Nanosilver coated Chitin derived membrane as an Eco-friendly Dressing and Its Effect on Wound Healing

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Abstract. This study aims to prepare an eco-friendly dressing using Chitin derived membrane with Nanosilver included for anti-bacterial purpose,and also divided into five groups.Group Control was Chitin and Group A was Chitin+dopamine gule (methods for mussel-inspired adhesion of Nanosilver) , respectively, at 0.5mmol/L, 1mmol/L, 2mmol/L (Namely group B, C and D). We found Nanosilver spherical particles formed on chitin dressing. The contact angle test shows that the material was still hydrophilic after processing. Cell staining showed no obvious toxicity in each group.On day 7, the healing rates are 35.2 %, 45.8 %, 56.7 %, 67.9 % and 73.8% in Group Control, A, B, C and D, respectively.The wound healing rate is higher in the C and D groups than these in the other three groups ($P < 0.05$). Similarly, the lengths of the new epithelialm were 618.5 μm, 703.7 μm, 810.2 μm, 879.4 μm and 951.0 μm. in the five groups, respectively. These in group C and D were higher than those in the other three groups ($P < 0.05$).

Introduction

Chitin is widely used in the medical field, because of good biocompatibility, no toxicity, low cost, easy modification, good mechanical strength, broad-spectrum antibacterial activity and so on[1,2]. However, there are regular ring structures and strong hydrogen bonds among chitin molecules, so that it has high crystallinity and poor solubility in water, dilute alkali, dilute acid and general organic solvents, which greatly limits its application[3,4]. To overcome these shortcomings, chitin can be quaternized ammonium salt, which is very important to its chemical modification[5,6]. Therefore, the combination of chitin and Nanosilver has become a hot research field not only because of the wide range and environmental protection of natural chitin sources[7,8]. Here we prepared the chitin-amphipathic anion/quaternary ammonium salt 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.

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Materials and Methods

Preparation of materials

The silver nanoparticles are attached to chitin by dopamine cross-linking,also divided into five groups.Group Control was Chitin and Group A was Chitin+dopamine gule (methods for mussel-inspired adhesion of Nanosilver) , respectively, at 0.5mmol/L, 1mmol/L, 2mmol/L (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.

Assay of cytotoxicity

Normal primary neonatal mice were used to isolate primary fibroblasts, which were used when cells were transferred to the 2nd and 3rd generations. The cells were counted and the required amount was calculated at 2000 cells per well using DMEM medium in 96-well plates. Each group of materials was placed into 12 parallel wells (measured four times, on day 1, day 3, day 5, day 7). Each well was added 150 μl configured medium and placed in 37 ℃ incubator. The operations were repeated three times.

The wound healing rate

One day before the experiment, 1% pentobarbital sodium was injected intraperitoneally (70 ul/g) and then subjected to depilation. The next day, after the mice were anaesthetized again and disinfectedont the back, two a round full layer of skin defect wounds in diameter of 0.6mm on left and right back symmetrically with a hole punch.. S.aureus and E. coli which had been prepared by co-culture were infused about 5 μl for each wound. The bacterial concentration was 108/ml. Clean materials (disinfected with 75% alcohol, rinsed with PBS buffer to completely remove the alcohol) were fixed on the wound with the adhesive towel. On 0, 1, 3, 5, and 7 days, photos of the wound were captured and materials were refreshed.

Preparation Mice full-thickness skin defect wound model,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 nanosilver layer was visible under D3.

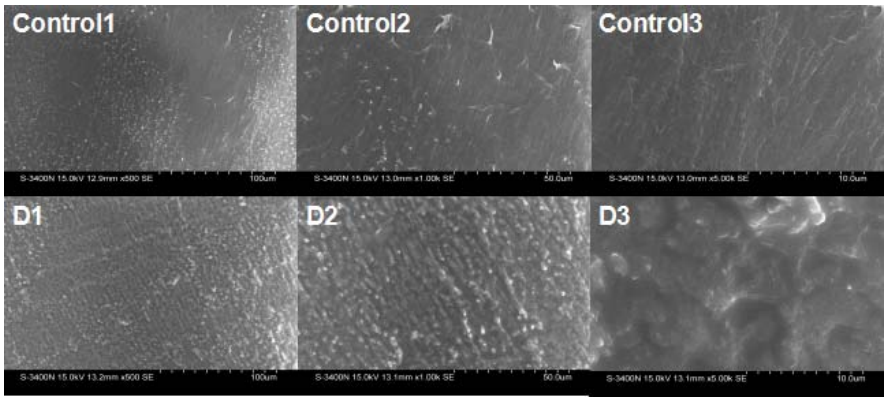

Figure 1. In the first row of chitin group (group Control), from left to right: Control 1 (5x 102 times), Control 2 (1 x 103 times), Control 3 (5x 103 times); In the second row of Chitin-nanosilver (group D), from left to right: D1 (5x 102 times), D2 (1 x 103 times), D3 (5x 103 times).

