

# **Preparation, post-modification and antibacterial application of gelatin electrospinning membranes**

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# **Preparation, post-modification and antibacterial application of gelatin electrospinning membranes**

**Abstract :** Two bis(diaryldiazomethane)s substituted with amino groups were synthesized and used for the surface modification of membranes electrospun from gelatin. These membranes were then reacted with tolylene-2,4-diisocyanate to give urea-functionalized materials, so that hydrogen peroxide could be reversibly bound onto their surface. These membranes were characterized by SEM, XPS, DSC, and tensile test to show their surface properties and bulk properties. The surface modification with amino-substituted diazomethanes and the subsequent cross-linking reaction with diisocyanates contributed to high loadings of hydrogen peroxide, and greatly increased the antibacterial activity of gelatin-derived membranes, which opened a new horizon in the preparation of high loading antiseptic/antibacterial biomacromolecular surfaces and interfaces.

**Key words:** Carbene insertion; Surface modification; Cross-linking; Antibacterial activity

## 1 Introduction

The gelatin that derives from the skin, bone, and sarolemma of animals is widely used in hemostatic agents and tissue adhesives, since it has the important advantage of renewability and biodegradability, and leads to only low levels of clinical inflammation<sup>[1, 2]</sup>. However, its applications are limited in many areas due to its poor antibacterial activity and mechanical properties. Chemical grafting or cross-linking of gelatin is an effective way to introduce stable covalent bonds between protein segments and improve their physical, chemical, and biologic properties<sup>[3, 4]</sup>. A great deal of research has created diverse antiseptic/antibacterial materials and surfaces by incorporating biostats/biocides into gelatins, such as nanosilvers<sup>[5, 6]</sup>, zinc oxides<sup>[7, 8]</sup>, tetralkyl ammoniums<sup>[9, 10]</sup>, and chitins/chitosans<sup>[11, 12]</sup>. In parallel with this work, a highly effective method to build biocidal polymers was reported by Sun<sup>[13-15]</sup> and Worley<sup>[16-18]</sup>, who have fully developed the applications of immobilized N-halamine for water purification<sup>[19]</sup> and antiseptic fabrics<sup>[20]</sup>.

Except for those methods, the materials that released hydrogen peroxide would also generate reactive oxygen species and have an effective bactericidal effect, which might represent an alternative solution to prepare antiseptic/antibacterial surfaces. Therefore, we reported a type of porous polystyrene beads<sup>[21, 22]</sup> that show antibacterial activity after the incorporation of hydrogen peroxide. Hydrogen peroxide was immobilized by its coordination with the urea groups, and a stabilized form, urea hydrogen peroxide (UHP), was formed<sup>[23, 24]</sup>. While this was a successful protocol to achieve antibacterial materials, the porous polymer beads were inappropriate for biological application.

In this work, a gelatin membrane, prepared by electrospinning in order to generate a porous structure, was surface modified with two bis(diaryldiazomethane)s. This modified gelatin membrane was a further example of the carbene modification of natural polymers, which had already been applied for the modification of colour<sup>[25]</sup>, hydrophobicity<sup>[26]</sup>, biocompatibility<sup>[27]</sup>, protein binding and adsorption<sup>[28]</sup>, and payload delivery<sup>[29, 30]</sup> effects on various surfaces. The subsequent cross-linking reaction between the end-group amines and diisocyanates gave a structurally robust surface, which was expected to bind hydrogen peroxide and largely endowed the antibacterial activity.

## **2 Experimental Section**

### **2.1 Materials and characterization**

Gelatin was purchased from Aladdin Co. with the grade of biochemical reagent. The other chemicals (A.R.) were purchased from Sinopharm Chemical Reagent Co., Ltd. All the reagents were used as received. Three bacterial strains, E.coli, L.monocytogenes and S.aureus, were purchased from J&K Scientific Ltd.

SEM photographs were taken on a Quanta-200 ESEM (FEI Co., Netherlands) after the samples were coated with gold (~20 nm thickness) with a Sputter Coater SCD-005 (TEC Co., UK).

XPS analysis was performed with a VG – Escalab X-ray Photo-electron Spectrometer VGX900. Casa XPS software was used to fit the spectra and the reference of C 1s was calibrated as 284.6 eV.

Static contact angle was measured using a DSA-100 contact angle instrument

manufactured by Krüss Co. with a 1  $\mu$ L water drop size. Three films of each type were analyzed, and each sample was measured in at least three areas.

The thermal property of the gelatin membranes were measured using a Perkin Elmer Pyris Diamond differential scanning calorimeter (DSC) at a heating rate of 10  $^{\circ}\text{C min}^{-1}$  under nitrogen atmosphere.

Tensile testing was performed on a Testometric AX Universal Strength Testing Machine at room temperature. “Dog bone” test strips were stamped from the transparent sample using gauge dimensions of 50 mm $\times$ 5 mm. The thickness of 0.6~0.8 mm was measured for each sample with SFJ digital thickness tester. Each sample was measured three times to take the average.

