Preparation of Chitin-graphene Eco-friendly Dressing and Its Effect on Wound Healing

Daijun Zhou[1,2], Tao Yang[1,2,#], Gaoxing Luo[1,2,*]

[1.]Institute of Burn Research; State Key Laboratory of Trauma,Burn and Combined Injury; Key Laboratory of Proteomics of Chongqing, Southwest Hospital, Third Military Medical University, Chongqing 400038, China.

[2.]Southwest Hospital, Third Military Medical University, 30 Gaotanyan Main Street, Shapingba District, Chongqing 400038, China.

#Co-first author: Tao Yang

*Corresponding to: Gaoxing Luo

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Abstract. The aim of this paper is to prepare the Chitin-graphene eco-friendly dressing, and also divided into four groups. Group Control was Chitin and Group A was Chitin+dopamine glue, respectively, at 0.2ug/ml, 1ug/ml, 5ug/ml (Namely group B, C and D). The precipitation of the adherent composite ions could be found under 5*103 times microscopy in group D. the contact angle test showed that the material was hydrophilic. The drug loading rate in group BCD was 30-40 (ug:mg), and entrapment efficiency was 70%-80%.there was no difference between groups (P>0.05).the cumulative release percentages were 86.7%, 88.4% and 89.6% after 72h in group B,C and D, respectively . After 7 days of injury, the wound healing rate was D > C > B > A > Control (P < 0.05), and the length of neonatal epithelium also showed the same trend.

Introduction

Chitin is a natural polysaccharide made of the N-acetyl-2-amino-2-deoxy-D-glucose linked through 13-1,4 glycosidic linkages. As a natural biopolymer, chitin is widely found in the shell of lower animals, especially crustaceans, and the cell wall of lower plants, bacteria, and algae. The production of chitin is only second to cellulose in nature[1,2].

Therefore, the combination of chitin and graphene has become a hot research field not only because of the wide range and environmental protection of natural chitin sources, but also the antibacterial effects[3,4] . Here we prepared the chitin-graphene eco-friendly dressing, and studied the anti-bacterial activity in vitro and effects on wound healing in mice, so that we could provide more reference for further study of natural dressings.

Materials and Methods

Ethical Statement

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) .

Preparation of materials

The graphene are attached to chitin by dopamine cross-linking,also divided into five groups. Group Control was Chitin and Group A was Chitin+dopamine gule , respectively, at 0.2ug/ml, 1ug/ml, 5ug/ml (Namely group B, C and D)

Scanning electron microscopy (SEM)

The samples of the material wafers were carefully washed with deionized water, dried and sprayed, and the aperture structures of the films were observed under vacuum condition by scanning electron microscope and photographed.

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.

Drug loading and encapsulation efficiency

(1) determination of standard curve

Weigh the graphene 50mg with a balance and dissolve it in the 5mlPBS buffer as the mother liquid (10mg/ml). Then the corresponding PBS were diluted to 3 concentration (i.e. 2.5mg/ml, 5mg/ml, 10mg/ml), the absorbance of graphene solution with different concentration at 450nm was determined by ultraviolet spectrophotometer, using graphene concentration as abscissa, absorbance as ordinate drawing standard curve, and the linear regression equation.

(2) determination of drug loading and encapsulation efficiency

Weigh the BCD group 50mg, liquid nitrogen, frozen powder, and then use ultrasonic dispersion to 5ml PBS buffer, time 60min. Fetch the supernatant after centrifugation, the absorbance of solution with different concentration at 450nm was determined by ultraviolet spectrophotometer, a standard curve to calculate the concentration , and thus calculate the entrapment efficiency and drug loading.

Drug loading = actual concentration * volume (UG) / dissolved total mass (mg);

Encapsulation efficiency = actual concentration / theoretical concentration

Drug release in vitro

Weigh the BCD group 50mg, added to the 5mlPBS buffer, and then placed in a constant temperature shaking table for culture (37 70r/min). At a specific point in time (0.5h, 12h, 24h, 48h, 72h,96h), the tubes were taken out. The upper release liquid 2ml was absorbed with a pipette and the absorbance was measured by UV spectrophotometer. 2ml fresh PBS solution was added to the test tube to keep the total volume of the system unchanged. During the test, a fresh PBS buffer solution was used in a cuvette as blank control.

