Antibacterial, efficient and sustainable CS/PVA/GA electrospun nanofiber membrane for air filtration

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Abstract

Since the outbreak of the COVID-19 epidemic, a large number of disposable protective masks have been manufactured and used, and the abandonment of masks has caused enormous pollution. In this paper the chitosan (CS), polyvinyl alcohol (PVA), and water were used as raw materials and the nanofiber membranes were prepared by electrostatic spinning. The CS/PVA fiber membranes were crosslinked by glutaraldehyde hydrochloric acid vapour. The fiber morphology, hydrolysis resistance, antibacterial properties, chemical structure, thermal stability and filtration performance of nanofiber membranes were characterized. Results shows that the antibacterial performance of the crosslinked composite nanofibers exceeds 97%, the thermal stability is improved, and the fiber morphology is not destroyed. The hybridized fiber membrane has high filtration performance, excellent antibacterial and hydrolysis resistance, which broadening the PVA fiber membrane application. It is expected to replace traditional protective materials and relieve environmental pressure.

1. Introduction

With the outbreak of the COVID-19 epidemic in 2019, as an important strategic material for protecting care workers and preventing the spread of the epidemic, the global demand for masks is 129 billion per month, of which 8% of the masks have not been appropriately handled and discarded in the sea, which causing the spread of disease and putting great pressure on the environment [1, 2]. The fiber membrane fabricated by electrospinning has the characters of a large specific surface area, high porosity, and small pore size. Therefore, they could be used for air filtration [3–7]. Since the organic solvents in the preparation process will cause damage to the environment, the organic solvents should be replaced with environmentally friendly solvents such as water [8]. The biodegradable nanofiber membrane manufactured by electrostatic spinning technology could be used as filter material, which can effectively alleviate the environmental pressure due to dispose of protective materials.

As one of the most abundant natural polysaccharides, chitosan (CS) contains a reactive amino group [9], which was widely used in tissue engineering and wound healing. It has excellent properties such as antibacterial, hemostasis, biodegradability, and biocompatibility etc. However, chitosan is difficulty used to manufacture nanofiber membranes by electrospinning due to its properties of polycations, rigid chemical structure, strong intermolecular force, and limited solubility in most organic solvents etc [10, 11].

The CS could be blended with a easily electrospinnable polymer, such as polyvinyl alcohol (PVA), polyethylene oxide, gelatin, silk fibroin [12] to overcome the spinning difficulties. As a kind of polymer with good biocompatibility, non-toxic, soluble in water, biodegradable and highly similar to human tissue, PVA has been widely used in the biomedical field due to its good spinnability. Nathan et al [13] prepared the fiber pad containing lysozyme with the blend of CS/EDTA acid and polyvinyl alcohol (PVA) solution by applying electrospinning technology for wound healing. Results showed that the fiber membrane had good antibacterial
properties. Yan et al [14] fabricated the PVA/CS hybrid nanofibers by electrospinning and applied them in biomedical-related fields. PVA is a water-soluble polymer that could be spun directly with water as a solvent. It is friendly to personnel and the environment. However, its hydrophilicity limits its application in protective equipment [15]. The chemical crosslinking method can improve its stability in aqueous media. There are many types of crosslinking agents for PVA, such as glutaraldehyde (GA), Glyoxal, sulfosuccinate and hexamethylene disocyanate [16]. Chen et al [17] modified MCS/PVA nanofibers with disulfides, and the fibers have good water stability. Soyekowo et al [18] prepared an electrically neutral nonfiber compositied membrane, whose selective layer is composed of a crosslinked polyvinyl alcohol-polyethylenemine polymer network integrated with zirconiums and is deposited on the intermediate membrane of polydopamine. It has an adjustable surface charge and could be used to purify textile waste water containing salt. Crosslinking can improve the application range of PVA, contribute the nanofiber membrane to insoluble in the solvent, and increase the mechanical properties of the fiber membrane. Glutaraldehyde (GA) is a highly active and effective crosslinking agent with anticorrosive properties [19]. It is widely used in CS and PVA crosslinking agents [20]. Since the non-specific binding ability of GA to biomolecules, it has aroused great interest as a PVA crosslinking agent. In addition, it has been confirmed that the film without residual GA can be produced by crosslinking PVA with GA vapor. The steam crosslinked PVA of GA does not need further detoxification, which can effectively delay the dissolution of physiological fluid and broaden the application range of PVA [21].

