# Fabrication of flexible self-standing all-cellulose nanofibrous composite membranes for virus removal

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## ARTICLE INFO

## ABSTRACT

All-cellulose nanocomposite membranes with excellent performance were successfully fabricated as novel filtration system to remove nanoparticles and virus from aqueous medium. These membranes were composed of two combined layers: an electrospun cellulose nanofabric layer treated by hot-pressing to provide mechanical support and a coating of regenerated cellulose gel with tiny inter-connected pores as barrier. Hot-pressing did not affect the fiber shape of electrospun nanofabrics, but significantly improved their mechanical properties due to increased hydrogen bonds. The regenerated cellulose gel formed a porous coating that tightly attached to electrospun nanofabrics, and its pore size varied depending on cellulose source, solution concentration, and drying process. By assembling these two layers together, the nanocomposite membranes showed the notable retention of negatively charged 100 nm latex beads (99.30%). Moreover, the electronegative nature of cellulose membranes imparted the rejection ratio of 100% and $(98.68 \pm 0.71)\%$ against positively charged 50 nm latex beads and Hepatitis C Virus, respectively.

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## 1. Introduction

Virus contamination outbreak can often occur in medical or biotechnology products (*e.g.*, vaccine, monoclonal/polyclonal antibody, plasma, immunoglobulin, etc.) (Levings & Wessman, 1990). It presents a serious health hazard and results in large economic losses as well as erosion of public trust. In order to reduce the risk of virus contamination, it is necessary to purify biotechnology products. On the other hand, some researchers need to enrich virus for specific experiments. However, the size of virus is extremely small, for example, the swine influenza virus has a typical particle size of 80–120 nm in diameter (Elford, Andrewes, & Tang, 1936). It is difficult to separate virus from liquid media by simple filtration. Nano-filtration is a convenient method to isolate small particles like virus (Asper, Hanrieder, Quellmalz, & Mihranyan, 2015; Quellmalz & Mihranyan, 2015; Rautenbach & Gröschl, 1990), and has been used to convert sea water into drinking water by filtering salt (Han, Xu, & Gao, 2013). Nano-filtration removal or enrichment of virus is a promising technology because it is non-destructive and non-interfering (Dishari et al., 2015).

Recently, the thin film nanofibrous composite (TFNC) membrane consisting of an ultrathin selective barrier layer (top layer), an electrospun nanofibrous scaffold (middle layer), and a non-woven fabric support (bottom layer) has become popular in ultrafiltration systems (Kaur, Barhate, Sundarrajan, Matsuura, & Ramakrishna, 2011), because it not only can block nanoscale substances but also has strong mechanical properties and high water flux (Ma et al., 2010a). For example, poly(ethylene terephthalate) (PET) non-woven mats are usually employed as the bottom layer to provide mechanical support, and electrospun poly-acrylonitrile (PAN) or polyvinyl alcohol (PVA) nanofibrous membranes constitute the middle layer (Ma et al., 2010b; Yoon et al., 2006). Many studies have focused on the fabrication of top barrier layer from a series of polymers, such as cellulose regenerated from ionic liquids (Ma et al., 2010a), cellulose nanocrystals (Ma, Burger, Hsiao, & Chu, 2014), chitin membrane (Ma, Hsiao, & Chu, 2011), chemically cross-linked PVA (Ma, Burger, Hsiao, & Chu, 2012; Ma et al., 2010b), polyamide (Yoon, Hsiao, & Chu, 2009), and graphene oxide (Yeh, Wang, Mahajan, Hsiao, & Chu, 2013). To the best of our knowledge, TFNC membranes with only two layers that are completely developed from natural polymers have never been reported.

Electrospinning is a versatile and easy technology to fabricate nonwoven and continuous nanofibers with diameters ranging from 50 nm to 500 nm by applying a high voltage (Kaur, Sundarrajan, Rana, Matsuura, & Ramakrishna, 2012; Liao et al.,

2015; Unnithan, Gnanasekaran, Sathishkumar, Lee, & Kim, 2014). Electrospun nanofibrous membranes have been widely used in filtration due to their highly porous network structure (Filatov, Budyka, & Kirichenko, 2007; Greiner & Wendorff, 2007). Among them, cellulose acetate has been successfully electrospun and then deacetylated to prepare cellulose nanofabrics (Liu & Hsieh, 2002; Rodríguez, Renneckar, & Gatenholm, 2011; Rodríguez, Sundberg, Gatenholm, & Renneckar, 2014; Son, Youk, Lee, & Park, 2004; Zhou et al., 2016). The obtained cellulose membranes exhibited superior filtration performance in aqueous system due to their high porosity and hydrophilicity. Moreover, cellulose is biodegradable and has good chemical resistance. It would not react with majority of components in feed solution. Until now, the most common filter membrane for aqueous system is made from cellulose. However, the mechanical properties of electrospun nanofibrous cellulose membrane were poor that limited their applications in filtration. It is reported that the hot pressed nanofibrous membranes revealed better pressure tolerance and mechanical performance when compared to untreated membranes (Kaur et al., 2011; Wang et al., 2013). Generally, electrospun nanofibers are randomly oriented and not interconnected at joints. Hot press technique compresses the nanofibers together and generates new joints or interactions under high temperature and pressure, thus enhances the structural integrity and mechanical properties of electrospun nanofibrous membranes (Asper et al., 2015; Lalia, Guillen-Burrieza, Arafat, & Hashaikeh, 2013). Nevertheless, the hot pressed electrospun cellulose nanofabrics have never been reported.