According to the standard curve, the measured concentration was calculated, and the release amount in each period was calculated. Finally, the cumulative release amount was calculated.

Mice full-thickness skin defect wound model

Mice (male, weighing about 25 g) were obtained from the Third Military Medical University Institute of Animal Research, a total of 25, each group of 5. The animals were all housed in a SPF grade rearing room at room temperature of 25 ℃, in which the relative humidity is 50 % and the circadian rhythm is 12 hours. Before the start of experiments, the mice were housed in a single cage

and adapted to it one week in advance. All experiments were conducted in accordance with the relevant ethical requirements of the laboratory animal ethics committee of Third Military Medical University.

The wound healing rate

The wound healing rate refers to the wound area of the origin and each phase after the injury, which could be measured through IPP6.0 software, using the AOI function to select the wound area, measuring its pixel area by “ count size " and calculating out the wound area. Wound healing rate = (original wound area - wound area on residual day after injury) / original wound area x 100%

HE staining

At 3 and 7 days post injury, the specimens of the wound tissue were obtained, and the paraffin sections were prepared. HE staining was performed as universal methods. The neonatal epithelial length was measured by different pathologists.

Results

Electron microscopy scanning

As shown in figure 1, the chitin in group Control retained the complete structure of the shell and membrane with clear texture under different magnification. In group D, precipitated and bedding graphene was visible under D3.

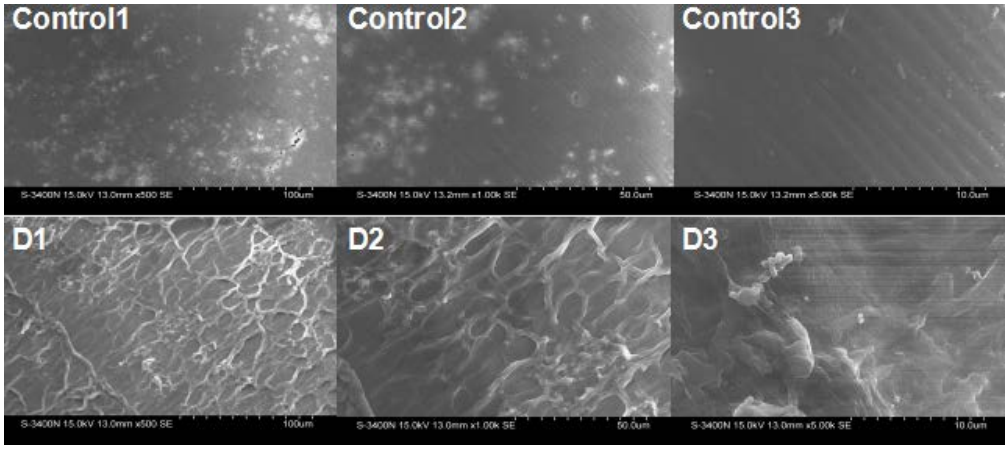

Figure 1. In the first row of chitin group (group Control), from left to right: Control 1 (5x 102 times), Control 2 (1 x 103 times), Control 3 (5x 103 times); In the second row of Chitin-nanosilver (group D), from left to right: D1 (5x 102 times), D2 (1 x 103 times), D3 (5x 103 times).

Contact angle test

As shown in Figure 2, the average contact angle (θ) of Control is 75.8±11.3°,A is 71.4 ± 10.6°,D is 68.3±10.1°, the difference was not statistically significant (P>0.05).

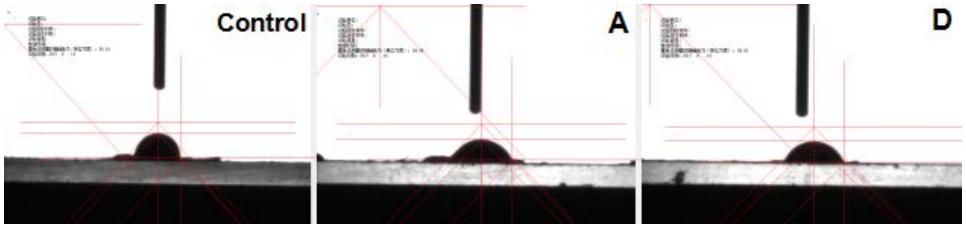

Figure.2 Compared with the the average contact angle of three groups, the difference was not statistically significant (P>0.05).