## 2.2 Synthesis of bis(aryldiazomethane)s

The synthesis of compounds **2a**, **3a**, **4a**, and **5a** were reported before<sup>[22]</sup>. Other compounds were synthesized as below.

4-Phenoxy-*N*-(1H,1H,2H,2H-perfluorooctyl)aniline (1b).

1H,1H,2H,2H-perfluorooctyl iodide (4.74 g, 10.0 mmol) and 4-phenoxyaniline (7.40 g, 40.0 mmol) was added into a 100 mL flask. The mixture was heated to 140 $^{\circ}\text{C}$  and stirred for 12 h. The crude product was purified by chromatography on a silica gel column eluting with petrol ether / ethyl acetate (1:10) to give the product **1b** as a yellow liquid.(48%).  $\delta\text{H}$  (400.3 MHz,  $\text{CDCl}_3$ ): 2.22(t, 2H), 3.37(m, 3H), 6.84-7.41(m, 9H),  $\delta\text{C}$  (100.1 MHz,  $\text{CDCl}_3$ ): 30.44, 36.39, 114.01, 117.30, 121.37, 122.20, 129.60, 143.44, 148.47, 158.90. HRMS:  $m/z$  calcd for  $\text{C}_{20}\text{H}_{15}\text{ONF}_{13}$ : 532.09406; found: 532.09409  $[\text{M}+\text{H}]^+$ .

2,2,2-Trifluoro-*N*-(4-phenoxyphenyl)-*N*-(1H,1H,2H,2H-perfluorooctyl)acetamide

(**2b**). **1b** (2.00 g, 3.76 mmol) and pyridine (0.89 mL, 11.3 mmol) was dissolved in dichloromethane (20 mL) and cooled down to 5°C in an ice/water bath. A solution of trifluoroacetic anhydride (0.79 mL, 5.65 mmol) in dichloromethane (5 mL) was added dropwise to the flask. The mixture was then heated to room temperature and stirred overnight. After that, the mixture was washed with water (50 mL×3), dried with MgSO<sub>4</sub>, and concentrated under vacuum to acquire a light yellow liquid (95%).  $\delta$ H (400.3 MHz, CDCl<sub>3</sub>): 2.48(t, 2H), 4.04(t, 2H), 7.06-7.45(m, 9H).  $\delta$ C (100.1 MHz, CDCl<sub>3</sub>): 28.10, 44.59, 118.72, 119.95, 124.54, 129.51, 130.09, 132.77, 155.72, 158.68. HRMS: *m/z* calcd for C<sub>22</sub>H<sub>14</sub>O<sub>2</sub>NF<sub>16</sub>: 628.07636; found: 628.07649 [M+H]<sup>+</sup>.

*N,N'*-((isophthaloylbis(4,1-phenylene))bis(oxy))bis(4,1-phenylene))bis(2,2,2-trifluoro-*N*-(1H,1H,2H,2H-perfluorooctyl)acetamide (**3b**). Aluminum chloride (0.949 g, 7.06 mmol) and **2b** (1.50 g, 2.82 mmol) was added to dichloromethane (20 mL) and the solution was cooled to 5°C with an ice/water bath. A solution of isophthaloyl chloride (0.287 g, 1.41 mmol) in dichloromethane (5 mL) was added drop wise to the flask. After the addition was finished, the ice/water bath was moved away and the reaction mixture was stirred for 10 h. Then, the mixture was washed with HCl aq. solution (2 M, 50 mL×3), dried with MgSO<sub>4</sub>, and concentrated under vacuum. The crude product was purified by chromatography on a silica gel column eluting with petrol ether / ethyl acetate (5:1) to give the product **3b** as a yellow solid (80%). Mp: 64-68°C;  $\delta$ H (400.3 MHz, CDCl<sub>3</sub>): 2.51(t, 4H), 4.07(t, 4H), 7.14-7.21(m, 18H).  $\delta$ C (100.1 MHz, CDCl<sub>3</sub>): 28.21, 44.69, 118.36, 120.48, 129.93, 132.66, 134.56, 137.97, 156.75,

160.46, 194.41. HRMS:  $m/z$  calcd for  $C_{52}H_{28}O_6N_2F_{32}Na$ : 1407.13286; found: 1407.13369  $[M+Na]^+$ .