In this paper, the CS/PVA nanofiber membrane was prepared by electrospinning. The nanofiber membrane was chemically crosslinked with glutaraldehyde hydrochloric acid vapor to improve the hydrolysis resistance. The CS/PVA/GA composite nanofiber membrane has 98% antibacterial effect on E. coli and staphylococcus aureus, and the filtration efficiency to particulate matter is over 95%. The CS/PVA/GA composite fiber membrane is a green, biodegradable, breathable and stable filtration material, effectively reducing secondary pollution to the environment. Meanwhile, it was found that PVA had antibacterial behavior after crosslinking with GA, and the aldehyde group of glutaraldehyde could inhibit and kill bacteria. The specific technological process and crosslinking mechanism are shown in figure 1.

2. Experiment

2.1. Materials

Polyvinyl alcohol (PVA, 98% alcoholysis degree) and Glutaraldehyde (50% solution) were supplied by Shanghai McLean Biochemical Technology Co., Ltd. Chitosan (CS, degree of deacetylation ≥95%) was supplied by Shanghai Aladdin Biochemical Technology Co., Ltd. Hydrochloric acid (HCl, 37% reagent grade) was supplied by Tianjin Fuyu Fine Chemical Co., Ltd. All received chemicals were used without further purification.

2.2. Fabrication of membranes

2.2.1. Preparation of spinning solution

The PVA precursor spinning solution with 10% mass fraction was prepared by adding 10 g PVA into 90 g of ultra-pure water and stirred at 80 °C water bath for 6 h. Different weights (0 g, 1 g, 2 g) of CS was added into the PVA solution and stirred at constant temperature until dissolved. The spinning solution with mass fraction of 0 wt%, 1 wt% and 2 wt% was prepared.

2.2.2. Preparation of nanofiber membrane

The CS/PVA nanofiber membrane was fabricated by electrospinning. The CS/PVA spinning solution was injected into the 20 ml syringe and driven by the injection pump at the flowing rate of 1 ml h⁻¹. The voltage DC power was 20 kV. The 30 g m⁻² melt-blown polypropylene (PP) nonwoven was wrapped on a collecting roller rotating at the speed of 100 rpm, which was used as the receiving base cloth to collect the fiber film. The distance between the tip to the drum receiver is 12 cm. The needle (No. 22) was fixed on a sliding table, moving back and forth with a speed of 20 cm min⁻¹. The 3 g m⁻² CS/PVA nanofiber membrane was prepared at 30 ± 3 °C and 40 ± 5% relative humidity. The fiber membrane was placed in a blast drying box and treated at 35 °C for 12 h to fully volatilize the solvent.

2.2.3. Cross-linking of nanofiber membrane

The fiber membrane was modified by steam crosslinking with a glutaraldehyde hydrochloric acid mixed solution. The fiber membrane with dimension of 18 cm × 30 cm was fixed on the wall of an airtight container, and the mixed solution of 10 ml HCl, 10 ml GA, and 20 ml distilled water was placed in a petri dish and fumigated at room temperature for 12 h. Fiber membranes A, B, and C were placed in a blast drying box and treated at 35 °C for 12 h to obtain fiber membranes D, E, F, respectively. The processing parameters was shown in table 1.
2.3. Characterization

2.3.1. Morphology
The surface morphology of fiber membrane was observed by a field emission scanning electron microscope (FEI QUANTA 650 FEG). The fiber diameter was measured by Image Pro Plus software.

2.3.2. Hydrolysis property
The hydrolysis resistance of pristine fiber membrane and modification with GA was investigated. The 4 × 4 cm nanofiber membranes were treated with distilled water for 12 h at 30 °C, 60 °C, and 90 °C, respectively. And then, the fiber membranes were dried at 35°C for 12 h. The weight reduction rate $F$ was calculated according to equation (1).
\[ F = \frac{G_1 - G_2}{G_1} \times 100\% \]

Where, \( G_1 \) and \( G_2 \) are the initial weight of the fiber membranes and the weight after the hydrolysis resistance test, respectively.

2.3.3. Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) (TENSOR) was used to determine whether chitosan was successfully added to PVA fiber, and the modification mechanism of glutaraldehyde on CS/PVA fiber was also analyzed.