Enzyme loading and encapsulation efficiency

As shown in Table 1, The drug loading rate in group BCD was 30-40 (ug:mg), and entrapment efficiency was 70%-80%.there was no difference between groups (P>0.05).

Table 1 drug loading and encapsulation efficiency in different groups

	B	C	D
Drug loading（μg/mg）	31.66±3.82	34.50±3.97	39.17±4.30
Encapsulation efficiency（%）	70.28±6.85	74.20±7.08	78.63±7.27

Release in vitro

As shown in figure 3, The release rate of BCD group tended to be stable and maximum at 72h, and the cumulative release percentages were 86.7%, 88.4% and 89.6% after 72h in group B,C and D, respectively .

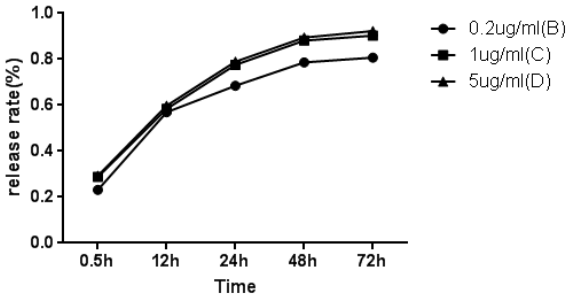

Figure 3, The release rate of BCD group tended to be stable and maximum at 48-72h.

Effect on Infectious wound healing

As shown in figure 4 , the wound healing rates of the Control, A, B, C and D groups respectively were 18.2 %, 25.6 %, 30.7 %, 36.2 % and 40.8 % after 3 days of injury, and 36.7 %, 47.5 %, 58.3 %, 66.7 % and 76.0 % respectively after 7 days of injury, namely, D > C > B > A > Control($P < 0.05$).

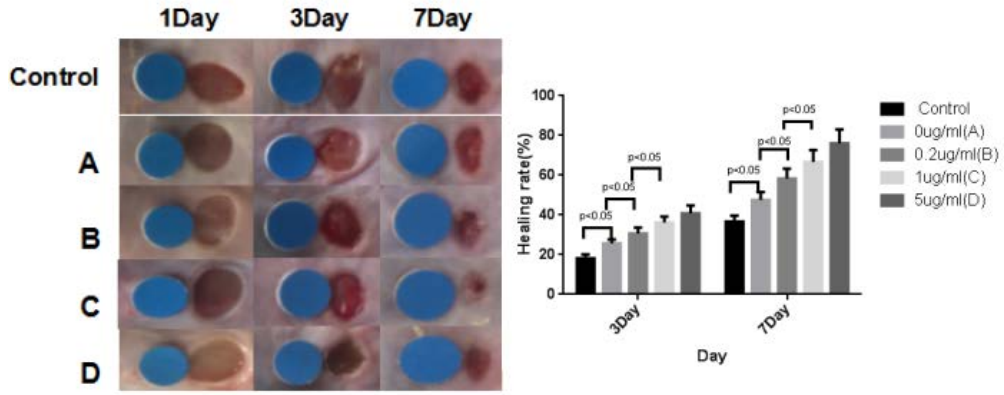

Figure 4. After 7 days of injury, the wound healing rates of five groups were D > C >B > A > Control

New epithelial length

As shown in figure 5, there were no length difference among groups after 3 days of injury ($P > 0.05$), while the wound epithelial lengths of blank, A, B, C, D groups after 7 days injury were respectively 618.0 μm, 689.7 μm, 749.3 μm, 814.4 μm and 890.5 μm, namely, D > C > B > A > Control, and the difference was not significant ($P > 0.05$).