4,4'-(((1E,1'E)-1,3-phenylenebis(hydrazonomethylene))bis(4,1-phenylene))bis(oxy))bis(*N*-(1H,1H,2H,2H-perfluorooctyl)aniline (**4b**). A suspension of **3b** (0.50 g, 0.36 mmol) in ethanol (35 mL) was treated with hydrazine monohydrate (0.35 mL, 7.22 mmol). Two drops of acetic acid were added as catalyst. The resulting mixture was heated to a gentle reflux for 45 h. After being cooled to room temperature, the solution was evaporated under vacuum. Dichloromethane (50 mL) was added to the residue, and the resulting solution was washed with NaCl aq. solution (0.9 M, 50 mL×3), dried with  $MgSO_4$ , and concentrated under vacuum to acquire a light yellow solid (90%). The crude product **4b** as a mixture of diastereoisomers was used without further purification. Mp: 110-114°C.  $\delta H$  (400.3 MHz,  $CDCl_3$ ): 2.30(t, 4H), 3.41(t, 4H), 3.55(s, 2H), 6.84-8.41(m, 18H), 7.02(s, 4H).  $\delta C$  (100.1 MHz,  $CDCl_3$ ): 30.67, 36.39, 113.94, 113.99, 117.51, 117.61, 121.82, 127.81, 130.30, 144.01, 147.50, 148.76, 159.43. HRMS:  $m/z$  calcd for  $C_{48}H_{35}O_2N_6F_{26}$ : 1221.24008; found: 1221.23900  $[M+H]^+$ .

4,4'-((1,3-phenylenebis(diazomethylene))bis(4,1-phenylene))bis(oxy))bis(*N*-(1H,1H,2H,2H-perfluorooctyl)aniline (**5b**). **4b** (0.22 g, 0.18 mmol) was dissolved in dichloromethane (10 mL) in a 100 mL flask. A mixture of manganese dioxide (81 mg, 0.94 mmol), sodium sulfate (72 mg, 0.5 mmol), and potassium hydroxide (22 mg, 0.50 mmol) was added to the flask and stirred for 24 h in the dark at room temperature. Then the mixture was filtered through a pad of Celite. The filtrate was

concentrated under vacuum to yield a purple solid (92%) and then stored at sub-ambient temperature to avoid decomposition. Mp: 126°C (Decomposed).  $\delta$ H (400.3 MHz, CDCl<sub>3</sub>): 2.43(t, 4H), 3.59(t, 4H), 3.76(s, 2H), 6.84-8.41(m, 18H),  $\delta$ C (100.1 MHz, CDCl<sub>3</sub>): 31.16, 61.22, 113.49, 117.61, 117.64, 120.60, 126.91, 143.07, 147.69, 156.92.

### 2.3 Preparation of gelatin nanofibrous membranes

Gelatin (8.0 g) was dissolved in a solution (42 g) of acetic acid / water (9:1, v/v) at room temperature to give a light yellow liquid. The solution was added into a syringe and pumped to a self-made electrostatic spinning machine with a flow rate of 5 mL h<sup>-1</sup>. The voltage was 20 kV and the collection distance was 10 cm. After the membrane was obtained, it was stored in a vacuum drier. After that, a solution (0.05 g mL<sup>-1</sup>) of **5a** or **5b** in dichloromethane was dropped onto the surface of the electrospun gelatin membrane. After the solution was evaporated at room temperature in the dark, the gelatin fiber was covered with the diazomethane. The mixture was then irradiated under UV light for 10 min until the color of the membrane changed from pink to orange. The mixture was filtered, the solid was washed by DCM for 5 times, and dried to yield the amino-modified gelatin membrane **6a-b**. Finally, tolylene-2,4-diisocyanate (2.0 mL) and dibutyltin dilaurate (2 drops) were dissolved in DCM (20 mL) and mixed with **6a-b** (0.5 g). The mixture was gently shaken at room temperature for 10 hours and filtered. The solid was washed by DCM for 3 times, and dried to obtain urea-functionalized gelatin membrane **7a-b**.

### 2.4 Loading of the hydrogen peroxide



The required urea-functionalized membrane **7a-b** were immersed in hydrogen peroxide solution (30% in water) for 2 hours. The solid was collected, washed with water (100 mL), acetone (10 mL), and dried at room temperature to obtain hydrogen-loaded gelatin membrane **8a-b**. A titration method with KI- $\text{Na}_2\text{S}_2\text{O}_3$  was used to determine the loadings of hydrogen peroxide. KI aq. solution (10%, m/m, 20 mL) and HOAc (10 mL) were mixed together, to which was added the sample of required **8a-b** (30 mg). 5 minutes later, aqueous starch solution (1%, m/m, 2 mL) was added and left to stand for 2 h. The resulting dark-blue solution was titrated with  $\text{Na}_2\text{S}_2\text{O}_3$  (0.001 M, 5.0-10.0 mL) to a slightly yellow end-point. The gelatin membrane without loading  $\text{H}_2\text{O}_2$  was tested as a reference.

## 2.5 Bioassay

*S.aureus* and *E.coli* were chosen to quantify the antibacterial activity of gelatin membranes. The bacterial solution was diluted to 1:50 with distilled water, to which was added the molten agar (bacterial solution (1:50) / agar = 1 / 100, v/v). The inoculated agar (25 mL) was pipetted from the culture bottle to the empty Petri dishes (90 mm) and these dishes were stored in the fridge.