2.3.4. Thermal properties

The thermal properties of the fiber membrane were tested by the synchronous thermal analyzer (STA 449 F4 Jupiter, Germany). The sample size is approximately 10 mg. The samples were heated at the rate of 20 °C·min\(^{-1}\) from 35 °C to 800 °C under a nitrogen atmosphere. The weight reduction process, residual weight percentage, and heat flux of the fiber film during heat treatment were measured.

2.3.5. Aerosol filtration

The automatic filter material detector (SLGLY-2626) was used to test the filtration efficiency and airflow resistance of fiber membrane to 0.24 ± 0.06 µm sodium chloride (NaCl) aerosol particles at the 85 l min\(^{-1}\) gas flow rate.

2.3.6. Antibacterial activity

Standard T/CIAA003-2020 'Antibacterial Mask' requires that the inhibition rate of Staphylococcus aureus (Gram-positive bacteria) and Escherichia coli (Gram-negative bacteria) should be more than 90%.

(1) Experiment

Blank control was set, PP nonwoven fabric was selected as the control group. The D, E, and F fiber membranes modified with CS/PVA crosslinked were used as the experimental group. After ultraviolet sterilization, the experimental samples were added with appropriate amount of LPBS buffer and bacteria solution. The samples of the blank control group and the control group were treated at 24 °C ± 1 °C and 250–300 r min\(^{-1}\). After 1 min shaking, 10 µl of the solution was cultured on nutrient agar plates and incubated for 24 h at 37 °C ± 1 °C. The original bacterial solution was diluted and transferred to a sterilized plate and incubated at 37 °C for 24 h and the bacterial colonies were counted.

(2) Calculation and evaluation of results

① Verification of test effectiveness

The growth value of test bacteria is characterized with F value, which is calculated according to equation (2). As F value beyond 1.5, and the living bacteria concentration in the control flask is higher than that of inoculation, i.e. the specimen is effective.

\[ F = \lg W_t - \lg W_0 \]

Where, F is the growth value of the test bacteria; \( W_t \) is the average concentration of viable bacteria in the flask after 18 h shock exposure to the control sample (CFU/mL); \( W_0 \) is the average concentration of viable bacteria in the control sample '0' contact time flask (CFU/mL).

② Calculation of bacteriostatic rate

The bacterial inhibition rate was determined by comparing the concentration of living bacteria in the flasks of control and antimicrobial samples after 18 h of oscillatory contact, as follows.

\[ Y = \frac{W_t - Q_t}{W_t} \times 100\% \]

Where, Y is bacteriostatic rate, \( W_t \) is the average concentration of viable bacteria in the flask after 18 h shock exposure to the control sample (CFU/mL); \( Q_t \) is the average value of the concentration of live bacteria in the flask after 18 h shaking contact to the antimicrobial sample(CFU/ml).
3. Results and discussion

3.1. Microstructure analysis
The microstructure of the fiber membrane after CS doping and GA crosslinking was observed by field emission scanning electron microscope. Figure 2 shows that the diameter of the fiber decreased from 306 nm to 268 nm with CS addition to the spinning solution. Since CS has the amino groups in the molecular chain, which could be ionized under acid or neutral conditions [22]. The electrical conductivity of the spinning solution could be improved due to CS addition [23]. With the increase of charge carried by the jet, a higher drawing force is applied to the jet. It is well known that the total tension in the fiber depends on the self-repulsion of the excess charge on the jet [24]. Therefore, the diameter of the nanofibers becomes finer with the increase of charge density. After GA crosslinking, the average diameter of composite nanofibers increased, and the fiber morphology was also changed into flat fibers.

This is attributed to the increase effective molecular entanglement and molecular weight due to GA addition, and the PVA/CS composite nanofibers swelled slightly during the crosslinking process. The addition of chitosan to PVA and crosslinking reaction with GA will affect the morphology of PVA fiber. The morphology of CS/ PVA/GA-1 fiber is better uniform, so the optimum mass fraction of CS is 1 wt%.

