Balsa-graphene Dressing for Wound Healing: a green and anti-infection matrix

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Keywords: Balsa; wound dressing; graphene; anti-infection matrix

Abstract. This study aims to prepare an eco-friendly dressing using balsa derived membrane with graphene included for anti-bacterial purpose. The balsa-graphene was prepared using delignification (group control) and dopamine (group A) methods for mussel-inspired adhesion of graphene, respectively, at 0.2, 1, 5ug/ml (group B,C,D). We found graphene spherical particles formed on the dressing. FTIR and contact angle test show that lysozyme adhered to the membrane.In vitro antibacterial effect was significant on both E. coli and S.aureus. The cytotoxicity of the balsa dressing was not found until day 7.

Introduction

The balsa is the lightest commodity timber, due to its minimum dissolved heavy, material uniformity, low thermal conductivity, and volume stability. Balsa can do a variety of special structures[1,2]. When applied as the wound dressing substrate, it may have the effect of low cost, good air permeability（the pore size of balsa fiber can reach several microns）and may be commonly used[3,4].

To date, the combination of graphene and dressing has become a popular research field. The ideal wound dressings should have the following characteristics: good mechanical properties, proper water vapor permeability and fine biocompatibility[5,6]. More importantly, infection, inflammation, etc. will cause a slow down or failure of the wound healing, the ideal wound dressing should also be able to provide a local surface suitable for antibacterial effect[7,8] .Therefore, we will combine delignification balsa and graphene to design a new economic and efficient antimicrobial dressing.

Materials and Methods

Materials and animals ethical Statement

Dopamine hydrochloride and graphene was purchased from Solarbio Science & Technology Co.,Ltd. (Beijing, China). Balsa was purchased from Lego. BALB/c mice (male, 20–25 g) was

obtained from the Experimental Animal Department of the Tird Military Medical University. (room temperature:25 °C; relative humidity: 50%; and circadian rhythm: 12 h) .

In this study, all animal protocols were permitted by the Institutional Animal Care and Use Committee of the Third Military Medical University (TMMU) Chongqing China, and all animal experiments followed the Regulation on the Management of Laboratory Animals, which was issued by Chinese Association for Laboratory Animal Sciences (CALAS) .

Delignification

The balsa with the density about 160 kg/m3 were punched and produced discs (diameter 0.6mm, thickness 0.8 mm). The discs were dried at 105 ± 3 ° C for 24 hours and then immersed in 1% the NaClO2-acetate buffer (pH 6.4, 80 °C) for 12 h. The samples were carefully washed with deionized water and then washed three times. Then dehydration was performed by ethanol: ethanol and acetone-acetone (1： 1) solution via three steps (10 min each step).

Dopamine glue with graphene

Tris hydrochloric acid (131.14 mg) was solved in 100 ml of deionized water, and then 200 mg of dopamine powder was added to prepare a Tris-dopamine solution (2 mg / ml, pH 8.5). The balsa was then immersed in the dopamine solution for 12 h, placed in a shaker at 37 °C (100 rpm). graphene powder was dissolved in deionized water and generated as 5ug/ml graphene solution, and then diluted to 0, 0.2, 1and 5ug/ml (ie, the A, B, C and D group). The samples were washed with deionized water carefully, immersed in different concentrations of graphene solution for 12 h on a 37 ℃ shaker (100 rpm).

Scanning electron microscopy (SEM)

The sample of the material wafer was carefully washed with deionized water, dried and sprayed, and the aperture structure of the film was observed under vacuum condition by scanning electron microscope and photographed.

Fourier infrared spectrum

Different materials characterization and chemical structure was Testing by Fourier transform infrared spectroscopy (Nicolet-460 Thermo Fisher) ,wavenumber scan range is 600-4000 cm-1.

Contact angle test

The film of different materials is placed on the horizontal surface, and the 1ul deionized water is dripped down from the top to the surface of the material. The contact angle size of the droplet is measured, and the average value of each sample is measured 3 times.

Bacteria co-culture

S.aureus and E.coli strains were obtained from the Institute of Burns, Southwest Hospital, Third Military Medical University. The bacteria (1*104 CFU/ml) were first detected by the microplate reader (An OD value about 0.07 met the standard) and then added 200 ul into each well of the 96-well plates. Each group of materials was placed in the wells. Incubation was performed at 37 °C for 12 h and 24 h.

Assay of cytotoxicity

Normal neonatal mice were used to isolate primary fibroblasts as routine method,(reference) and the 3rd passage cells were used to assay the cyto toxicity of the prepared balsa-graphene dressing. After cells attached, materials were placed into wells. Each group contained 12 wells (for day 1, 3, 5 and 7 cell proliferation assessment, triple for each time point). Each well had 150 ul medium and cells were cultured in the 37 ℃ incubator.