Contact angle test

As shown in Figure 2, the average contact angle (θ) of Control is 75.8±11.3° ,A is 79.3 ± 11.9° ,D is 85.6±12.5° , the difference was not statistically significant (P>0.05).

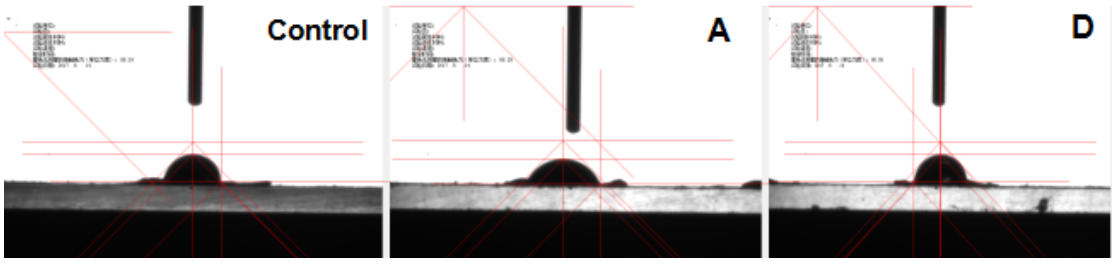

Figure.2 Compared with the the average contact angle of three groups, the difference was not statistically significant (P>0.05).

Test of cytotoxicity

Known from table 1 and figure 3, the 5 groups basically showed no proliferation inhibition (P > 0.05) to cells during 1-7 days.

Table 1. Changes of OD value in dressings affecting the proliferation of mice fibroblast cells.

	1Day	4Day	7Day
Control	0.263±0.018	0.507±0.038	0.705±0.044
A	0.257±0.017	0.518±0.032	0.695±0.041
B	0.273±0.016	0.517±0.036	0.688±0.040
C	0.255±0.015	0.510±0.034	0.680±0.040
D	0.266±0.014	0.512±0.035	0.672±0.038

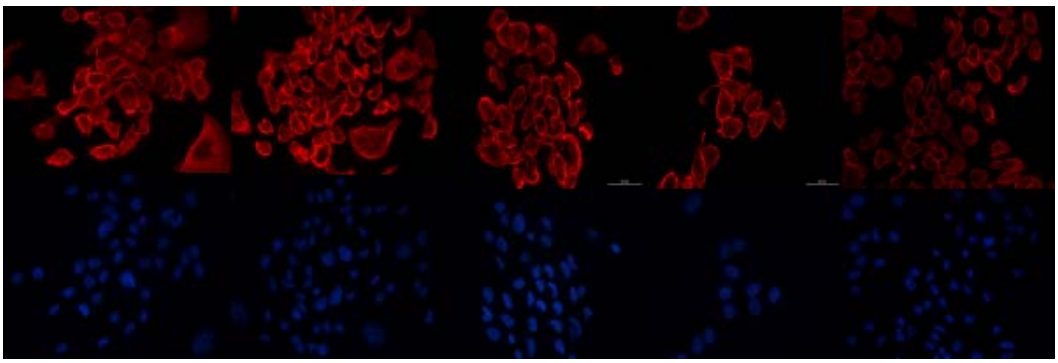

Figure 3. There was no significant proliferation inhibition in 4 groups during 1-7 days *(P >0.05)*.

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 18.5 %, 24.6 %, 30.1 %, 37.8 % and 41.3 % after 3 days of injury, and 35.2 %, 45.8 %, 56.7 %, 67.9 % and 73.8% respectively after 7 days of injury. namely, D > C > B > A > Control (P > 0.05).