Using a sterile method, 15 mm-diameter circles were punched in the bacteria-seeded agar plate to produce empty wells. The required membrane (20 mg) and water (300  $\mu\text{L}$ ) were added into the wells. Some molten agar was then added to the wells so that a uniform layer of agar was obtained. The agar plates were incubated for 24 h to encourage bacterial growth. The diameter of the antimicrobial clear zone around each well was measured and recorded. The gelatin membrane without loading  $\text{H}_2\text{O}_2$  was

tested as a reference.

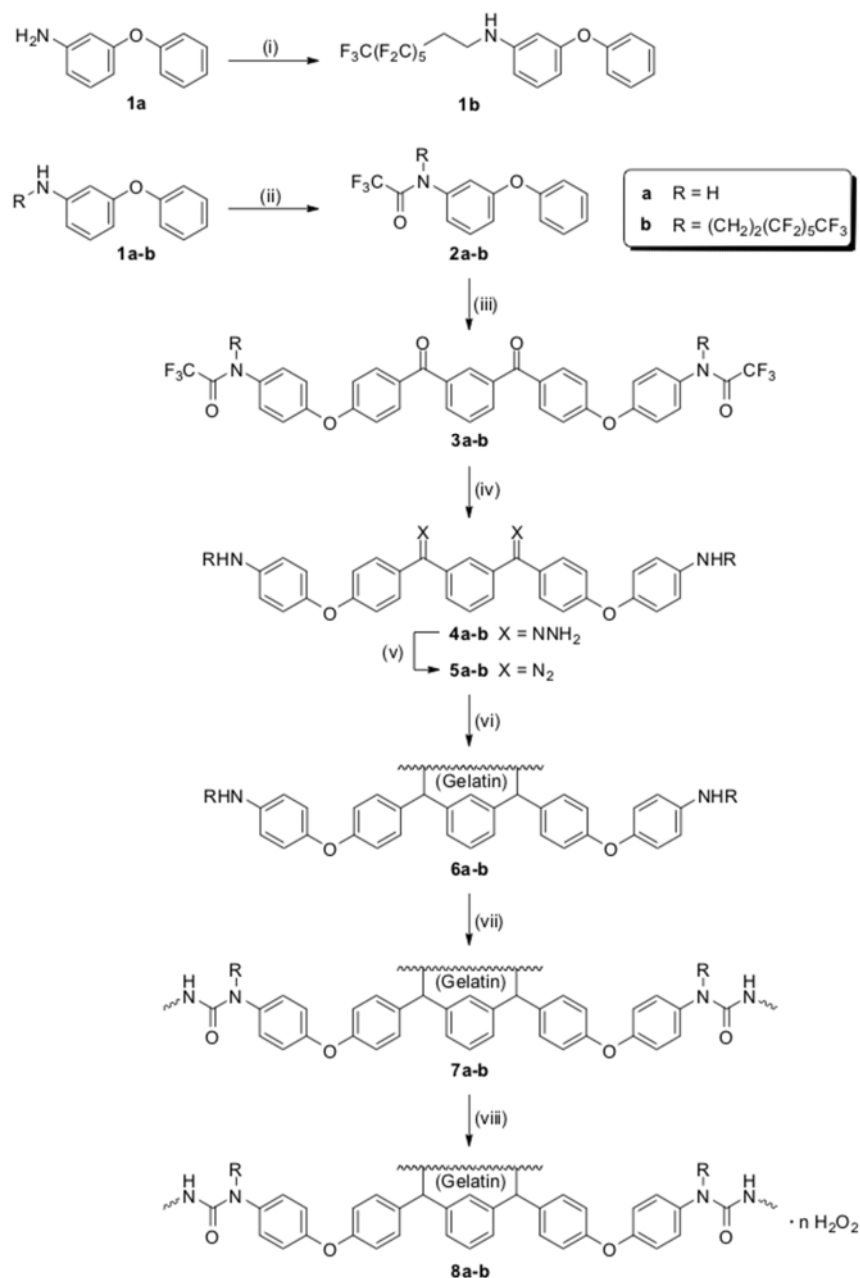
An aq. solution of Penicillin G potassium salt ( $1.0 \text{ mg mL}^{-1}$ ) was used for calibration.

The volume of the solution in each well was 50  $\mu\text{L}$ , 100  $\mu\text{L}$ , 150  $\mu\text{L}$ , 200  $\mu\text{L}$ , 250  $\mu\text{L}$ , and 300  $\mu\text{L}$ . The well was then fulfilled to 300  $\mu\text{L}$  with distilled water. The agar plates were incubated for 24 h to encourage bacterial growth. The diameter of the inhibition zone around each well was measured and recorded.