Results and Discussion

Electron microscopy scanning

As shown in Figure. 1, the balsa retained the ordinary balsa structure (A). The balsa-graphene group shows some dopamine layer and graphene (as crystals precipitated on the surface) were visible.

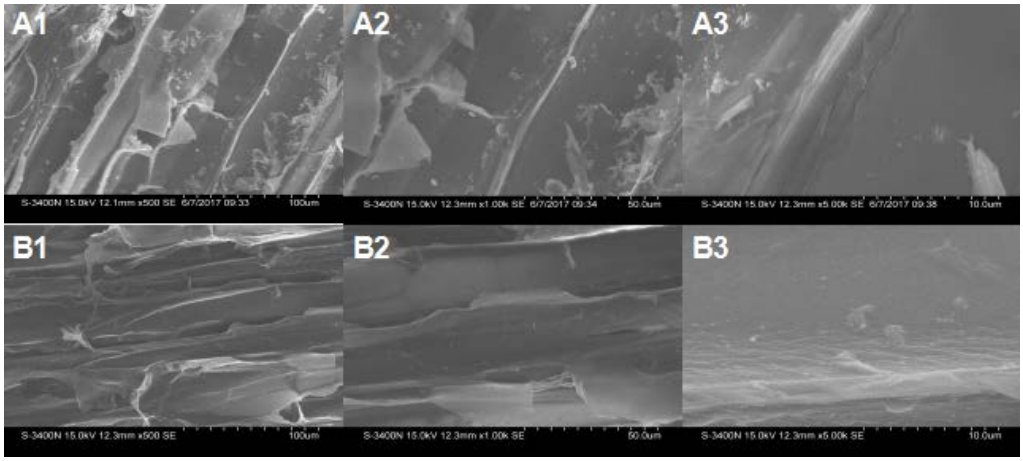

Figure 1. In the first row of balsa group (group A), In the second row of 0.2ug/ml balsa-graphene (group B), from left to right: 5x 102 times, 1 x 103 times, 5x 103 times.

Fourier infrared spectrum

As shown in Figure 2, the infrared spectra of the 3 groups (Control, A, D) are approximately similar. The Control group region of 1030.54 cm-1-1364.01cm-1 exhibits broad and strong peaks. As shown in D, graphene has two distinct characteristic peaks, corresponding to C=0 stretching vibrations of amide I (1618.99cm-1) and N-H, flexural vibrations and C-N stretching vibrations of amide II (1031.45cm-1).

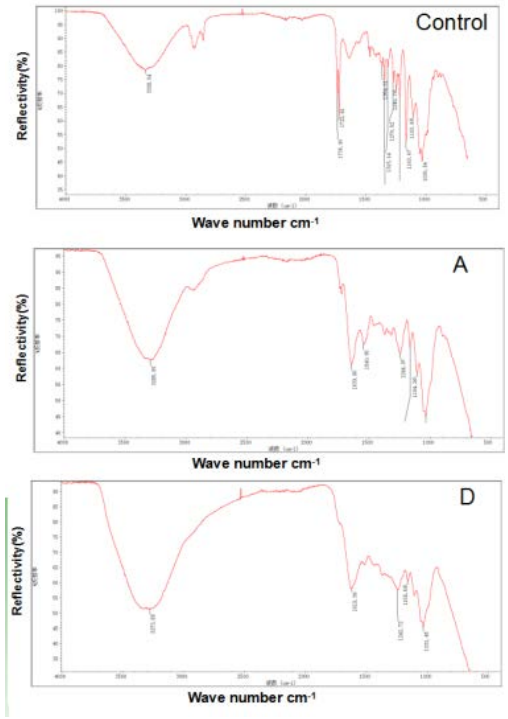

Figure.2 The infrared spectra of the Control, A and D groups

Contact angle test

As shown in Figure 3, the average contact angle (θ) of Control group is 3.90± 0.85°, A is 4.15 ±0.87°,D is 57.28±6.21°.Compared with group A and D, the difference was statistically significant (P<0.05).

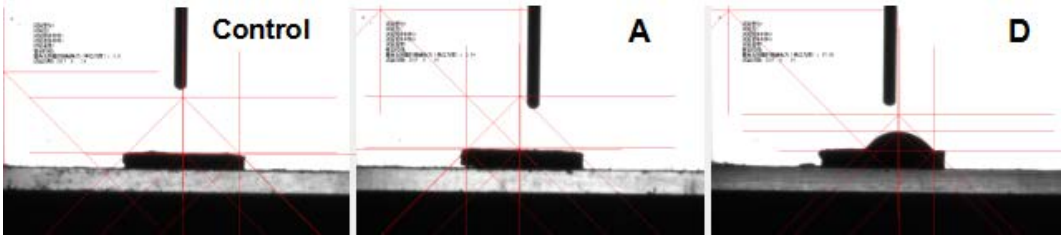

Figure 3 the average contact angle (θ) of Control group is 3.90± 0.85°, A is 4.15 ±0.87°,D is 57.28±6.21°.

