotubes are hydrophobic, so a surfactant is needed to decrease the surface tension of water which will allow for the carbon nanotube's dispersion in water (Jiang et al. 2003, and Rastogi et al. 2008). Bentonite clay is not classified as a surfactant but was tested for its ability to easily disperse in a solution and the potential to disperse other materials (Liu et al. 2008). All solutions were produced using distilled water. Four different samples were tested: a 10% w/v solution of sodium lauryl sulfate, 1% w/v solution of bentonite clay, 10% w/v solution of PEG 400, and distilled water. To create 1% w/v solutions with carbon nanotubes, 5 mL of each solution were placed in a test tube along with 50 mg of carbon nanotubes. All 4 solutions were placed in an ultrasonic bath for 1 hour. Because low temperatures allow for maximum dispersion of the carbon nanotubes (Shi et al. 2013), low temperatures were ensured by simultaneously taking out water from the ultrasonic bath with a pipette and replacing it with ice water. The solutions were left undisturbed for 14 days to settle. The sodium lauryl sulfate solution worked the best and was used in the preceding experiments.

Synthesis of copper oxide nanoparticles

I modeled copper oxide synthesis from Wang, et al. (2001). I ground 5.035 grams of copper (II) chloride dihydrate in a mortar for 5 minutes and 3.061 grams of sodium hydroxide was grounded in an herb grinder for 5 minutes. Afterwards, both were combined together in a mortar where 6 mL of polyethylene glycol (PEG) 400 was added to the mixture and ground for 30 minutes using a pestle. The mixture was then washed in an ultrasonic bath three times with 60 mL of distilled water and then with 60 mL of ethyl alcohol to remove the PEG. The solution was left to settle for 8 hours and washed 2 more times with 60 mL water and ethyl alcohol. Each of the washes were for 25 minutes. The solution was then left to settle for 2 hours and the remaining liquid was pipetted out. The copper oxide solution was then placed in the microwave at high power for 4 minutes and 30 seconds to dry.

Fabrication of the sponges

I cut a standard melamine sponge into 1cm x 1cm x 0.5cm blocks and then weighed and cut them to size to obtain 5 mg. The sponge blocks were placed in an ultrasonic bath with distilled water for 30 minutes to remove dust and other particles that will prevent the nanomaterials from binding to sponge's surface. The melamine sponges were then placed in an oven at 190℃ for 10 minutes to dry.

I produced the carbon nanotube coated sponges by combining two 5mg melamine blocks in 10 mL of 1% w/v carbon nanotubes in 10% w/v sodium lauryl sulfate and distilled water. I soaked and squeezed the melamine blocks in each solution then placed them in an oven at 190℃ for 10 minutes. Afterwards, they were placed back in the solution and the process of saturating and drying was conducted until all the solution was used up. These 2 sponges were named CNT, for carbon nanotube.

I produced the copper oxide sponges by dispersing 200 mg of copper oxide nanoparticles in 10 mL of the 10% w/v sodium lauryl sulfate solution via ultrasonication for 1 hour. To ensure maximum dispersion, water was constantly replaced with ice cold water. The sponges were named CNP, for copper oxide nanoparticle. The process of saturating and drying was repeated until the solution was used up.

I produced the carbon nanotube and copper oxide sponges by dispersing 100 mg of copper oxide nanoparticles in 10 mL of 0.5% w/v carbon nanotubes in 10% w/v sodium lauryl sulfate. The mixture was placed in an ultrasonic bath for 1 hour. I soaked and squeezed the melamine blocks then placed them in the oven to dry. Afterwards, they were placed back in the solution and the process of saturating and drying was conducted until all the solution was used up. The sponge were named CNM, for carbon/copper oxide nanomaterials.

After all the sponges cooled they were washed with 25 mL of distilled water to extract any loose nanoparticles that did not properly adhere, then placed in an oven at 190℃ for 10 minutes to dry.

