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RESEARCH ARTICLE

Electrospun composite nanofibrous membrane as wound dressing with good adhesion

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A photopolymerizationable mimic mussel protein structure monomer, dopamine methacrylamide (DMA), was synthesized. The photopolymerization of DMA was analysed by series real time near infrared spectroscopy (SRTIR). Dopamine methacrylamide/poly (ethylene oxide) (DMA/PEO) nanofibers were successfully prepared by electrospinning of aqueous DMA/PEO solution. Biocompatible nanofibrous membrane with good adhesion was produced by photocuring from the DMA/PEO nanofibers. The surface characterization and structure of the composite nanofibrous membrane were characterized by a scanning electron microscopy (SEM) and contact angle measurements. For identifying the potential crystalline of curing, a XRD method was used through comparing diffraction data. In the cell adhesion test we utilized the mouse fibroblast (L929) to exam the various use of the nanofibrous membrane as scaffolding materials for skin regeneration.

Keywords electrospinning, wound dressing, adhesives

1 Introduction

Skin is a very important human organ that covers the entire external surface and forming about 8% of the total body mass [1]. Recently, more and more tissue engineered skin substitutes are demanded in the treatment of dermal wounds caused by various diseases [2]. Among the techniques of fabricating the wound dressing electrospinning is an attractive method to produce nanofibrous membrane for tissue engineering scaffolds [3]. But these membranes usually have no adhesion with the surfaces of the tissue. Various synthetic biopolymers have been electrospun for the use of tissue engineering, which include poly(ethylene oxide) (PEO), poly(lactide) (PLA), poly(glycolic acid) (PGA), polyethyleneimine, poly(vinyl alcohol) (PVA) and so on [4,5]. As one of the most extensively studied polymers for electrospinning, poly(ethylene oxide) (PEO) has been widely used with/without the addition of other composition [6–8]. Some inorganic molecule composition and polymer blended fiber is produced in order to get some special function materials [9,10].

Mussel adhesive proteins is considered an adhesive that can reliably and durably bond to various target surfaces in a wet environment [11]. It consists of conserved decapeptide repeats that are rich in dihydroxyphenylalanine (DOPA) residues. Both natural and synthetic adhesives containing DOPA and its derivatives have good performance in long-term adhesion in whatever environment [12,13]. So we considered that the group of catechol forms extraordinarily strong bonds with the surface. And further, many chemicals were synthesized for the use as adhesives for various fields [14–16].

In this paper, a photopolymerisable mimic mussel protein structure monomer was blended with the PEO in electrospun solution, producing a composite fiber. After photocuring, we gain a nanofibrous membrane with good adhesion. It has demonstrated a new way to produce a wound dressing with an adhesive surface, unlike other wound dressing that has no bonding strength with different materials.

2 Experimental

2.1 Materials

PEO with molecular weight of 600 kg/mol was supplied by Acors Organics. Dopamine·HCl and methacrylate anhydride was purchased from Sigma Co., Ltd. Compounds. Photoinitiator 2959 (Darocur 2959, 2-hydroxy-1-[4-(hydroxyethoxy) phenyl]-2-methyl-1-propanone) was supplied by Ciba-Geigy Chemical Co. (Tom River, NJ). Other reagents were provided by Beijing Chemical Reagents. All of the materials were used without further purification.

2.2 Synthesis of dopamine methacrylamide (DMA)

5 g of dopamine·HCl was dropped in the solution that was formed by 19 g of sodium carbonate decahydrate and 4 g of $NaHCO_3$ dissolved in 100 mL of deionized water and bubbled with N_2. 4.8 mL of methacrylate anhydride in 30 mL of THF was then added dropwise. The aqueous mixture was stirred for 8 h at room temperature, followed by the addition of hydrochloric acid to reduce the pH to less than 2, and extracted with 60 mL of ethyl acetate three times. The ethyl

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acetate were combined and dried over $MgSO_4$ and got the ethyl acetate removed through reduced pressure distillation until the volume of the mixture got to 30 mL. 200 mL of hexane was poured into the mixture with stirring and the suspension was held at 4°C for 8 h. The product was recrystallized from hexane and dried, yielding 4 g of grey solid. The structure of DMA was investigated by 1H-NMR, and the result has been reported previously in literature [13]. The preparation scheme of DMA is shown in Fig. 1.

