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Non-enveloped virus reduction with quaternized chitosan nanofibers containing graphene

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ABSTRACT

Membranes are an accepted technology for water purification. Membrane filtration can remove pathogens, including bacteria and viruses, by size. For small viruses that can have a diameter <25 nm, removal by size leads to large membrane areas, high transmembrane pressures, low water flux, and frequent changing of membranes. In this work, we discovered that electrospun nanofibers made of chitosan and functionalized with a quaternary amine (HTCC) have the ability to adsorb a model non-enveloped virus, porcine parvovirus (PPV). To improve the virus removal of HTCC, we added graphene. Graphene both enhanced the ability to form nanofibers with HTCC and improved the virus removal. The hydrophobicity of graphene and the high charge of the HTCC create a system that can bind 95% of PPV. The HTCC/graphene nanofibers could be incorporated into microfiltration membranes and remove virus by adsorption. This would create a low pressure system that is more likely to benefit areas in need of fresh water.

1. Introduction

The World Health Organization estimates that 1 billion people lack access to safe drinking water and 2 million deaths each year are attributed to unsafe drinking water, mainly caused by pathogen contamination.[1] Women and children are disproportionately affected by the harmful and deadly costs of drinking water contaminates. Currently, municipal systems treat water in a multistep process of coagulation and flocculation, sedimentation, rapid sand filtration, and disinfection.[2] The most common disinfection agent is chlorine, which is known to react with natural organic matter (NOM) and create disinfection by-products that are suspected to be carcinogenic.[3,4] Advanced systems of purification include ozone treatment, coagulation, activated carbon filtration, and ultra- and nano-filtration are currently employed in select municipal water systems.[2,5] These methods have many of the same difficulties, including high backpressures of small pore-sized membranes, the ability of small viruses and heavy metals to escape the system, high cost, and the requirement for a large, multistep municipal system. Smaller systems can use photo and catalytic reduction of pathogens, often termed SODIS (solar disinfection), but require long disinfection times and the results can be variable.[6,7]

Membrane filtration is an accepted technology for the reduction of contaminants from drinking water. Bacteria are easily removed with an ultrafiltration membrane, although higher than desired backpressures are still required.[8] Antibacterial surfaces made of silver and other metal ions have been created, but these surfaces still need long contact time to reduce microbial titer.[9] Viruses have been removed by nanofiltration membranes using the size-exclusion principle, but they have high backpressures and quickly foul.[10,11] Viruses have generally been captured with strong anion membranes[12,13] and specifically captured with receptors for influenza[14] and smallpox.[15] Carbon nanotubes (CNTs) are also becoming popular to use as a pore forming material in water filtration, but due to the small pore diameter of CNTs, the transmembrane pressure is high in CNT membranes.[16] A recent electrically-driven carbon nanotube filter removed a bacteria phage (a virus that infects bacteria), but concerns remain over the release of viruses after the voltage is turned off.[17] As new methods to remove viruses are explored, there are still many disadvantages to the current methods, with high transmembrane pressures being the largest disadvantage. For this reason, we have explored the adsorption to nanofibers for the reduction of viruses from drinking water. Nanofibers allow high flux and low fouling filtration, while the functionalization will target a large range of potentially deadly contaminants.

Electrospinning has shown great promise as a method to create high surface area filter material.[18,19] A large electrical potential between the collector and the polymer solution creates a Taylor

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cone. A small jet of nanofiber material can be ejected from the jet when the viscosity and surface tension are overcome by the electrostatic force. One material that is easy to electrospin is chitosan.[20] We have chosen to use chitosan because it (i) is easily functionalized, (ii) can form nanofibers, and (iii) is biocompatible and hydrophilic. Hydrophilicity is an important characteristic of low fouling surfaces. Low fouling surfaces have the potential to have greater water fluxes and less need for regeneration, making them ideal for water purification applications. Since chitosan is also easily functionalized, we explored the ability of chitosan to adsorb viruses by adding a quaternary amine group to chitosan to form N-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride (HTCC) (see the structure in Scheme 1).

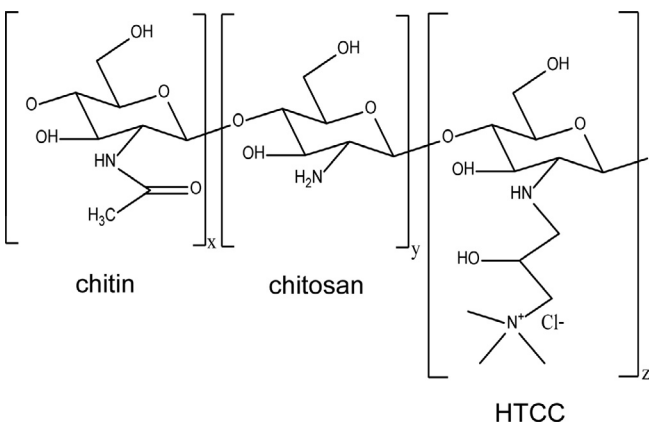
Biopolymers are a sustainable method for creating virus reduction surfaces. One important characteristic of virus reduction surfaces is positive charge[12] and it is also possible that a hydrophobic surface could also attract viruses.[21,22] Chitosan has a positive charge and has been shown to have antibacterial,[19] antifungal,[23] and antiviral[24] activities. It has been shown that the positive charge on chitosan can cause bacterial cell permeation and cell death.[25] This makes chitosan a desirable biopolymer for water purification. The antibacterial activity of chitosan can be increased by increasing the positive charge by functionalization with a quaternary amine.[26]

In this work, we compare the ability of chitosan and a quaternary derivative of chitosan for virus reduction applications. Here, we use the model virus, porcine parvovirus (PPV). PPV is often used in virus reduction applications due to its small size and chemical resistance to many disinfection technologies.[27] Additives were tested to improve the ability to electrospin the quaternary amine, and it was found graphene, with its combination of conductivity and hydrophobicity, was able to improve both the ability to electrospin HTCC and also the reduction of virus.

