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# Effect of vapor-phase glutaraldehyde crosslinking on electrospun starch fibers

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ABSTRACT

In this work, we have proven that starch nanofibrous membranes with high tensile strength, water stability and non-cytotoxicity can be produced by electrospinning of starch solution and post-treatment with GTA in vapor phase. GTA vapor phase crosslinking plays a key role in forming water-stable nanofiber membrane and improving the mechanical properties. Comparing with non-crosslinked starch fibers, the crosslinked fibers are increased by nearly 10 times in tensile strength. The crosslinked starch fibrous membranes are non-cytotoxic. They may find applications in the fields of tissue engineering, pharmaceutical therapy and medical.



## 1. Introduction

Starch is an abundant, inexpensive biopolymer widely existing in plant. Apart from as food source, starch has been widely used in pharmaceutical and medical fields (Lingyan & Ziegler, 2014; Zhang & Venugopal, 2006; Zongqiang, Lijun, & Hui, 2014). For example, ARISTA™ AH an absorbable surgical hemostatic powder is a derivative of starch. Starch is chiefly used in the form of powder, film or blend with other materials. Processing starch into fine fibers could open up novel applications especially as tissue engineering scaffolds, wound dressings, and drug release. However, difficulties still remain in making starch fibers using conventional fiber-making techniques. Starch contains three hydroxyl groups in each repeat unit, which are easy to form inter- and intra-molecular hydrogen bonds. Hydrogen bonding between the O3 and O2 of sequential residues allows the starch to form a helical conformation, which is relatively stiff with a hydrophobic surface. The hierarchical structure also leads to the formation of starch granules, reducing chain mobility and therefore increasing the processing difficulty (Moran, Vazquez, & Cyras, 2013).

Electrospinning is a simple, versatile, and scalable technique to produce fine polymeric fibers. Electrospun fibers possess a large surface to mass ratio and small pore size. They have shown enormous potential for applications in the fields of filtration, tissue scaffolds and wound dressing. Recently, starch nanofiber membranes have been prepared by electrospinning (Huang, Zhang, Kotaki, & Ramakrishna, 2003; Reneker et al., 2002). However, the electrospun starch reported has issues with water solubility and low mechanical strength.

Crosslinking is an effective technique to improve the stability and mechanical properties of polymers. Crosslinking agents, such as formaldehyde, sodium trimetaphosphate (Carmona-Garcia et al., 2009; Mao, Wang, Meng, Zhang, & Zheng, 2006), epichlorohydrin (Kittipongpatana & Kittipongpatana, 2013), and phosphorus oxychloride (Kim, Hwang, & Byung-Yong, 2012) have already been used to crosslink starch granules, mostly through blending the crosslinking agent with starch in an aqueous solution (Cao, Chen, Chang, Muir, & Falk, 2008; Phattaraporn, Waranyou, & Thawien, 2011). However, these methods are unsuitable for crosslinking electrospun starch fibers because the addition of the crosslinking agent into electrospinning solution could affect electrospinning process and fiber morphology.

Glutaraldehyde (GTA) has been used to crosslink hydroxyl-containing polymers (e.g. PVA) and gelatin through a vapor phase

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crosslinking reaction with high efficiency, short reaction time and low cost (Ramires & Milella, 2002; Zhang, Venugopal, Huang, Lim, & Ramakrishna, 2006). In comparison with other crosslinking agents, GTA has lower cytotoxicity and the crosslinked materials are biocompatible and non-thrombogenic, and have good mechanical properties. However, vapor phase GTA crosslinking of starch nanofibers has not been reported in research literature.

Here, we prove that water resistant starch nanofiber membranes with enhanced thermal and mechanical properties can be prepared by electrospinning and by subsequently crosslinking the as-spun starch nanofibers in GTA vapor. The effect of GTA crosslinking on the structure, thermal and mechanical properties, and hydrophilicity of starch nanofiber membranes was examined. Our study has indicated that GPA is an effective crosslinking agent that can improve the thermal and mechanical properties of starch membranes without altering the fiber morphology. We further studied the cytotoxicity of the nanofiber membranes using *Escherichia coli* (*E. coli*) as a model (Azami, Rabiee, & Moztarzadeh, 2010; Bigi, Cojazzi, Panzavolta, Rubini, & Roveri, 2001; Scotchford, Cascone, Downers, & Giusti, 1998). The starch fibers with improved water resistance and mechanical properties may find applications in biological field.

## 2. Experimental

### 2.1. Materials

Starch HYLON VII (an unmodified high amylase corn starch containing approximately 70% amylose) was purchased from Ingredion Incorporated, USA. Dimethyl sulfoxide (DMSO) from Tianjin Kemiou Chemical Reagent and aqueous glutaraldehyde (50%) from Sigma were used as received. The starch-DMSO solution for electrospinning was prepared by dissolving starch HYLON VII in DMSO, and then stirred at 70 °C for 20 min.