### 3 Results and discussion

#### 3.1 Synthesis of bis(diazomethane)s

As shown in Scheme 1, 4-phenoxyaniline **1a** was reacted with 1H,1H,2H,2H-perfluorooctyl iodide to give the compound **1b**. The amino group in **1a-b** were protected by trifluoroacetic anhydride (TFAA) to give amides **2a-b**, followed by the subsequent Friedel-Crafts reaction to generate bis(arylketone)s **3a-b**<sup>[31]</sup>. The ketone groups of **3a-b** were reacted with hydrazine monohydrate to form the hydrazones **4a-b**, and their oxidation with  $\text{MnO}_2$  gave the bis(diaryldiazomethane)s **5a-b**. These diazomethanes are suitable for conversion into carbenes under UV light, and the derived active carbene intermediate reacts with most polymers and introduces diverse chemical functionality onto the substrate efficiently<sup>[32]</sup>.



**Scheme 1** Synthesis of precursor and modification of gelatin membrane. Conditions: (i)  $\text{I}(\text{CH}_2)_2\text{C}_6\text{F}_{13}$ , 140°C, 12h, (48%) (ii) TFAA, pyridine, DCM, 5°C-r.t., overnight (95-96%); (iii) isophthaloyl chloride,  $\text{AlCl}_3$ , DCM, 5°C-r.t., 2-10h (80-89%); (iv)  $\text{NH}_2\text{NH}_2$ ,  $\text{H}_2\text{O}$ , HOAc, EtOH, reflux, 40-45h (87-89%); (v)  $\text{MnO}_2$ ,  $\text{Na}_2\text{SO}_4$ , KOH, DCM, r.t., 5-24h (89-92%); (vi) UV light; (vii) tolylene-2,4-diisocyanate, dibutyltin dilaurate, r.t., 10h; (viii) 30%(m/m)  $\text{H}_2\text{O}_2$ , r.t., 2h.

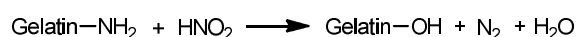
### 3.2 Preparation and modification of gelatin electrospinning membrane

From the known structure of gelatin, there are only a few amino groups on the end of the polymer chains, which can react with isocyanates to bind hydrogen peroxide. The amino-substituted carbenes generated from diazomethanes **5a-b** were used to react with gelatins to increase the amino groups. However, natural polymers like gelatin are very hard to dissolve in organic solvents, and diazomethanes cannot be dissolved in water, so carbenes and gelatins are not able to be mixed homogeneously and reacted thoroughly. In order to activate the heterogeneous reaction, the best way is to expand the surface area of gelatin. Therefore, nanofibers derived from gelatin were prepared via electrospinning, and these were subsequently immersed into the bis(diaryldiazomethane) solution. After the gelatin membrane was collected and dried, the bis(diaryldiazomethane) was physisorbed on the surface of the gelatin. Irradiation of this material under UV light was used to generate carbenes and the insertion reactions of carbene with suitable surface X-H bonds ( $X = C, N$ ) gave the modified gelatin membrane.

### 3.3 Urea-functionalization of modified gelatin membrane

The Van Slyke nitrogen method was used to quantify the primary amino groups of **6a**. As shown in Scheme 2, amino-modified gelatin was reacted with nitrous acid to generate nitrogen gas, and the volume of nitrogen gas was quantified. The amount of  $-NH_2$  could then be calculated as widely reported<sup>[33, 34]</sup>. Both pure gelatin and the modified gelatin **6a** was tested, and the amounts of  $-NH_2$  were 0.49 mmol/g and 1.38 mmol/g, respectively. This obvious change indicated that the surface modification

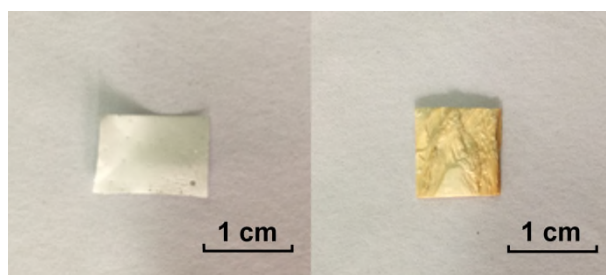
with amine groups was successful. Tolyene-2,4-diisocyanate (2,4-TDI) was then reacted with the amino-modified gelatin membranes **6a-b**. The reaction between amines and isocyanates functionalized the surface of gelatin membrane, giving the urea-substituted gelatin membranes **7a-b**.