2.3 Electrospinning

We prepared PEO aqueous solutions by dissolving the PEO into chloroform and stirring the mixture at room temperature for 24 h. DMA in ethanol was added to the PEO solution before it was used. Each sample took 6 mL of the solution. Although the PEO and DMA were blended with different weight ratios, the PEO concentration was kept at 0.1 g in all experiments except the 10/0 sample. And the D-2959 concentration was kept at 1% in both P5/5 and P8/2 system for initiated polymerization. The composition of each sample was listed in Table 1.

The detailed electrospinning procedure can be found in our precious work [17]. The solution was supplied by a syringe pump at a constant feed rate of 0.02 mm/s. And the solution was electro spun at a high voltage of 20 kV generated by a power supply (BGG4-21, BMEI CO., Ltd.). The distance between the aluminum foil collector and the spinneret with a needle having an inner diameter of 0.47 mm was 15 cm. All the experiments were carried out at room temperature.

Every 5 min during the electrospinning, the sample P5/5 and P8/2 were irradiated with UV light source (30 mW/cm^2, 320–480 nm, EXFO lite, EFOS Corporation, Mississauga, Canada) for 15 min to form a film containing the group of catechol as soon as possible. The spinning process lasted for 60 min.

2.4 Real time near infrared spectroscopy

Polarized FT-IR spectroscopy (Nicolet FTIR-560) was used to investigate the polymerization process of the DMA in the dimethyl sulfoxide (DMSO). We dissolved the DMA in DMSO, and prepared the DMA solution with two different concentrations of 30% and 40% (w/w). And all solutions kept the D-2959, photoinitiator, concentration at 1% (w/w). The polymerization process of the DMA was monitored by using series real time near infrared spectroscopy (SRTIR, Nicolet5700 instrument, Thermo Company, USA) to help quantify the conversion [18].

2.5 SEM characterization

The surface of the nanofibrous membrane were characterized by a scanning electron microscopy (SEM, S-4700 Hitachi) using an accelerating voltage of 20 kV. Before observation, each specimen sputter was coated with a thin layer of gold.

2.6 Contact angle measurement

The contact angle measurements were performed on the cast films using a sessile drop contact angle measuring device (JC2000C1, Powereach Digital Technology Co., Ltd., Shanghai, China) with distilled water droplets (about 0.5 μL in size) at room temperature. Besides, the samples of 0/10, 5/5, P5/5 were measured, and the contact angle was measured for each spot after a contact for 3 s.

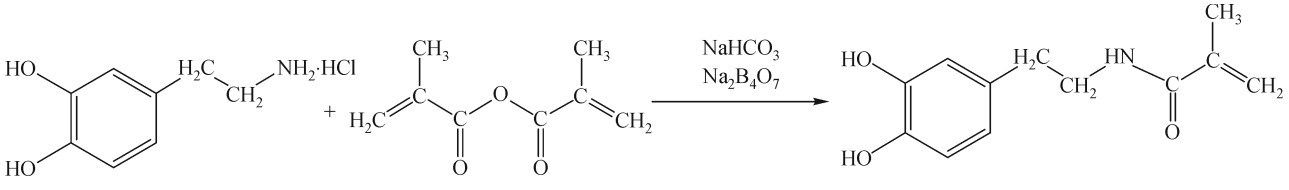

Figure 1 Reaction equation of the DMA

Table 1 Composition of each sample

Sample/g	0/10	2/8	4/6	5/5	6/4	8/2	10/0[a)]	P5/5	P8/2
DMA	0	0.025	0.067	0.100	0.150	0.400	0.100	0.100	0.400
PEO	0.100	0.100	0.100	0.100	0.100	0.100	0	0.100	0.100
Chloroform	1	1	1	1	1	1	1	1	1
Ethanol	5	5	5	5	5	5	5	5	5
D-2959	0	0	0	0	0	0	0	0.001	0.004

a) The sample of 10/0 cannot form nanofibers; P5/5 and P8/2 were irradiated after electrospinning.