### 2.2. Electrospinning

Electrospinning was performed using a purpose-built apparatus consisting of a syringe with a metal needle, a syringe pump, a high voltage power supply, and a metal mash collector. The starch-DMSO solution was placed in the syringe. 20 kV DC voltage was charged to the syringe needle and the metal collector which covered with an aluminum foil was grounded. The tip-to-collector distance was kept at 25 cm, the flow rate of the starch-DMSO solution was controlled at 1 ml/h during electrospinning. After electrospinning, the nanofibrous membrane obtained was dried in vacuum for 24 h to remove solvent residue. The sample was then stored in a dry cabinet at room temperature.

### 2.3. GTA vapor-phase crosslinking

The starch nanofibrous membrane was placed in an airtight desiccator containing 15 ml of 25% aqueous glutaraldehyde solution in a petri dish. The desiccator with the nanofiber samples was kept at 37 °C for a different time periods. After crosslinking, the samples were exposed in a fume hood for one hour to remove extra glutaraldehyde, and then dried in vacuum for 24 h.

### 2.4. Characterizations

Fibrous morphology was observed by a field emission scanning electron microscope (FESEM, Hitachi s4800, Japan). Fourier transform infrared (FTIR) spectroscopy was measured on an attenuated reflectance Fourier transform spectrometer (Nicolet iS 10, USA). Thermal properties were measured by Differential Scanning Calorimeter (DSC 200 F3, German). Dynamic mechanical analysis (DMA) was performed by Netzsch Company (DMA242, Germany) at frequency 1 Hz with a rate of 5 °C/min. The tensile properties of the fibrous membranes were measured by Instron 3369 (USA). The starch fibrous membranes (0.1–0.2 mm thick) were cut into length × width of 40 mm × 10 mm, and the samples were tested at a speed of 5 mm/min. Water contact angle was measured by YH-168A(YHDO) with water droplets of 50 μl in volume.

### 2.5. Antibacterial property

*E. coli* was used for cytotoxicity evaluation of the fibrous membranes. The method was in reference of the literature reports (Chen, Luo, & Sun, 2007; Yang, Wu, Hong, Liang, & Hu, 2012). In brief, nanofibre samples ($1 \times 1\ cm^2$) were placed on agar plate and $10^6$ to $10^7$ CFU/plate of *E. coli* were spread. The samples were sterilized under UV for 3 h and wetted with an additional sterile water (0.1 ml). The agar plates were incubated for 24 h at 37 °C.

## 3. Results

### 3.1. Morphology

During electrospinning, the spinning temperature was controlled at 60 °C to decrease the molecular interaction and increase the flexibility of starch chains. Fig. 1a shows a photo of the as-spun starch fiber membrane. The fiber membrane was soft and flexible. SEM imaging indicated that the starch fibers were continuous in length with an average diameter of 200–700 nm (Fig. 1b and c).

After GTA vapor crosslinking, the fiber membrane turned yellow slightly and shrank dimensionally (Fig. 1d). The starch fibers still maintained the fibrous morphology, expect that the fibers swelled with the diameter changed to 300–700 nm (Fig. 1e and f).

### 3.2. FTIR

FTIR was used to examine the structural change of the starch before and after GTA crosslinking treatment. As shown in Fig. 2a, the raw starch material shows a broad band at 3326 $cm^{-1}$, corresponding to hydroxyl groups, and the band at 1047 $cm^{-1}$ represents the C—O stretching vibration, which comes from the β phase crystalline structure (Bernazzani, Peyyavula, Agarwal, & Tatikonda, 2008). The band 1022 $cm^{-1}$ was assigned to bending of C—O bond (Bernazzani, Chapados, & Delmas, 2001; Flores-Morales, Jimenez-Estrada, & Mora-Escobedo, 2012; Van Soest, de Wit, Tournois, & Vliegenthart, 1994a, 1994b).

For the as-spun starch fibrous membranes without crosslinking, the peak at 1047 $cm^{-1}$ decreased considerably, suggesting the loss of some β crystalline content. After crosslinking treatment, the starch fibers showed changes in FTIR spectrum (Fig. 2a). The O—H variation peak at 3326 $cm^{-1}$ became broad and weaker. A new peak appeared at 1000 $cm^{-1}$ (C—O—C), indicating the success of chemical crosslinking, whereas the crystalline areas at 1047 $cm^{-1}$ had no significant change after crosslinking (Fig. 2a).

Fig. 2b shows the XRD patterns of the starches. For the raw material, XRD pattern reveals the main crystalline features of V-type starch with a $2\theta$ diffraction peak at around 20° and type β crystalline with a diffraction peak at 25°. For the as-spun starch fibers, there were no obvious crystal peaks and the β crystalline disappeared. Vapor-phase crosslinked starch fibers showed no obvious crystal peak either. For comparison, we also crosslinked the starch fibers using a liquid crosslinking method. However, the liquid crosslinked starch fibers show a sharp crystal peak at $2\theta = 23$. This suggests that liquid phase crosslinked was in favor of starch recrystallization. Vapor phase crosslinking allowed the starch to restrain