**Scheme 2** The mechanism of Van Slyke nitrogen method

### 3.4 Surface morphology of gelatin membrane

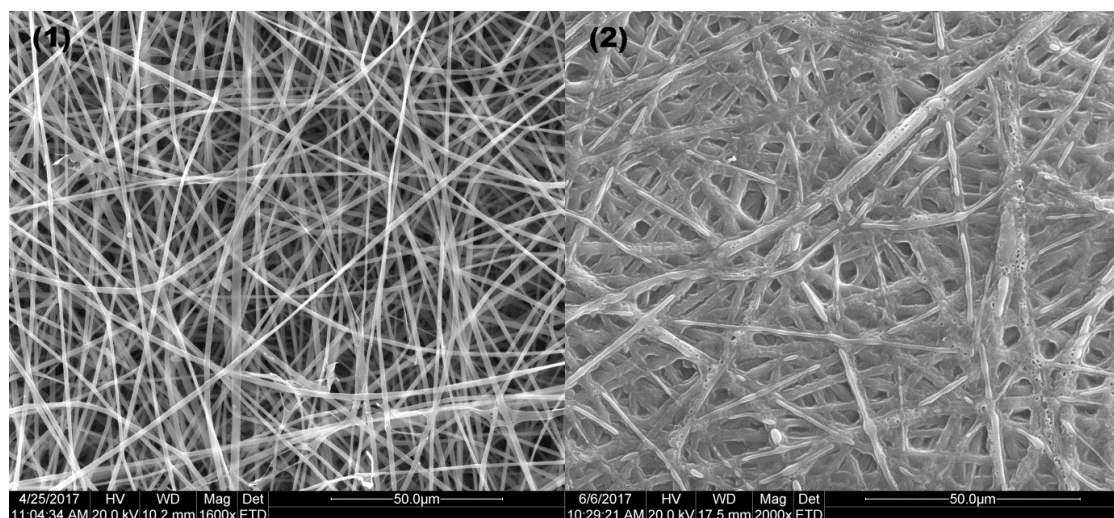
It is interesting that the gelatin membrane appears to be a yellow colour after modification by the diazomethane, which is very different from the white color of unmodified gelatin membrane. The colour changes of typical samples are shown in Figure 1. This immediately suggested that the modification had been successful, but extra characterization was made to provide additional confirmation.



**Figure 1** Photographs of gelatin electrospinning membrane: (1) pure gelatin; (2) **6a**.

In order to understand the morphology of the gelatin membrane, the surface structure was observed by scanning electron microscopy (SEM) and the results were shown in

Figure 2. From Figure 2(1), the nanofibers showed a homogeneous length, along with an interconnected and continuous fibrous morphology. After the gelatin membrane was treated with diazomethanes **5a-b**, it changed into a unique network morphology (Figure 2(2)). It suggested the cross-linking between gelatin fibers had resulted, which was characterized and analyzed below.

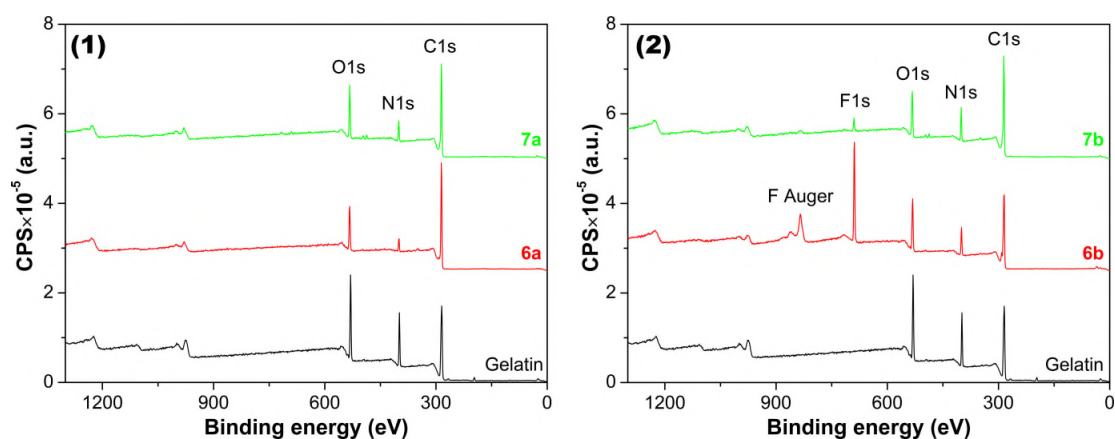


**Figure 2** SEM pictures of gelatin electrospinning membrane: (1) pure gelatin; (2) **6a**.

### 3.5 Surface Analysis of gelatin membranes

The chemical composition on the surface of gelatin membranes might be different after being modified by carbenes, as well as the subsequent functionalization by 2,4-TDI. Therefore, XPS analysis was carried out to explore the difference between these membranes (Figure 3). As shown in Figure 3(2), the characteristic peaks of fluorine (F 1s) of **6b** at 688.60 eV confirmed that the pure gelatin had been successfully modified by perfluoroalkyl groups. After the subsequent reaction with 2,4-TDI, the cross-linking between amines and isocyanates formed a urea layer on the

surface of membrane, which led to a clear decrease of the fluorine peak in **7b**. Moreover, the percentage of carbon, nitrogen, oxygen, and fluorine varied on the surface of **6a-b** and **7a-b** (Table 1) when compared to the pure gelatin membrane. This outcome proved that the gelatin had been successfully modified by carbene and cross-linked by 2,4-TDI.