Imaging and elemental analysis

Copper oxide nanoparticles were adhered onto an aluminum plate with carbon tape and a carbon coating was placed on the edges of the sponges in order to prevent the accumulation of electrons which could result in the formation of a static electric field when taking the images. I used the Zeiss EVO-10 Variable Vacuum Scanning Electron Microscope (SEM) coupled with Energy Dispersive X-ray Spectrometry (EDAX), located in the SEM Lab at the Earth and Planetary Science Department at UC Berkeley, in order to obtain the images and perform elemental analysis on the surface of the nanoparticles. EDAX was performed at 20 kV and with a count size of 47 seconds. SEM and EDAX were operated at a low vacuum pressure and an accelerated voltage to obtain the highest quality images possible.

System set up

The enclosure was printed out of Poly Lactic Acid (PLA), and a CNT sponge was placed inside, followed by a plastic mesh and then the other CNT sponge (Figure 1). A mesh was placed in between the sponges to prevent the system from short-circuiting (Huo et al. 2017). An insert was placed under the bottom sponge to limit the flow rate and ensure the sponge did not fall out. The postive terminal of the battery was connected to the top sponge, meanwhile, the negative termial of the battery whas connected to the bottom sponge.

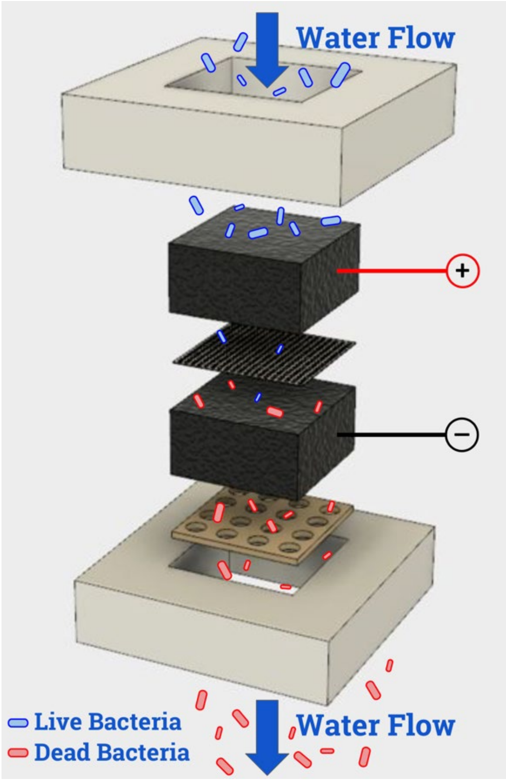

Figure 1: System set up

Electroporation

Carbon nanotubes have a high electric conductivity and aid in increasing the force of the electric field. Gram negative bacteria are attracted to the positive surface of the nanomaterials created by the electric field. When bacteria come in contact with the electric field enhanced by the nanomaterials the bacteria's membrane will begin to dissolve (Figure 2).

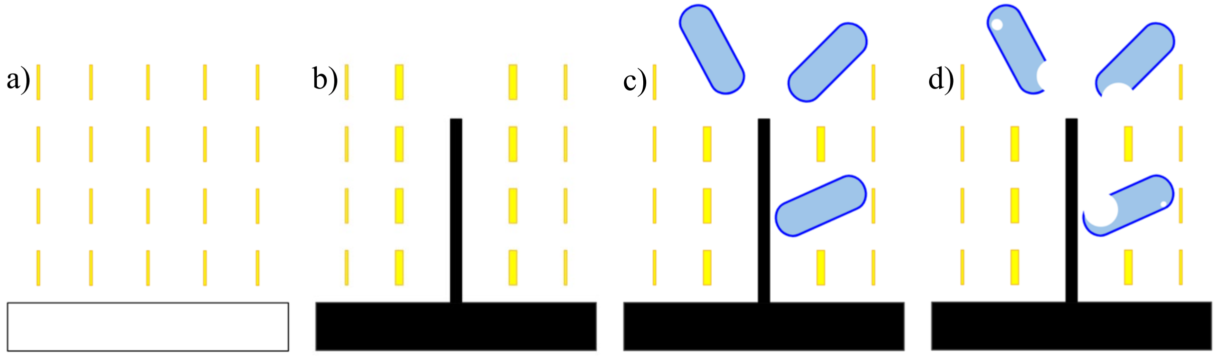

Figure 2: Electroporation mechanism. Visualization of (a) an electric field applied on sponge without nanomaterials, (b) electric field enhanced by nanomaterials, (c) gram-negative bacteria attracted to the nanomaterials, (d) electroporation and rupture of bacterial membrane.