Cotton and wood are good sources of cellulose because of the high yield and affordability (Klemm, Heublein, Fink, & Bohn, 2005). Canada is the largest world producer of newsprint and northern bleached softwood kraft pulp, and the main component of paper and pulp is wood-based cellulose (Kuhlberg, 2005). As a good cellulose solvent, the NaOH/urea aqueous solution developed by Zhang's group provides a 'green' and economical way to rapidly dissolve cellulose at low temperature (Cai & Zhang, 2005). Recently, this technology has been successfully adapted for dissolving wood cellulose after partial acidic hydrolysis (Gong, Wang, Tian, Zheng, & Chen, 2014). A series of functional cellulose materials have been fabricated based on this solvent system, such as hydrogels (Zhou, Chang, Zhang, & Zhang, 2007), microporous membranes (Zhou, Zhang, Cai, & Shu, 2002), multifilament fibers (Cai et al., 2007), and microspheres (Luo & Zhang, 2013). Particularly, cellulose solution can form ultrathin gel membrane when being cast on a substrate (Zhou et al., 2002). The regenerated cellulose gel membrane exhibits a dense surface with interconnected nanoscale pores (Wang & Chen, 2011). This unique structure provides the possibility to coat electrospun cellulose nanofabrics with regenerated cellulose gel membrane to act as a selective barrier layer to block virus and other microorganisms. Furthermore, the expected excellent compatibility between two cellulose layers would be beneficial to increase water permeation and separation efficiency (Lau, Ismail, Misdan, & Kassim, 2012). The two-layer all-cellulose nanofibrous composite membranes were fabricated in current study, where the hot pressing treated electrospun cellulose nanofiber provided the mechanical support and the regenerated cellulose gel coating worked as the separation layer. The structure, mechanical properties and filtration performance of composite membranes were investigated, and the retention rate of Hepatitis C Virus (HCV) was tested to evaluate the practical and functional performance.

## 2. Experimental methods

### 2.1. Materials

Spruce cellulose (bleached kraft pulp) with α-cellulose content of 87.3% was provided by Alberta-Pacific Forest Industries Inc. (AB, Canada). It was hydrolyzed by 20 wt% sulfuric acid at 30 °C for 24 h and the viscosity-average molecular weight ($M_\eta$) was $5.8 \times 10^4$ (Gong et al., 2014). Cotton cellulose (cotton linter pulp) with $M_\eta$ of $1.0 \times 10^5$ was supplied by Hubei Chemical Fiber Group, Ltd. (Xiangfan, China). Cellulose acetate (CA, average $M_N$ Ca. 30,000, 39.8 wt% acetyl content) and fluorophore tagged polystyrene latex beads (L9902, 100 nm in diameter, sulfonate-modified; fluorescent red; ex ~ 575 nm; em ~ 610 nm and L0780, 50 nm mean particle size, amine-modified, fluorescent blue, ex ~ 360 nm; em ~ 420 nm) were purchased from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada). Commercial cellulose filter paper (CM) was purchased from Whatman™ (GE Healthcare, Buckinghamshire, UK). Dulbecco's Modified Eagle Medium (DMEM), Non-essential amino acids (NEAA), penicillin and streptomycin were obtained from Life Technologies (Burlington, ON, Canada). Acetic acid and all other chemical reagents were purchased from Fisher Scientific (Markham, ON, Canada) and were used as received unless otherwise described.

### 2.2. Preparation of all-cellulose nanofibrous membranes

#### 2.2.1. Hot pressed electrospun cellulose nanofabrics

Nonwoven cellulose acetate nanofabrics were fabricated by a customized digital electrospinning apparatus EC-DIG (IME Technologies, Eindhoven, Netherlands) at room temperature (22 °C). Briefly, 8 g cellulose acetate was dissolved in 42 mL acetic acid/water (75/25, v/v) solution, and then it was forced through a blunt needle with a diameter of 0.8 mm at the rate of 1 mL h⁻¹. The applied voltage was fixed at 23 kV. A rotating drum with a diameter of 10 cm was chosen as the collector, and the distance between the tip and collector was set as 15 cm. The obtained cellulose acetate mats were subsequently immersed in 0.5 M KOH ethanol solution at room temperature for 1 h to generate cellulose nanofabrics. The cellulose nanofabrics were washed with excess deionized water and cut into square pieces (3 in. × 3 in.). Several square pieces (5, 10 and 15 pieces) were piled up and sandwiched between two plane white PTFE plates. They were pre-heated at 110 °C for 10 min and then hot pressed by a Carver benchtop laboratory press (model 3851, Carver Inc., Wabash, IN) under the pressure of about 7.66 MPa (10,000 pounds/3 in. × 3 in.) at 110 °C for 50 min. The resultant cellulose nanofabrics were coded as L5, L10 and L15, corresponding to the different number of pieces used to pile up the nanofabrics. The cellulose sample without hot pressing was coded as RC.

#### 2.2.2. Regenerated cellulose gel coating

Cotton cellulose (CC) and hydrolyzed spruce cellulose (SC) solutions with cellulose concentration of 1, 2 and 3 wt% were prepared as described by Cai and Zhang (2005). Briefly, 7 wt% NaOH/12 wt% urea aqueous solution was precooled to −12.6 °C, and desired amount of cellulose was added in the solution with mechanical stirring at 2000 rpm for 3 min. The cellulose solutions were degassed by centrifugation at $805 \times g$ and 4 °C for 5 min. To form the regenerated cellulose gel coating, the hot pressed electrospun cellulose nanofabric L5 was firstly soaked in 75% acetic acid aqueous solution for 1 min, and then its one face was quickly dipped in the above prepared cellulose solutions to obtain the one-side coated all-cellulose nanofibrous composite membrane. The resultant samples were either directly washed using deionized water, or immersed in pure ethanol for 1 h (solvent exchange) and then thoroughly washed. All the membranes were dried at room temperature and pre-immersed in water before the filtration test. The detailed preparation processing parameters of each sample is shown in Table 1.