**Figure 3** XPS spectra of pure gelatin, modified gelatin by perfluoroalkyl carbene, and functionalized gelatin by 2,4-TDI: (1) modified with **5a**; (2) modified with **5b**.

**Table 1** XPS data of pure gelatin, modified gelatin, and functionalized gelatin

Sample	C %	N%	O%	F%
Pure gelatin	64.9	17.0	18.1	-
<b>6a</b>	81.9	5.5	12.6	-
<b>6b</b>	63.4	9.3	12.1	15.2
<b>7a</b>	77.3	7.9	14.8	-
<b>7b</b>	74.8	11.3	11.6	2.3

Water contact angles were measured by the Sessile drop method in order to evaluate the variation of surface wetting properties after modification. Pure gelatin is hydrophilic (water contact angle,  $57^\circ$ ), and may even dissolved by water drops if test time is prolonged. However, the water contact angle of modified gelatin membrane **6a** was increased to  $110^\circ$ , and the water contact angle of modified gelatin membrane **6b** was even higher ( $132^\circ$ ). The reason for this lay in the presence of the perfluoroalkyl groups which have a low surface free energy and make gelatin more hydrophobic. Moreover, it was noteworthy that the modified gelatin membranes could not be dissolved in water, which were very different from the pure gelatin membrane.

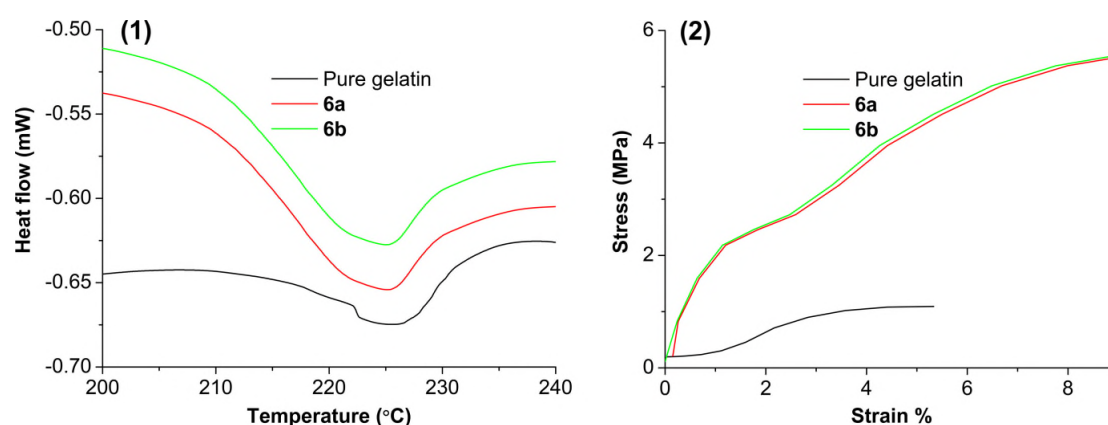
### **3.6 Bulk analysis of gelatin membranes**

The thermal behavior of gelatin membranes was measured by DSC and the glass transition temperature was determined (Figure 4(1)). The glass transition temperature of all the membranes was very similar, which was about  $225^\circ\text{C}$ , and the treatment with diazomethanes only gave a small change of this peak. In contrast, the curves for tensile test via a static mechanical analyzer indicated a large change after diazomethane treatment. The stress-strain curves of all gelatin membranes at room temperature are shown in Figure 4(2). Thus, the tensile strength of the pure gelatin electrospun membrane was very low, but after being treated with diazomethanes, the gelatin membrane showed typical hard polymer behavior, namely, high elastic modulus and relatively small elongation at break.

Although the stress-strain curves demonstrated that the treatment by diazomethanes led to great improvement of the mechanical properties, it was noteworthy that the



glass transition temperature of all the membranes did not change very much. It indicated that the increase of tensile strength might not be attributable to the chemical reaction of the carbene insertion modification, but lay in the transformation of the supramolecular aggregation of the membranes. That is, the treatment with diazomethanes mainly changed the physical structure of the gelatin surface, while the bulk property of the polymer has been maintained during the modification process.