2. Results and discussion

2.1. Preparation of HTCC

HTCC, as shown in Scheme 1, was prepared by the epoxide addition of a quaternary amine.[28] The FTIR spectra of chitosan and HTCC are shown in Figure 1A. The peak at 1590 cm^{-1} in chitosan, representing the primary amine N-H bending, is reduced in the HTCC. A new peak at 1478 cm^{-1} is found in the HTCC, and this is the C–H bending of the methyl group on the quaternary nitrogen. The results are the same as has been previously found.[29–31] The structure of the HTCC was also confirmed comparing the structure to chitosan by NMR,[32] shown in Figure 1B and C.



Scheme 1. Structure of chitin, chitosan, and HTCC.

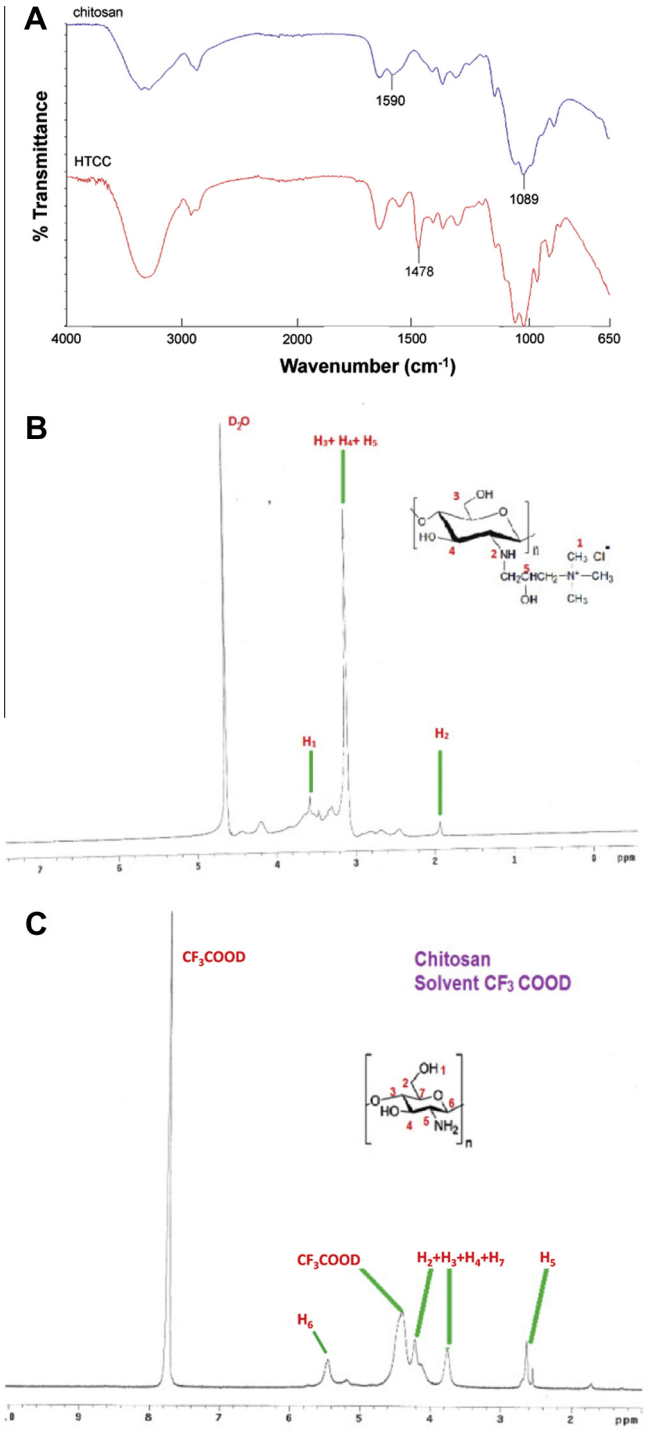


Figure 1. Characterization of HTCC. (A) FTIR of chitosan and HTCC. (B) ^1H NMR of HTCC in HCl/D_2O. (C) ^1H NMR of chitosan in CF_3COOD.

2.2. Electrospinning of HTCC blends

Chitosan is easy to electrospin, but HTCC is more difficult to find the best conditions to form a continuous nanofiber jet during electrospinning. HTCC has been electrospun as a 1:1 blend of HTCC and polyvinyl alcohol (PVA), but pure HTCC could not be electrospun.[28] We explored a wide range of additives, with select properties shown in Table 1, that have been shown to improve the ability to electrospin biopolymers, including PEO,[20] PVA,[28] and SDS.[33] We also added graphene, due to its unique properties of high