3.2. Hydrolysis resistance
The hydrolysis resistance of fiber membrane was tested at different temperatures, as shown in figure 3. The results show that the hydrolysis loss rate of the fiber membrane increased with the increase of temperature, and
the uncrosslinked fiber membrane was completely hydrolyzed when the temperature reached 90 °C. With the addition of CS, the hydrolysis loss rate of CS/PVA fiber membrane has been increased, which is attributed to the increase of hydrophilic groups. The higher the temperature, the more active the hydrophilic groups [25], and the greater hydrolysis loss rate of fiber membrane. The hydrolysis resistance of the fiber membrane has been improved after GA crosslinking. The hydrolysis loss rate of 1 wt% CS/PVA/GA fiber membrane was maintained at about 10%, which is the lowest at 90 °C, and the fiber has the best anti-hydrolysis effect. After GA crosslinking, the hydrolysis loss rate of fiber membrane decrease by 87.6% at 90 °C in comparison to the pristine one. This is due to the aldehyde group reacting with the hydroxyl group to form the acetal ring and ether bond [26], and the hydrophilic group on the polymer molecular chain is reduced, which makes the crosslinked fiber membrane has the anti-hydrolysis behavior. The results are consistent with the interaction mechanism proposed by FTIR analysis [27]. This technology could be applied to protective materials such as masks.

3.3. Antibacterial analysis
The antibacterial behavior of the nanofiber membrane has been improved with CS addition, as shown in figures 4 and 5. Result shows that the inhibition rate of nanofiber membrane to the staphylococcus aureus and the Escherichia coli are more than 98% and 97% with CS addition, which is higher than the relevant standards of protective material masks [28]. The bacteriostatic rates of Staphylococcus aureus and Escherichia coli are shown in tables 2 and 3, respectively. Morphology of CS/PVA/GA-1 fiber is better than that of CS/PVA/GA-2, which results in the uneven distribution of CS in PVA fiber. Thus, the nanofiber membrane with 1 wt% CS has the best antibacterial effect on glucose aureus and Escherichia coli, and the optimum amount of CS is selected for study. The PVA fiber membrane prepared by electrospinning has good fiber continuity. Chitosan is uniformly dispersed on the fiber, adsorbing the bacterial cell wall by electrostatic action, which results in variation of the structure of the bacterial cell wall and thus kills bacteria [29]. Since the aldehyde group in GA has a bactericidal effect on bacteria, the unreacted aldehyde group after crosslinking has an inhibitory effect on bacteria, and PVA/GA fiber membrane also has a particular antibacterial impact.

3.4. FTIR analysis
The variation in functional groups of PVA nanofiber films doped with CS and crosslinked with GA were studied by FTIR spectroscopy. As shown in figure 6, for the CS/PVA blend, the absorption band from 3600–3200 cm⁻¹ is assigned to –OH and –NH₂ stretching vibrations. Characteristic peaks of PVA observed at 2918 cm⁻¹ and 2852 cm⁻¹, 1633 cm⁻¹, 1426 cm⁻¹, 1090 cm⁻¹, 840 cm⁻¹ are attributed to C–H, C=C, –OH, C–O and C–C stretching vibrations, respectively [29, 30]. The absorption peak at 1729 cm⁻¹ represents the C=O bond, which has characteristic peak of GA. The absorption peaks at 1657 cm⁻¹, 1580 cm⁻¹ and 1334 cm⁻¹ have characteristic peaks of CS and are attributed to amide 1, II and III, respectively. Moreover, the glycoside group of CS appeared at 1135 cm⁻¹. Compared with the PVA nanofibers, the absorbance peak intensity at 3200–3600 cm⁻¹ of crosslinked PVA/GA composite nanofibers decreases with the addition of GA. This is attributed to the reduction of hydroxyl during crosslinking reaction. After CS/PVA fiber membrane was crosslinked with GA, the peak at 1580 cm⁻¹ corresponding to NH₂ was almost eliminated, which is attributed to the consumption of amino groups in CS due to GA crosslinking. According to [31, 32], the crosslinking mechanism for GA is
considered to form covalent bond between PVA, CS, and GA since the hydroxyl and amino groups react with aldehyde groups through hemiacetal and acetal reaction. There is a strong absorption peak at 1018 cm\(^{-1}\) because the aldehyde group reacts with the hydroxyl group in the PVA molecular chain, the hydrophilic and hydroxyl group decrease, and the C–O–C bond is formed during the crosslinking reaction. The variation of absorption peak indicated that the CS/PVA fiber membrane was successfully crosslinked with GA.