Analysis

Dispersion Methods

After 14 days of settling, the dispersions were analyzed. Water and PEG were ineffective and had an insignificant effect in dispersing carbon nanotubes (Figure 3). Bentonite clay is electrically conductive when placed in a solution to form an aqueous solution; thus, the positive charge on the clay particles attracted carbon nanotubes to its surface, which allowed the carbon nanotubes to disperse. However, this type of dispersion is not ideal for this application because having a large aggregate of carbon nanotubes on the surface of a clay particle could clog the sponge, reducing the flow rate.

I chose to proceed with sodium lauryl sulfate and not bentonite because the clay particles will prevent the carbon nanotubes from properly adhering to the surface of the melamine sponge, leading to a decrease in the life expectancy of the filter. In addition, since sodium lauryl sulfate is soluble in water (Jiang et al. 2003, and Rastogi et al. 2008), it is expected to eventually be released from the filter, leaving the carbon nanotubes adhered to the surface of the sponge. Sodium lauryl sulfate has great dispersion properties because it contains a polar hydrophilic head and a non-polar hydrophobic tail. The hydrophobic tail binds with the carbon nanotubes and exerts a force while undergoing sonication to pull the carbon nanotubes apart to individual segments, resulting in a superior dispersion (Jiang et al. 2003, and Rastogi et al. 2008)

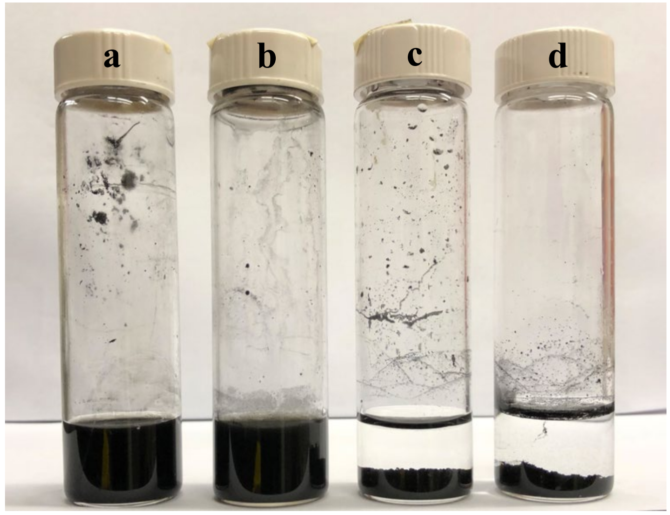

Figure 3: Photograph of the dispersions after 14 days of settling. Dispersions of 50 mg carbon nanotubes in 5mL of: (a) 10% w/v solution of sodium lauryl sulfate, (b) 1% w/v solution of bentonite clay, (c) 10% w/v solution of PEG 400, and (d) distilled water.

Energy Dispersive X-ray Spectrometry

The main components of the nanoparticles were copper and oxygen, resulting in the successful synthesis of copper oxide (Figure 4). There was a minor carbon concentration but that could either be due to the carbon coating, or through cross contamination in the laboratory when I was measuring out the carbon nanotubes. In addition, there is a small peak at 6 keV which corresponds to a minor impurity of iron, but none of these substances should have a significant impact on my results in the proceeding experiments since their concentration are miniscule and insignificant.