2.7 XRD analysis

The DMA monomer and samples of 0/10, 5/5, P5/5 were recorded on an X-ray diffractometer (D/Max 2500VB2 +/Pc, Rigaku, Japan) with Cu Kα characteristic radiation (wavelength = 0.154 nm) at a voltage of 40 kV and a current of 50 mA. The scanning rate was 10°/min and the scanning scope of 2θ was from 5° to 60° at room temperature.

2.8 Cell culture and adhesion

The nanofibrous membranes were extensively washed three times with sterile phosphate buffered saline (PBS) and were transferred to individual 24-well tissue culture plates. 1 mL of the aliquots of mouse fibroblasts (L929) suspension of 1.5×10^4 cell/mL were seeded on the samples. After 48 h of culture, cellular constructs were harvested and rinsed twice with PBS to remove non-adherent cells, and were subsequently fixed with anhydrous ethanol at room temperature for 4 h. Dry samples were sputtered with gold for cell morphology observation on the surface of the nanofibrous membranes by SEM [1].

3 Result and discussion

3.1 FT-IR analysis

In order to avoid the monomer loss, the electrospun solution containing DMA was used to produce nanofibrous membrane, and the membrane was photocured with UV light source. Therefore, we determined the rate and the equilibrium conversion rate of photopolymerization of DMA in solution. DMA is a photopolymerization monomer soluble in several kinds of organic solvents. Although the concentration of the monomer dissolved in ethanol is too little to be detected by the SRTIR. It is more easily dissolved in DMSO, so the photopolymerization with the monomer in DMSO could be detected more clearly. That said, the photopolymerization process of DMA was monitored in DMSO instead of in ethanol. It could be seen that the photopolymerization of DMA in DMSO led to high conversion of double bond for a few minutes as shown in Fig. 2. The sample of 40% DMA has a higher level of conversion and rate of polyerization than the other sample.

3.2 SEM characterization of nanofibrous membranes

In the conventional electrospinning process, the electrospun solution should be composed of one or several kinds of polymer. But in this research, a functionalized organic small molecule was composed with the polymer. So in this

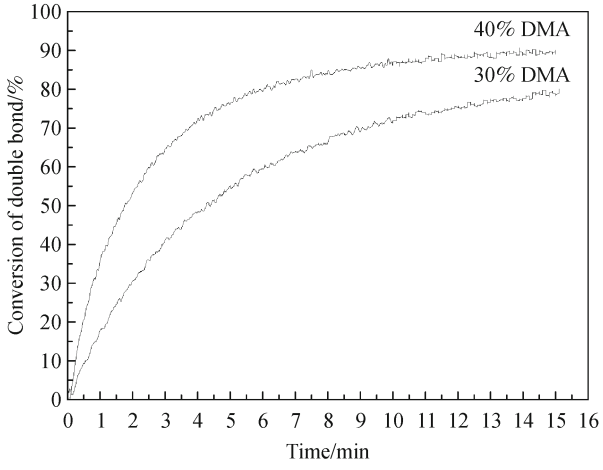

Figure 2 Double bond conversion vs. irradiation time of photopolymerization of DMA in DMSO with different weight ratios

electrospinning process, some phenomena like crystallization of small molecule, the diversification of the microstructures of the fibers before and after irradiated under UV light should be seen.

