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Adsorption of a non-enveloped mammalian virus to functionalized nanofibers

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ABSTRACT

In the pursuit of finding superior methods to remove pathogens from drinking water, this study examines the adsorption of a non-enveloped, mammalian virus to highly charged nanofibers. N-[(2-Hydroxyl-3-trimethylammonium) propyl] chitosan (HTCC) nanofibers were synthesized by the addition of a quaternary amine to chitosan. HTCC was blended with polyvinyl alcohol (PVA) to produce nanofibers by electrospinning. The nanofibers were stabilized against water by crosslinking with glutaraldehyde. When studied in the range of 100–200 nm in diameter, larger fibers were able to adsorb about 90% more virus than smaller fibers. The kinetics of the adsorption was modeled with pseudo-first order kinetics and equilibrium was achieved in as little as 10 min. Equilibrium adsorption was modeled with the Freundlich isotherm with a Freundlich constant of 1.4. When the Freundlich constant deviates from 1, this demonstrates that there is heterogeneity at the adsorption surface. The heterogeneity likely occurs at the nanofiber surface since a polymeric blend of two polymers was used to electrospin the nanofibers. The model mammalian virus, porcine parvovirus (PPV), has a fairly homogeneous, icosahedral protein capsid available for adsorption. The fast adsorption kinetics and high capacity of the nanofibers make HTCC/PVA a potential filter material for the removal of pathogens from drinking water.

1. Introduction

Diarrheal diseases caused by pathogens in drinking water lead to the death of over a million people each year [1]. These pathogens are typically bacteria or viruses. Bacterial pathogens can be removed by disinfection with UV light [2] or removal by ultrafiltration [3]. However, viral pathogens are more difficult to remove. The small size of many viruses makes nanofiltration a requirement for sized-based removal [4] and leads to high transmembrane pressures. Viruses can be removed by chemical treatment, the most common is chlorine. However, the interaction of chlorine with natural organic matter (NOM) is suspected to create carcinogenic byproducts [5]. Chlorination also requires a coordinated supply system, which is often lacking in underdeveloped countries. Due to the many limitations of providing clean drinking water in underdeveloped countries, we are interested in engineering methods of virus removal to purify drinking water that do not rely on chemical disinfection or size-based removal.

Adsorption of virus to a solid surface is another option to purify drinking water. An electrochemical carbon nanotube filter has been shown to both adsorb and inactivate MS2 bacteriophage particles [6]. Although the power requirements for this filter are low, many places in underdeveloped countries do not have adequate access to electricity to continually run a filter of this type. The adsorption of viruses to alumina [7], iron [8], and clays [9] has shown that many different surfaces can adsorb viruses and there is a complex relationship between electrostatic and hydrophobic interactions. The hydrophobic interaction of super-powdered activated carbon (S-PAC) and MS2 bacteriophage achieved a 4 log reduction value (LRV), equivalent to 99.99% removal, after contact for 8 h [10]. In general, chemicals with low-solubility are well adsorbed by activated carbon [11]. Up to this point, activated carbon adsorption has not been considered a typical virus removal step. The hydrophobic and polycationic coating of N,N-dodecyl,methyl-polyethylenimine (PEI) was able to quickly and efficiently disinfect aqueous solutions containing the non-enveloped poliovirus and rotavirus [12] and the enveloped influenza virus [13]. A novel ion adsorber was capable of greater than 4 LRV of mouse minute virus (MMV), xenotropic murine leukemia virus (MLV) and simian virus 40 (SV40) [14]. The adsorber contained eight layers of hydrophilic PVDF base membrane, derivatized with a quaternary amine ligand providing an anion exchange surface [14]. Also explored were trimeric peptide ligands (WRW and KYY), which removed all detectable porcine parvovirus (PPV) from solutions by a minimum of 4.5 LRV [15]. The

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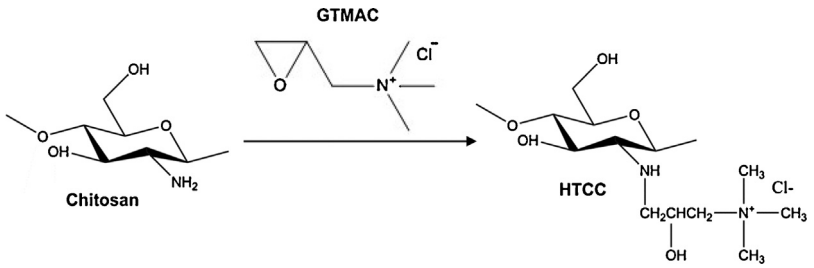

Fig. 1. Synthesis of HTCC from chitosan using GTMAC.

small peptides had two hydrophobic and one positively charged amino acid [15]. However, for negatively charged adsorbers, low virus removal was found. A 0.22 μm pore size, modified PVDF membrane filter with a negatively-charged surface was reported to remove less than 0.5 LRV MS2 bacteriophage [3]. One negatively-charged commercial GS9034 microfilter was reported to remove only 1 LRV of MS2 bacteriophage from water [16]. The major disadvantages of ion absorbers are low pathogen capacity and long contact times. To increase the adsorption capacity and decrease the needed contact time, we are exploring high surface area nanofibers with a high charge density.