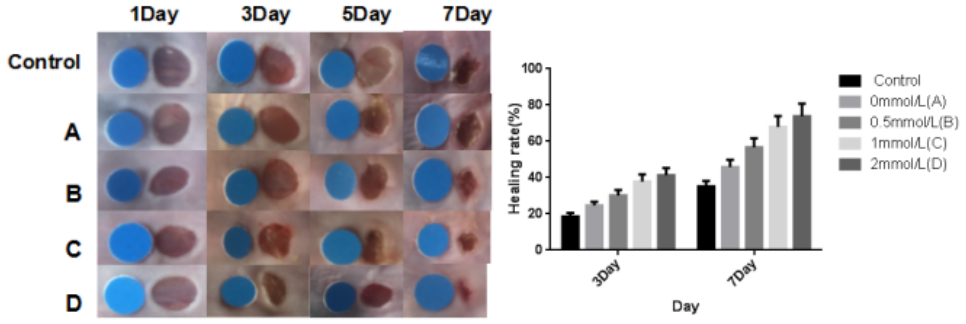

Figure 4. After 7 days of injury, the wound healing rates of five groups were D > C >B > A > Control

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 618.5 μm, 703.7 μm, 810.2 μm, 879.4 μm and 951.0 μm, namely, D > C > B > A > Control(P<0.05).

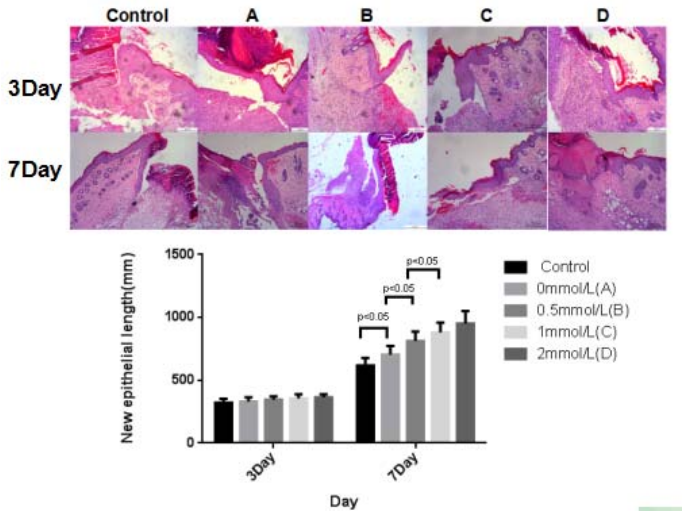

Figure 5. HE staining, the length of the arrow represented the length of the New epithelial.

Discussion

In recent years, the use of chitin and nanosilver in medicine has attracted much attention, which can be used to develop absorbable surgical sutures, medical dressings, artificial skin, cell-bound drugs , anticoagulants and artificial kidney membranes and so on [9,10]. 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 nanosilver layer 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 nanosilver is effectively attached to it, which is consistent with the structure observed by Gopi and Sahraee using scanning electron microscopy [11,12].the average contact angle (θ) of Control is 75.8±11.3° ,A is 79.3 ±11.9° ,D is 85.6±12.5° , 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. This may be related to the internal hydrophobicity of chitin and the basic structure of external hydrophilic [13,14].

As for cytotoxicity, the 5 groups basically showed no proliferation inhibition (P > 0.05) to cells during 1-7 days. This may be related to some characteristics of chitosan and nanosilver, for example, they are all safe, and non-toxic, so that they can be widely used in the food industry [15,16]. the wound healing rates of the Control, A, B, C and D groups respectively were 18.5 %, 24.6 %, 30.1 %, 37.8 % and 41.3 % after 3 days of injury, and 35.2 %, 45.8 %, 56.7 %, 67.9 % and 73.8% respectively after 7 days of injury. namely, D > C > B > A > Control (P > 0.05). This suggested that the nanosilver played a major antimicrobial role in the wound surface during the initial period (0-3 days), that is, to mainly control the infection. If the two materials acted synergistically, such as in group D, the bacterial growth ccould be maximized inhibition. The process of wound healing consists of two parts, namely, wound contraction and wound epithelialization. For compact skin species (e.g. human beings), the wound healing mainly depends on the re-epithelialization of the wound surface[17]. There was no difference in the length of neonatal epithelium in each group after 3 days of injury. This was mainly due to the fact that the edge of the wound was still mainly in the preparation period of cell proliferation and migration, and the neonatal epithelium was not obvious[18]. 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 618.5 μm, 703.7 μm, 810.2 μm, 879.4 μm and 951.0 μm, namely, D > C > B > A > Control(P<0.05). The

results above suggested that chitin compound nanosilver could accelerate the healing of the wound by controlling infection and antibacterial adhesion at the same time in the late period of injury (7 days after injury).

In summary, in this study Nanosilver coated Chitin derived membrane as an Eco-friendly Dressing and Its Effect on Wound Healing was successfully prepared.this providing new means and ideas for wound application of natural dressing.

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