The diameter of the fibers tended to increase with the rise of DMA concentration when it is under 40%. As shown in Figs. 3(a)–(c), the diameter of the fibers ranged from 200 nm to 2 μm. With the increase of the concentration of DMA, some aggregation of needle-like crystals structure was seen, as shown in Figs. 3(c)–(f). The more the DMA concentration increased, the rougher the surface the nanofibrous membranes got to be, because the increase of the DMA concentration resulted in more crystal structure. And we could not see the edge of the nanofibers clearly, which may also result from the crystallization of DMA around the fibers.

As shown in Figs. 3(g) and (h), after irradiated under UV light, the microstructures of the fibers appeared more smooth, like a polymer film coating on the fiber, and the aggregation of needle-like crystals structure had disappeared. It is because after photopolymerization the DMA had formed into polymer film around the fiber, and the polymerization prevented the crystallization of DMA on one hand. The newly formed polymer adhered to the fibers, making the surface more smooth than the uncured samples. The polymer formed by DMA coated the nanofibrous membranes which had gained the adhesion of the group of catechol as mussel protein.

3.3 Water contact angles

Water contact angle measurements for the fibrous membranes were performed, and the results are shown in Fig. 4. It can be seen that the addition of DMA increases the contact angle value. So the introduction of DMA made the membrane more