Assay of the antibacterial activity

As shown in Table 1,Table 2, the growth of S.aureus and E.coli in the Control group was in accordance with the normal trend. When the concentration of graphene increased gradually, the inhibitory effect on the bacteria gradually increased, and the difference was statistically significant (P <0.05).

Table 1 OD values of co-culture of different concentrations of balsa-graphene dressings and S.aureus.

	Control	A	B	C	D
12h	0.518±0.064	0.502±0.060	0.453±0.049	0.391±0.042	0.337±0.035
24h	0.864±0.089	0.858±0.087	0.754±0.073	0.683±0.066	0.605±0.059

Table 2 OD values of co-culture of different concentrations of balsa-graphene dressings and E.coli.

	Control	A	B	C	D
12h	0.484±0.048	0.470±0.041	0.402±0.038	0.339±0.034	0.265±0.030
24h	0.722±0.068	0.709±0.065	0.631±0.060	0.568±0.054	0.497±0.048

Test of cytotoxicity

As shown in Figure. 4, graphene in different concentrations was hardly inhibited the cell growth (P> 0.05). Only on day 7, group D showed a light inhibitory effect (P <0.05).

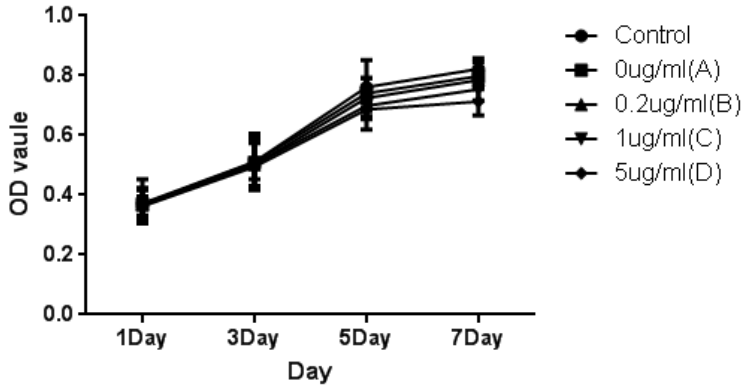

Figure.4 Until day 7, group D had inhibitory effect on cell proliferation (P <0.05)

Discussion

The defense barrier on body surface of the burns were destroyed, and the immune capacity decreased significantly. Extensive tissue necrosis and invasion of bacteria in vivo will lead to wound infection. Various means to prevent bacterial invasion and the loss of body fluids are needed. As shown in Figure. 1, the balsa retained the ordinary balsa structure (A). The balsa-graphene group shows some dopamine layer and graphene (as crystals precipitated on the surface) were visible. We think that is because the graphene concentration of 5ug/ml is much larger than that 1ug/ml of other related research [9,10]. As shown in Figure 2, the infrared spectra of the 3 groups (Control, A, D) are approximately similar. The Control group region of 1030.54 cm-1-1364.01cm-1 exhibits broad and strong peaks. As shown in D, graphene has two distinct characteristic peaks, corresponding to C=0 stretching vibrations of amide I (1618.99cm-1) and N-H, flexural vibrations and C-N stretching vibrations of amide II (1031.45cm-1). It shows that the graphene molecule has been immobilized on the carrier, but the secondary structure has not changed significantly[11,12]. As shown in Figure 3, the average contact angle (θ) of Control group is 3.90± 0.85°, A is 4.15 ± 0.87°,D is 57.28±6.21°.Compared with group A and D, the difference was statistically significant (P<0.05).The D group still has hydrophilicity.

In recent years, there have been many studies to prepare graphene into microcapsules, nanoparticles, pellets and other forms, and combined with different materials to improve its stability and its bacteria bactericidal effect [13,14]. As shown in Table 1,Table 2, the growth of S.aureus and E.coli in the Control group was in accordance with the normal trend. When the concentration of graphene increased gradually, the inhibitory effect on the bacteria gradually increased, and the difference was statistically significant (P <0.05). In this study, the graphene was fully adhered to the pores by dopamine glue, which provides a stable releasing microenvironment towards bacteria[15,16]. As shown in Figure. 4, graphene in different concentrations was hardly inhibited the cell growth (P> 0.05). Only on day 7, group D showed a light inhibitory effect (P <0.05).This may be related to graphene, which is natural and safe . The safety of graphene makes it widely used for Materials Science [17,18].

In summary, this study prepared a Balsa-graphene Dressing for Wound Healing: a green and anti-infection matrix. But High concentration with long-term contact might inhibit cell proliferation. This dressing provides a new means for wound healing via natural materials.

Acknowledgements

This study was supported by The Key Foundation of Southwest Hospital (SWH2016ZDCX2014).

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