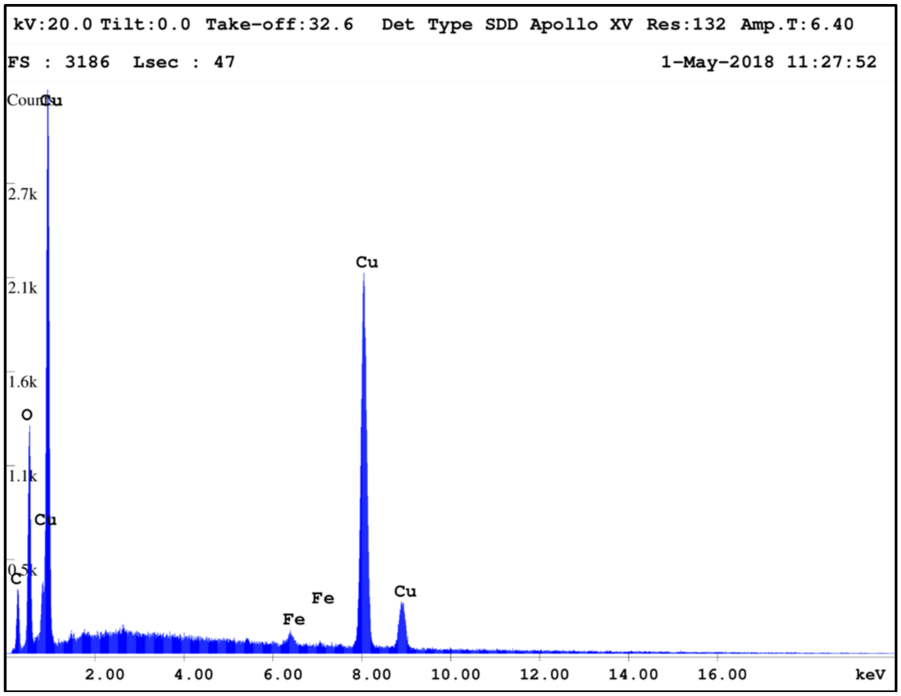

Figure 4: EDAX of copper oxide nanoparticles.

Scanning Electron Spectrometry Images

SEM was used to visualize the copper oxide nanoparticles and the surface of the sponges. The copper nanoparticles were properly formed, in which they were around 500 nm in diameter (Figure 5). As a result of the small size of the copper oxide nanoparticles they did not become entrapped in the pores of the sponge, resulting in the nanoparticles not properly adhering to the surface of the melamine sponge, causing a large amount of copper oxide nanoparticles to be released in the initial washing.

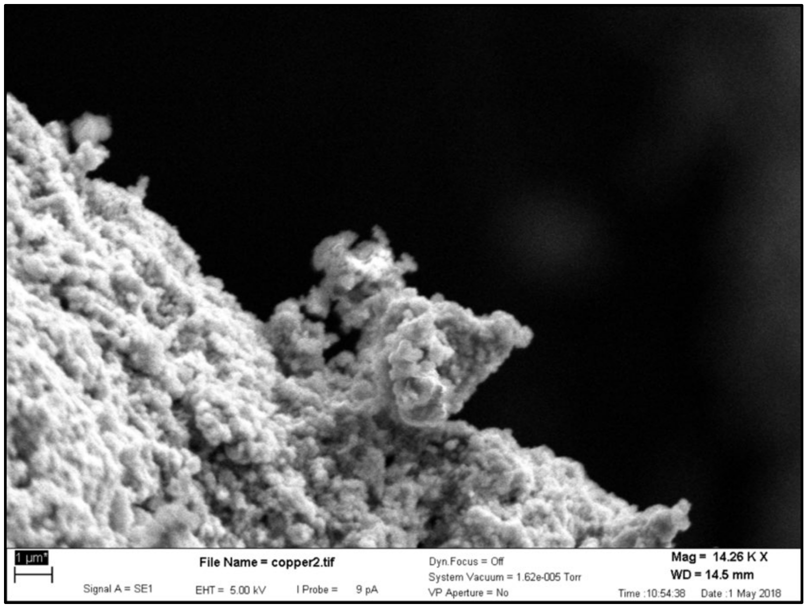

Figure 5: SEM images of the copper oxide nanoparticles. The copper oxide nanoparticles were magnified to 14.26 KX.

The large length of the carbon nanotubes allowed them to be better entangled and adhered within the walls of the melamine sponge. This suggests that there is a positive correlation between the adherence rate and the length of the nanomaterial. Thus, to increase the adherence rate of copper oxide nanoparticles they must be made to be longer in size, but not longer than the smallest pore size on the melamine sponge.