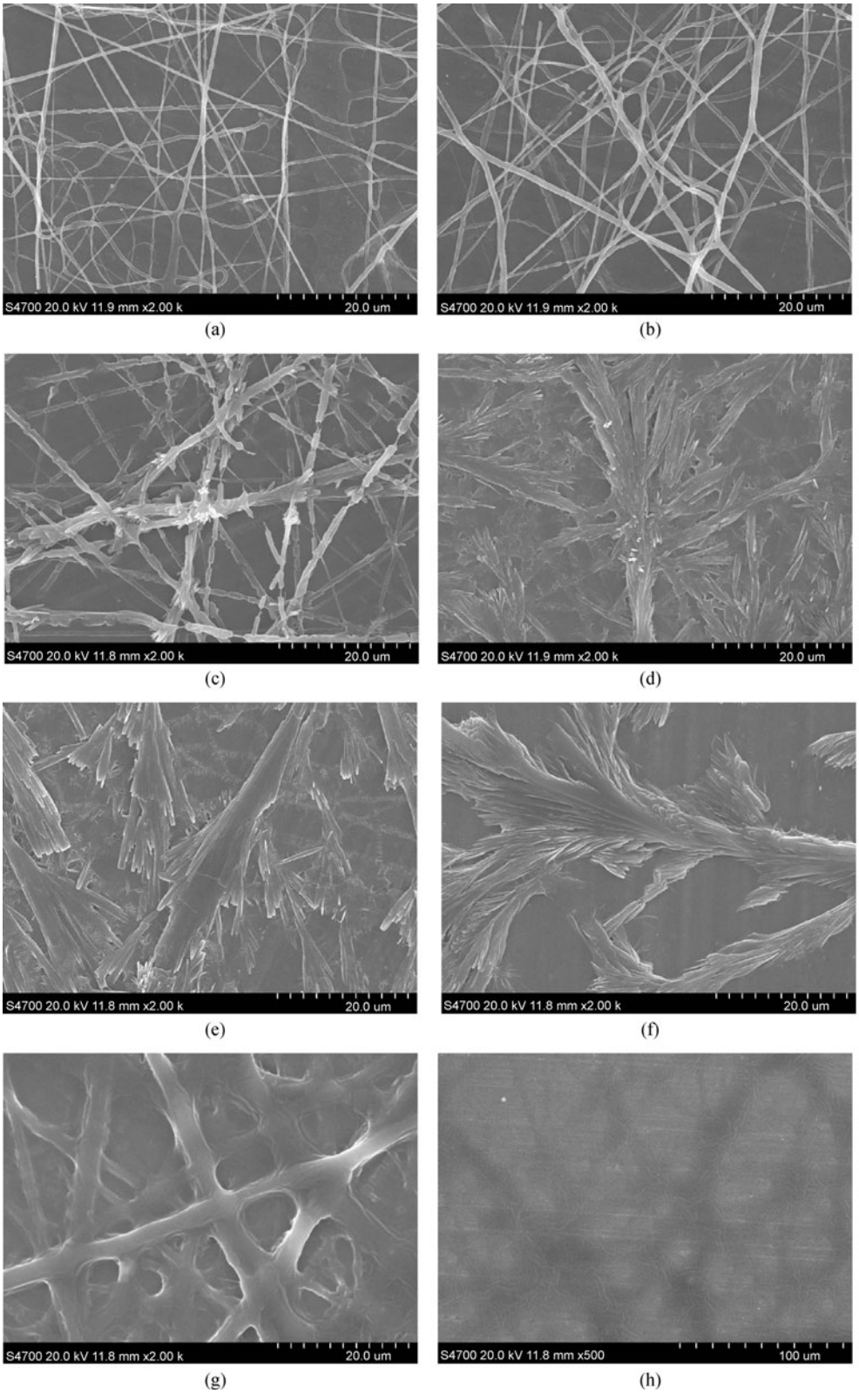

Figure 3 SEM image of nanofibrous membranes made by DMA/PEO

(a)–(f) nano-micro fibers made by various weight ratios of W_{DMA}/W_{PEO}: (a) 0/10, (b) 2/8, (c) 4/6, (d) 5/5, (e) 6/4, (f) 8/2; (g) and (h) nanofibrous membranes produced by DMA and PEO with the weight ratio at 5/5 and 8/2. After electrospinning the film was irradiated with UV light source. (g) P5/5, (h) P8/2

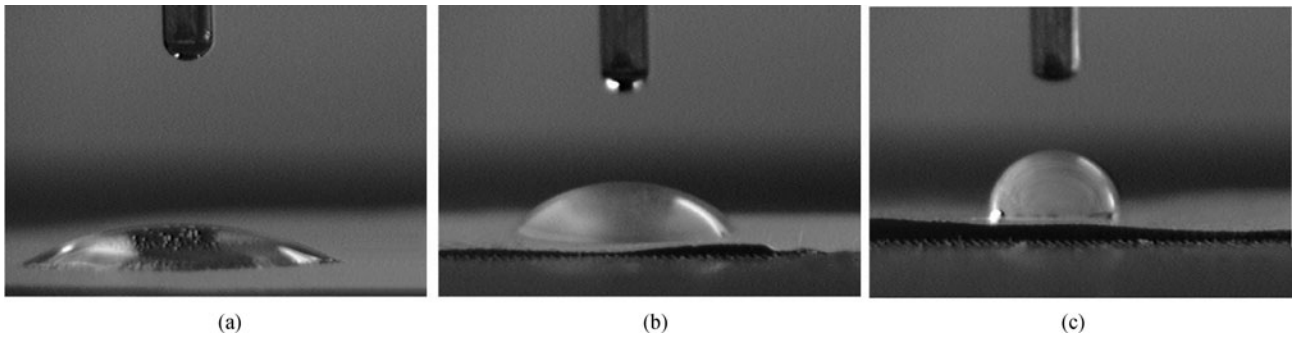

Figure 4 Contact angle images of nanofibrous membranes made by DMA/PEO (a) 0/10, contact angle = 15.1º; (b) 5/5, contact angle = 53.0º; (c) P5/5, contact angle = 81.5º

hydrophobic. In addition, the photocuring strengthens the hydrophobic of the membrane. And the different contact angle values between the sample 5/5 and P5/5 have also proved the occurrence of the photopolymerization.