**Figure 4** Bulk analysis of pure gelatin and modified gelatin: (1) DSC curve; (2) stress-strain curve.

### 3.7 Antibacterial activity of gelatin membranes

According to the literature<sup>[35, 36]</sup>, the urea groups may easily bind hydrogen peroxide, and this is an effective way to prepare antibacterial materials with controlled-release properties. Here, the cross-linking between amines and isocyanates formed a very dense layer with a large number of urea groups, and this was expected to achieve antibacterial gelatin membranes. In order to determine the loadings of the hydrogen peroxide, a titration method with KI / Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> was used, and the detailed results are shown in Table 2. By this method, it was shown that the original carbene modification

gave a surface layer that in turn allowed the introduction of large numbers of urea groups, which could easily bind hydrogen peroxide with high loading levels. After that, the antibacterial activity was tested upon incubation with bacteria (*S. aureus* and *E. coli*). Large zones of inhibition were found for the two membranes (Table 2), and demonstrated clear antibacterial activity.

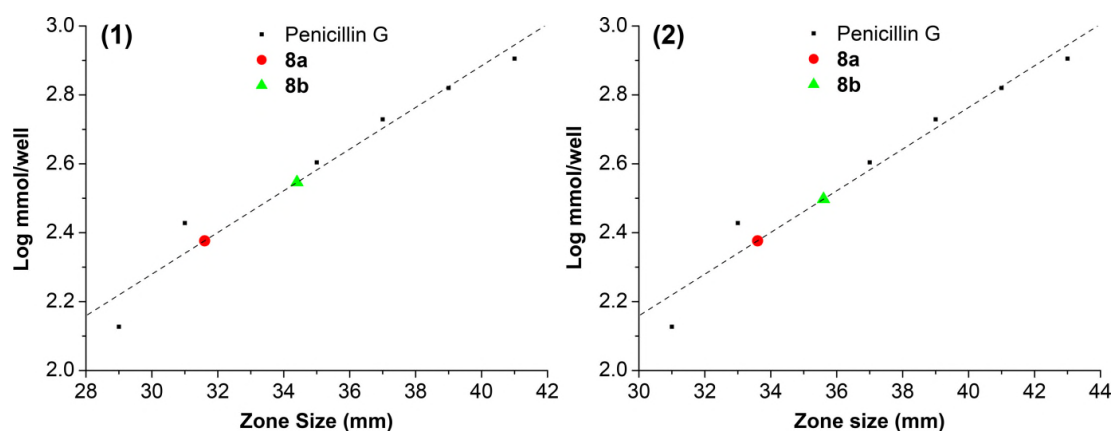
**Table 2** The loadings of hydrogen peroxide and the antibacterial activity

Membranes	Loading of H <sub>2</sub> O <sub>2</sub> (mmol g <sup>-1</sup> )	Activity against <i>S. aureus</i> (mm) <sup>1</sup>	Activity against <i>E. coli</i> (mm) <sup>1</sup>
<b>8a</b>	3.3	31.6	31.6
<b>8b</b>	3.5	34.4	33.6

<sup>1</sup> The diameter of inhibition zones

In order to give a comparison of the antibacterial activity between gelatin membranes and other materials, Penicillin G potassium salt in aqueous solution was used for calibration, giving log(nmole per well) versus diameter of inhibition zone as a straight line<sup>[22]</sup>. Using this calibration curve, the equivalent concentration of Penicillin G potassium salt for the membrane was determined according to the diameter of their inhibition zones (Figure 5). It was shown that the membrane **8b** had a better antibacterial activity than the membrane **8a**, which might derive from the loading amount of H<sub>2</sub>O<sub>2</sub> on the surface (Table 2). Moreover, this method allowed comparison and supplement with other antiseptic treatments<sup>[37, 38]</sup>, since Penicillin is widely used

in clinic for many years and has much less toxicity than most of other antibiotics.



**Figure 5** Log(nmole/well) versus diameter plot for Penicillin G and the equivalent of Penicillin G for gelatin membranes: (1) S. Aureus; (2) E. Coli

## 4 Conclusions

Two kinds of amino-bis(aryldiazomethane)s were synthesized, and were used to modify gelatin membranes by a bis(arylcarbene) insertion reaction. The detection of fluorine in the XPS spectra showed that the protocol is successful. DSC curves and stress-strain curves showed that the modification led to an obvious change of supramolecular aggregation instead of chemical cross-linking. The amino-loaded membranes were subsequently reacted with tolylene-2,4-diisocyanate to give cross-linked gelatin membranes with urea groups, which was used for binding hydrogen peroxide to give antibacterial activity. The discovery of this carbene modification followed by the reaction with diisocyanates opens a new horizon for preparing robust antibacterial gelatin membranes, which gives a general approach for the preparation of antibacterial biomacromolecules that cannot usually be dissolved in

organic solvents.

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## Table of Contents

Gelatin electrospinning membranes were prepared and modified through carbene insertion chemistry. The surface modification with amino-substituted diazomethanes and the subsequent cross-linking reaction with diisocyanates contributed to high loadings of hydrogen peroxide, and greatly increased the antibacterial activity of gelatin-derived membranes, which opened a new horizon in the preparation of high loading antiseptic/antibacterial biomacromolecular surfaces and interfaces.