Nanomaterials successfully adhered to the surface of the sponge (Figure 6b-d) apparent from the difference of the sponge's surface when compared to (Figure 6a). The average pore size of the nanoparticle treated sponge is about 200 um, resulting in a fast flow rate. *Escherichia coli* is 2 microns in length, thus the size of the sponge allows *E. coli* to easily penetrate through, reducing the chance of biofouling. Membrane, biosand and ceramic filters have small pore sizes and work by trapping bacteria. However, this can lead to biofouling and can reduce the quality of the water and lead to the degradation of the filter (Guo et al. 2012). Therefore, by reducing the quantity of pathogens that are trapped in the filter, the overall life expectancy of the filter is extended, and the water quality is improved (Guo et al. 2012).

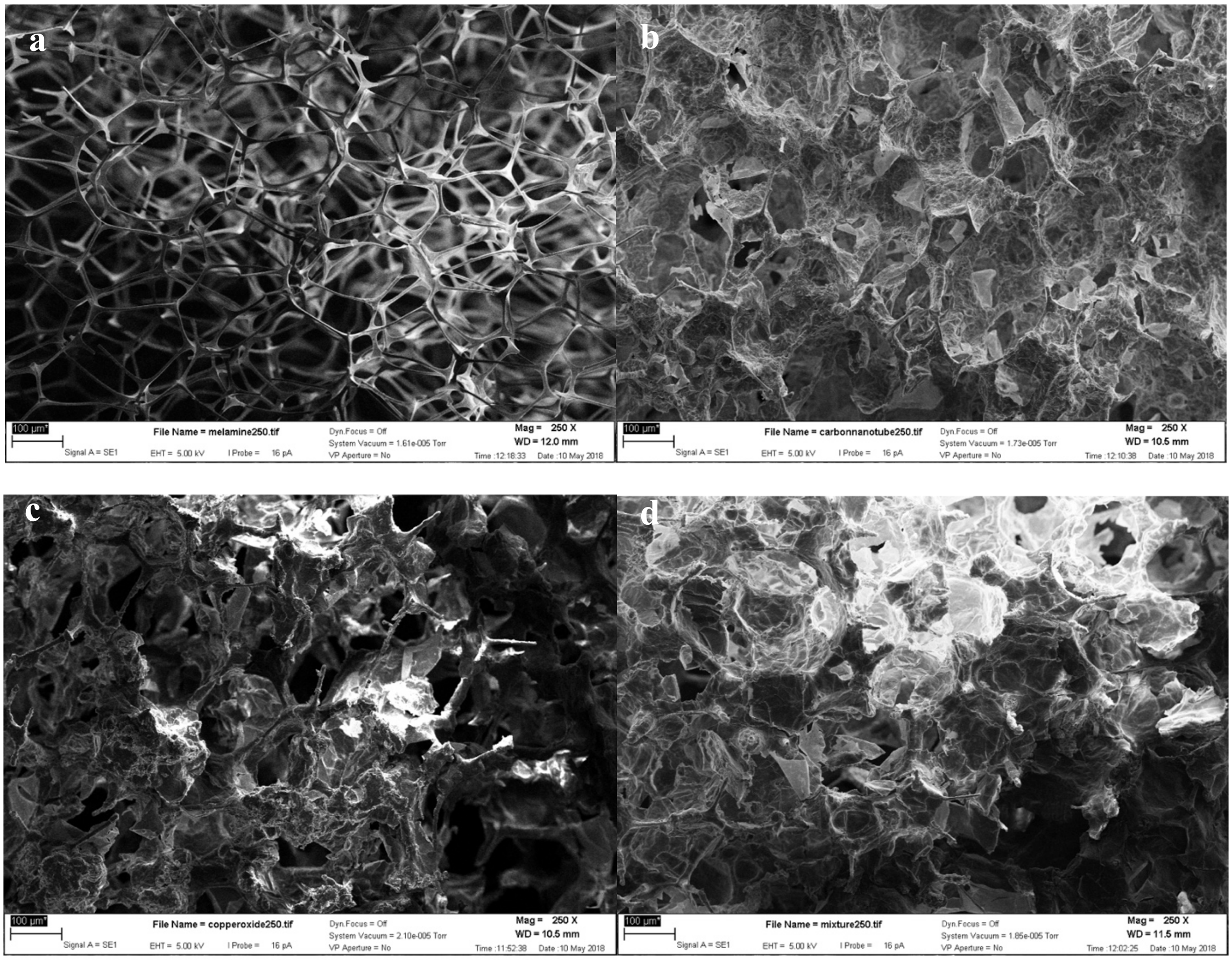

Figure 6: SEM images of the melamine sponge's surface. Images at a magnification of 250 X were taken of (a) standard melamine sponge, (b) CNT melamine sponge, (c) CNP melamine sponge and (d) CNM melamine sponge