3.4 XRD characterization

The XRD patterns evaluated the crystallinity of DMA monomer and fibrous membranes with different component and treatment methods in Fig. 5. The PEO fibrous membranes showed a characteristic peak at the 2θ region of 19.3º. The XRD patterns of the samples of P5/5 and 5/5 still had this peak caused by the crystal of PEO fibers, and also had diffused peaks from 12° to 26º as with the 0/10 sample. But the sample of 5/5 had another peak at the 2θ region of 14.5º caused by the crystal of DMA monomer. Because the DMA monomer had a characteristic peak at 14.5º, and the polymerization prevented the crystallization of DMA, the P5/5 did not have a peak at 14.5º at all. This was another evidence that has proved the occurrence of the photopolymerization.

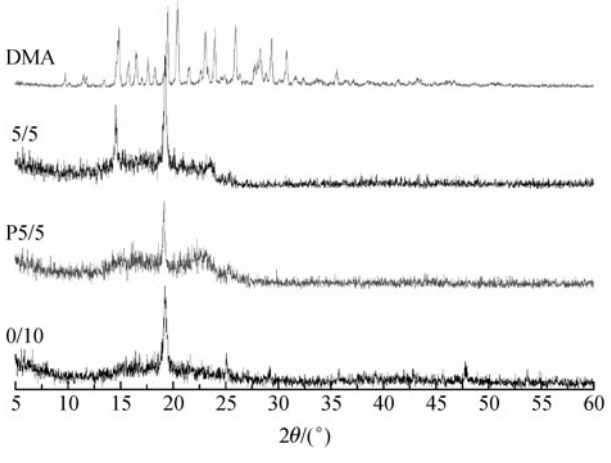

Figure 5 XRD patterns of DMA monomer, 5/5, P5/5 and 0/10

3.5 Cell adhesion and morphology

To examine the interaction of the nanofibrous membranes with tissue cells, the test was conducted using mouse fibroblasts (L929) cells in compliance with the ISO1993-5 standard test method. Fig. 6 shows the SEM of L9293 cultured on the nanofibrous membranes for 48 h on the sample of P5/5. The SEM images revealed that L929 cells could adhere and exhibite a normal morphology on the membrane. Interestingly, we noted that L929 cells tended to attach and grow along the nanofibers and suggest good cell viability on the nanofibrous membranes surface.

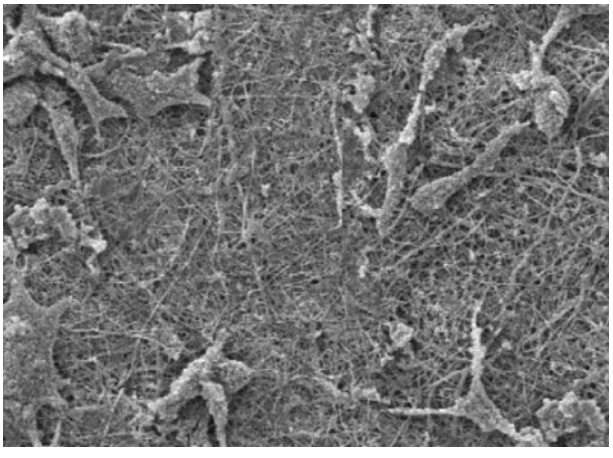

Figure 6 SEM images of L929 cell seeded on nanofibrous membrane after 48 h's culture (magnification: ×1000)

4 Conclusions

This study has shown that the biocompatible nanofibrous membranes were successfully prepared by electrospinning of aqueous DMA/PEO and photocuring with UV light. This process made the mimic mussel protein monomer, DMA, uniformly distributed between the fibers or on the surface of the fibers. Cell culture and adhesion test of the nanofibrous

membranes with mouse fibroblasts (L929) indicated that L929 cell could adhere to the DMA/PEO electrospinning nanofibrous membranes. At the same time, the introduction and curing of the DMA in situ let the nanofibrous membranes have the biological performance of mussel-inspired adhesion. These electrospun nanofibrous membranes have the potential to be used as wound dressing materials for skin tissue engineering.

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