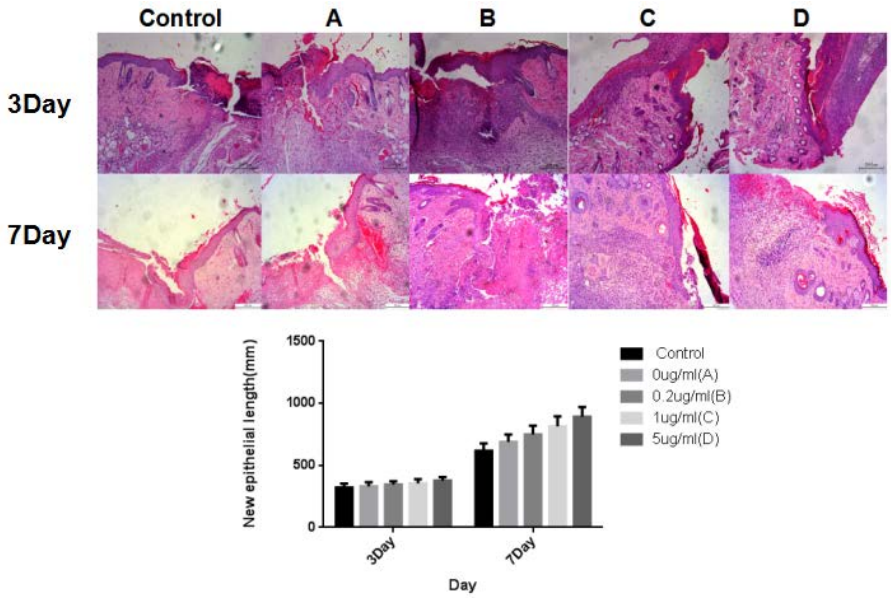

Figure 5. HE staining, the length of the arrow represented the length of the New epithelial.

Discussion

As shown in figure 1, the chitin in group Control retained the complete structure of the shell and membrane with clear texture under different magnification. In group D, precipitated and bedding graphene was visible under D3. The reason for this is that the structure of chitin itself has been changed by the composite reaction, so that the graphene is effectively attached to it, which is consistent with the structure observed by Gopi and Sahraee using scanning electron microscopy[5,6].the average contact angle (θ) of Control is 75.8±11.3° ,A is 71.4 ±10.6° ,D is 68.3±10.1° ,the difference was not statistically significant (P>0.05).all of them are hydrophilic, indicating that the Preparation has not changed the characteristics of the material itself[7,8].

As shown in Table 1, The drug loading rate in group BCD was 30-40 (ug:mg), and entrapment efficiency was 70%-80%.there was no difference between groups (P>0.05)., which showed that this method was effective for graphene embedding.The release rate of BCD group tended to be stable and maximum at 72h, and the cumulative release percentages were 86.7%, 88.4% and 89.6% after 72h in group B,C and D, respectively , As for the release of drugs,BCD release faster in the first 24h, and this obvious early release phenomenon may be possible18,20. After 72h, the amount of released from chitin remained almost unchanged, indicating that its release had stabilized and reached its maximum[9,10].

In this study, a mature mouse wound infection model was used, that is, mixed liquid of common gram-positive bacteria(Staphylococcus aureus)and gram-negative bacteria (Escherichia coli) dropped onto the wound infection[11,12]. the wound healing rates of the Control, A, B, C and D groups respectively were 18.2 %, 25.6 %, 30.7 %, 36.2 % and 40.8 % after 3 days of injury, and 36.7 %, 47.5 %, 58.3 %, 66.7 % and 76.0 % respectively after 7 days of injury, namely, D > C > B > A > Control($P < 0.05$). This suggested that the graphene played a major antimicrobial role in the wound surface during the initial period (0-3 days), that is, to mainly control the infection[13,14]. There were no length difference among groups after 3 days of injury ($P > 0.05$), while the wound epithelial lengths of blank, A, B, C, D groups after 7 days injury were respectively 618.0 μm, 689.7 μm, 749.3 μm, 814.4 μm and 890.5 μm, namely, D > C > B > A > Control, and the difference was not significant ($P > 0.05$).The results above suggested that chitin compound

graphene could accelerate the healing of the wound by controlling infection and antibacterial adhesion at the same time in the late period of injury[15].

In summary, Chitin-graphene Eco-friendly Dressing was successfully prepared and Its Effect on Wound Healing has been Confirm. However, whether it has other mechanisms to promote re-epithelialization remains to be further studied.

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