LIMITATIONS

I will use Coliscan Easygel to test for *E. coli* and total coliforms which may overshoot the total concentration present in the water (Chuang et al. 2011). IDEXX tests are far more accurate, but due to funding limitations Easygel is the next best option. Easygel was "recommended for testing improved water sources only" (Trottier 2010). Thus, it could overshoot the *E. coli* and total coliform concentrations present in the water.

Due to a lack of a proper binder, such as polydopamine, the copper nanomaterials did not properly adhere to the surface of the melamine sponge. Thus, there was a loss of copper nanomaterials, which resulted in an inefficient binding process. In addition, this may result in an inconsistent procedure, because every time the sponge is replicated different concentrations of copper nanomaterials will be left on the sponge after the initial washing.

In addition, to obtain a high inactivation rate, there must be a sufficiently long contact time, which leads to a slow flow rate. To increase the flow rate, in order to provide a sufficient quantity of water, the contact time must be decreased; as a result, the voltage must be increased. This increase in voltage will come at a cost, making it less affordable and less of a viable option for people in developing countries. In addition, the increase in voltage will drain out the batteries at a faster rate, and can damage the melamine sponge, decreasing the life expectancy of the filtration system.

FUTURE DIRECTIONS

To test the sponge, an electric current will be placed across the sponges as water is filtered through them at a constant rate. The voltages that will be tested are: 0, 4.5, 9, 13.5, and 18 Volts. This entire procedure will be conducted to test CNT, CNP and CNM. The filtered water for each test will be collected and placed in a petri dish with Coliscan Easygel media and left to settle for 48 hours at room temperature.

After analyzing the results, the next step would be to visualize what mechanism of bacterial inactivation was the most responsible for bacterial death. I will analyze: oxidative stress, the oligodynamic effect, impalement and electroporation. The sponges will have to be replicated twice and then compared in how effective the filter was in reducing *E. coli* and total coliforms.

Once the ideal voltage is known, the information can be used to continue with further experimentation to increase the efficiency and capabilities of the filter. The sponge size can be increased, and then compressed to a 1 x 1 x 0.5 cm size. The compression can be longitudinal, lateral, or both. Each mode of compression, as well as the size of the sponge, will produce different results. By compressing the sponge, the pore size of the sponge will be reduced, increasing its filtration capabilities by reducing the amount of pathogens that can pass through and by increasing the surface contact possibilities, which will lead to a higher rate of bacterial inactivation by electroporation. Compression will increase density, resulting in an increase in the contact between the nanomaterials to transport the electric current, which will further increase efficiency (Luo et al. 2017).

$$R = \frac{\rho L}{A}$$

where ρ = resistivity, L = length, and A = cross-sectional area. Thus, by increasing the cross-sectional area or decreasing the length, a sponge with a lower resistance and higher electrical conductivity can be achieved. If the shape of the sponge is enlarged, following compression to model the minimization of the resistance equation, the filter can possess greater disinfection capabilities at a lower voltage which can reduce the cost.

CONCLUSION

Copper oxide nanoparticles can be synthesized in a cost-effective and efficient manner, however it's efficacy is yet to be determined. If the materials are bought in bulk, the price of the filter will drastically decrease. A membrane filter typically costs around $20, where the melamine sponge filter costs between $10.40 - $13.01, depending on the amount of batteries used. Thus, this filter will able to compete with membrane filters, as well as ceramic filters, when it comes to cost. In addition, because the filtration system uses an electric current, that eliminates the possibility of disinfection byproducts, DBPs, from forming (Huo et al. 2016).

There is not a surplus of research being conducted for water filtration in developing countries, as such, my research helped in providing further information. Due to the low energy requirement, the filter can be powered by batteries or solar cells, further making it a viable option for developing country use. In addition, all of the components with exception to the carbon nanotubes, can be synthesized or purchased in developing countries, further reducing the overall cost of the filter.

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