Highly charged chitosan derivates that can be electrospun into nanofibers have been our material of choice for virus removal. Chitosan derivatives that contain a high positive charge are well-known for their antimicrobial properties [17–19]. We have shown that the chitosan derivative, N-[(2-Hydroxyl-3-trimethylammonium) propyl] chitosan (HTCC), also has the ability to reduce the infectivity of mammalian viruses [20,21]. In order to create a device to purify water, we have electrospun HTCC into nanofibers. Electrospinning is a common method of creating nanofibers for applications ranging from tissue engineering [22], electronics [23], and membrane materials [24]. Two main attributes of electrospun mats are the ease of fabrication and the high surface to volume ratio, which facilitates adsorption. Additionally, the pore size of the created filters was in the range of several microns. This allows for a high water flux with low transmembrane pressures. Ma et al. demonstrated that increasing the hydrophilicity of an adsorption filter also increased the water flux while maintain high bacteria and virus removal [24].

In the present study, we expanded on our past work that looked at the creation of water-stable, highly charged nanofibers for virus removal [20]. We evaluated the adsorption of the non-enveloped, mammalian virus PPV. PPV is a chemically stable and small virus, with a diameter of 18–26 nm [25]. This makes the virus difficult to remove either chemically or by size, which is why it was chosen as a model virus for removal.

2. Experimental

2.1. Synthesis and characterization of HTCC

HTCC was synthesized as described earlier [20,21], and is shown in Fig. 1. Briefly, chitosan (75–85% deacetylated, MW = 190,000–310,000 Da) and glycidyltrimethylammonium chloride (GTMAC) (≥90%) were mixed in Nanopure water (resistance ≥18 MΩ) at 85 °C for 10 h. The resulting HTCC mixture was dialyzed to remove the excess GTMAC and then filtered to remove the excess chitosan. The final product was purified with cold acetone precipitation and dried. The resulting product was characterized with FTIR in a PerkinElmer FT-IR Spectrum One Spectrometer (Shelton, CT).

The degree of quaternization (DQ) was measured by the titration of chloride using silver nitrate, as has been described previously [21]. The DQ was determined to be 76.4 ± 4.3%.

2.2. HTCC nanofiber formation and characterization

Blends of the synthesized HTCC and purchased polyvinyl alcohol (PVA) (99% hydrolyzed, MW = 89,000–98,000 Da) were created at a 10% (w/w) polymer and a ratio of 4:6 HTCC:PVA in Nanopure water. Nanofibers were created with a homemade electrospinning apparatus [21] consisting of a multi speed syringe pump (Braintree Scientific Inc., Braintree, MA), a Glassman positive DC high voltage power supply (High Bridge, NJ), capable of generating voltages in the range of 0–30 kV, and a rotating drum collector covered with aluminum foil run by an Electro Craft Torque power pump (Gallipolis, OH). The needle was 5 cm from the rotating drum collector, a 20 kV voltage was applied, the syringe pump was run at 4.5 ml/h, and a rotation speed of 1500 rpm was used for the drum collector. The voltage and pump speed were varied to create fibers of different diameters. The fibers were collected on Whatman filter paper that was attached to the collector.

HTCC nanofibers were crosslinked with 30% glutaraldehyde vapor at 37 °C for 4 h, as described earlier [20], to impart water stability to the nanofibers. The nanofibers were imaged with a Hitachi S-4700 cold-field emission scanning electron microscope (FE-SEM) (Tustin, CA) after sputter coating with 5 nm of platinum/palladium (Hummer Sputtering System, Union City, CA). The accelerating voltage for the FE-SEM was 5 kV, and the magnification was from 1000× to 80,000×. To determine the fiber diameter, 50 random fibers from three SEM-micrographs were calculated with Nano Measurer and OriginLab software.

2.3. Virus removal

Virus removal was accomplished with the model porcine parvovirus (PPV) (strain NADL-2) that was propagated and titrated on porcine kidney cells (PK-13), which has been described previously [26,27]. The virus was titrated with the cell viability assay, MTT. The log removal value (LRV) was used to determine the amount of virus that adsorbed to the nanofibers,

$$\mathrm{LRV} = -\log_{10}\left(\frac{c_f}{c_o}\right) \quad (1)$$

where c_f is the infectious virus concentration after adsorption and c_0 is the initial infectious virus concentration, both in MTT_{50}/ml.

For virus adsorption studies, nanofibers on a filter paper support were punched to create a 0.50 cm^2 circle. The nanofibers were incubated with 0.5 ml of virus of different concentrations and different times. For the kinetic studies, the initial virus concentration was 7 $\log_{10}(MTT_{50}/ml)$ in Nanopure water. For the equilibrium studies, the incubation time was 10 min with various starting concentrations diluted in Nanopure water. All studies had end-over-end rotation with a Roto-shake Genie rocker (Scientific Industries Inc., Bohemia, NY). The amount adsorbed to the nanofibers was calculated with Eq. (2):

$$q_i = \frac{(c_o - c_i)V}{M} \quad (2)$$