3.5. Thermal behavior
Thermal stability of fiber membrane is shown in figure 7. All samples exhibit three distinct stages from 30 to 800 °C. The first stage occurs below 200 °C, which is attributed to the evaporation of water associated with the polymers. After adding CS, a hydrogen bond is formed between CS and PVA. The PVA nanofiber membrane has the same trend as the CS/PVA nanofiber membrane. The uncrosslinked fiber membrane degrades rapidly at 230 °C ~ 340 °C due to the cleavage of the main molecular chains of PVA and CS, and the thermal decomposition of hydroxyl and amino polar groups. The GA crosslinking improves the thermal stability of fiber membrane, and rapid weight loss occurs when the temperature reaches 320 °C. This is attributed to the crosslinking reaction between the hydroxyl group of PVA and glutaraldehyde, forming a more stable acetal ring and ether bond [33, 34]. The results are consistent with the interaction mechanism obtained from FTIR analysis. When the temperature reaches 500 °C, the TG curve flattens, which indicates that the composite nanofibers have been completely burned.
3.6. Filtration performance

Figure 8 shows the filtration performance of the CS/PVA/GA fiber membrane. It is observed that the filtration efficiency of the fiber membrane is always more than 95%, and the filtration resistance is less than 343.2 Pa with the increase of cyclic loading of particles. The result meets the application requirements of the mask [35]. The

![Figure 5. The number of Escherichia coli after reacted for 18 hours and diluted 100 times (e) control group; (f) PVA/GA; (g) CS/PVA/GA-1; (h) CS/PVA/GA-2.](image)

**Table 2. Bacteriostatic rate of Staphylococcus aureus.**

| Experimental group | PVA/GA | CS/PVA/GA-1 | CS/PVA/GA-2 |
|--------------------|--------|-------------|-------------|
| Bacteriostatic rate(%) | 84.20  | 99.26  | 98.95 |

**Table 3. Inhibition rate of Escherichia coli.**

| Experimental group | PVA/GA | CS/PVA/GA-1 | CS/PVA/GA-2 |
|--------------------|--------|-------------|-------------|
| Bacteriostatic rate(%) | 79.68  | 99.90  | 97.00 |
diameter of the PVA fiber membrane became smaller, the density increased, the interfiber pore decreased, and the filtration resistance increased from 240 Pa to 258 Pa with the addition of CS. After crosslinking the PVA fiber membrane, the filtration resistance increased from 240 Pa to 290Pa. The filtration resistance of the CS/PVA/GA fiber membrane is 10.3% higher than that of the CS/PVA crosslinked fiber membrane since the fiber swelling after crosslinking to the fiber membrane [36], and the filtration resistance of the fiber membrane also increases.

Figure 6. FTIR spectrum of electrospun fiber membrane.

Figure 7. TG spectrum of Nanofiber membrane.

Figure 8. Filtration performance of fiber membrane.
4. Conclusion

In this paper, CS/PVA nanofiber membranes were prepared by electrostatic spinning by selecting water as the solvent, which reduced the secondary pollution to the environment during the preparation process. The fiber membranes were modified with glutaraldehyde hydrochloric acid vapor to improve the hydrolysis resistance of the fiber membranes. The fiber membrane properties were characterized and the following conclusions were obtained.

1. The PVA nanofibers doped with 1 wt% CS has the best performance. The CS/PVA/GA-1 composite fiber membrane inhibited *Staphylococcus aureus* and *Escherichia coli* by more than 97%. The fibers have free aldehyde groups after GA crosslinking, which also have good antibacterial properties.

2. The hydrolysis loss rate of crosslinked fiber membrane is maintained at about 18% at 90 °C. The thermal stability is improved and the fiber morphology is not destroyed, which broadening the PVA fiber membrane application.

3. The circulating filtration efficiency of the CS/PVA/GA fiber membrane is more than 95%, and the filtration resistance does not exceed 343.2 Pa, which meets the requirements of the filter material used in the mask.

4. The use of GA/HCl vapor for chemical crosslinking provides an idea to solve the instability of PVA electrospun fibers in an aqueous environment. The CS/PVA/GA composite nanofiber membrane has good hydrolysis resistance while maintaining good antibacterial properties and filtration efficiency. As a green and environmentally friendly material, it will be used to disposable protective products, e.g. masks.

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Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

Conflict of interest

We declare that we have no conflict of interest.

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