Chitin-based Wound Dressing

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Introduction

Many studies to modify natural polymers have been tried to utilize chemical modification or physical methods. Among these works, hydrogel types and interpenetrating polymer network(IPN) structures have been noted by many researchers.[1,2,3] Hydrogels are generally formed from water soluble polymers by crosslinking either by radiation and chemical method or by polymerizing hydrophilic monomers in the presence of crosslinkers. Semi-IPNs are defined as composition in which one or more polymers are crosslinked and are linear or branched.

In this study semi-IPNs hydrogels based on β-chitin were synthesized and characterized for application to wound dressing. [4, 5]

A variety of temporary wound dressings have been developed, both nonmedicated and medicated, and some have been successful clinically.[6,7,8] Commercially available synthetic wound dressings consisting of a polyurethane membrane are capable of minimizing evaporative water loss from the wound and preventing bacterial invasion and thus are useful in the management of superficial second-degree burns. They are of no use, however, in the treatment of deep second-degree and third-degree burns. The ideal structure of a bilaminated dressing consists of an outer membrane and an inner three-dimensional matrix of fabric or sponge. The outer membrane prevents body fluid loss, controls water evaporation, and protects the wound from bacterial invasion; the inner matrix encourages wound adherence by tissue growth into the matrix. In the management of deep second-degree or third-degree burns, the drainage of exudate must be taken into account. In addition, the concept of the drug delivery system has been introduced to burn care, and antibacterial drug-impregnated wound dressings are proven effective in controlling bacterial invasion even through a porous matrix. The present work focuses on the development of a synthetic wound dressing with bactericidal capacity and reports on a evaluation of *in vitro* and *in vivo* test for wound dressing.

Experimental Methods

Synthesis of semi-IPNs

2 x 10^{-3} mol of purified PEG (Mn=6000) was dissolved in 150ml of benzene in a 500ml of round-bottomed flask. 0.49 ml of triethylamine and 0.57ml of acryloyl chloride were added to the flask, and the reaction mixture was stirred for 3h at 80 °C. The reaction mixture was filtered to remove triethanolamine hydrochloride, and the macromer was obtained by pouring the filtrate in a large excess of n-hexane. Then, 2.4% (w/w) of formic acid solution of β-chitin and PEG macromer was bubbled for 30minutes with a nitrogen flow. To this solution was added the initiator solution(0.3g of 2,2-dimethoxy-2-phenylacetophenone dissolved in 1 ml of N-vinylpyrrolidone) and was poured onto sealed

glass mold and irradiated using a UV lamp until gelation occurred.

Sponge types of hydrogels impregnating AgSD

β-Chitin and antimicrobial agent, silver sulfadiazine (AgSD), is formic acid. This solution is cast on glass plate and precipitated into acetone. And then, gel obtained is immersed into EtOH-H_2O bath. After a day, gel is washed to remove residual solvents and sufficiently swelled to reach equilibrium. Finally, the product frozen at -70 °C is followed by freeze-drying. Another samples in this study are also prepared in a similar manner.

Antibacterial test on agar plate

The antibacterial efficiency of wound dressing material were studied on blood agar plate. A piece of wound dressing material are placed on a blood agar plate (90mm in diameter) seeded with Pseudomonas aeruginosa (Ps.a.) at a density of 10^7 Ps.a./cm^2 in a manner similar to a method that has been described in a previous article. A piece of wound dressing material (1cm x 1cm) are then placed on a bacterial-seeded agar plate and left in a incubator at 37 °C for 2 days. After period of 2 days, the samples are removed, and then 1 cm^2 of the agar immediately beneath the samples are cut out and homogenized in 10ml of a sterile saline at 15,000 rpm. The resulting solution is inoculated on a new blood agar plate. After 1 day of culture at 37 °C, the forming bacterial colony are counted, and the number of bacteria beneath the sample is expressed in the number of bacteria per 1cm^2. As a control, the number of bacteria on an agar without coverage is calculated in a similar manner.

In vivo animal test

The full thickness skin wound were prepared by excision (1cm in diameter) of the dorsum of wistar rat. An then, the excised wound is covered with different dressing materials. As a control, conventional vaseline gauze dressing is applied on the same experimental condition. The rats of each group are sacrificed 5 and 10 days after application, at which time each wound surface is observed and the central portion of underlying tissue is biopsied, fixed in 10 % formaldehyde, stained with hematoxylin-eosin, and the wound healing process is examined histologically.

Results and Discussion

Swelling kinetics of semi-IPN hydrogels are plotted in *Figure 1*. All hydrogels swelled so rapidly and reached an equilibrium within 20 minutes. In our previous studies, EWC of β-chitin was around 48%. As PEG macromer was incorporated in semi-IPN, EWC of semi-IPN increased to 60-81% and increased with β-chitin content. Since PC1-1 possesses rich crosslinkable end groups, crosslinking degree may be the highest among the samples, resulting in the lowest EWC of PC1-1. This behavior is in a general agreement with previous results for IPN hydrogels, which have shown that high crosslinking density leads to a low water content.

Mechanical strength of semi-IPN hydrogels is shown in *Table 1*. The tensile strength of semi-IPN hydrogels both at dry and wet state increase with β-chitin content. In the dry state, the mechanical strength of the semi-IPN hydrogels turned out to be relatively strong. In addition, their mechanical strength was retained even in the highly swollen state.

As might be expected, the lower amount of crosslinkable end groups led to higher EWC. However, regardless of increasing EWC, the tensile strength of semi-IPN hydrogels in the dry and wet states was still enhanced.

Water vapor transmission rates (WVTR) of some wound dressing materials are shown in Figure 2. In general, WVTR of natural human skin is 200-500 g/m^2/day. The evaporative water loss from burnt skin is 20 times the rate through intact skin. Wound surfaces contain relatively more water and therefore it is necessary to evaporative water through the wound covering in order for the covering to adhere to the wound surface. Fluid-filled pockets between the wound and covering lead to infection. The covering must have higher WVTR than normal skins. WVTR of samples prepared in this study is calculated in the range of between 2400 and 2800 g/m^2/day.

Figure 3 illustrates the bactericidal capacity of some wound dressing materials. It is noteworthy that antibacterial test of AgSD-impregnated ones on agar plate reveal perfect supression against bacteria, *pseudomonas aeruginosa*, up to 7 days. Moreover, histological studies confirm the proliferation of fibroblasts and the distinct reduction of infection cells.

Conclusions

To prepare chitin-based wound dressing material β-chitin and PEG diacrylate macromer were synthesized by UV irradiation method and their properties were studied. Hydrophilic PEG diacrylate macromer segments were crosslinked and formed a network structure with β-chitin. All hydrogels exhibited relatively high EWC in the range of 60-81%. Mechanical strength were much higher with increasing β-chitin content. WVTR of samples prepared in this study is calculated in the range of between 2400 and 2800 g/m^2/day. Note that antibacterial test of AgSD-impregnated wound dressing materials on agar plate reveal perfect supression against bacteria, *pseudomonas aeruginosa*, up to 7 days. Moreover, histological studies confirm the proliferation of fibroblasts and the distinct reduction of infection cells.

References

1. N. P. Desai and J. A. Hubbell, *Macromolecules*, **25**, 226, 1992

2. A. S. Sawhney, C. P. Pathak and J. A. Hubbell, *Macromolecules*, **26**, 581, 1993

3. S. S. Kim, Y. M. Lee and C. S. Cho, *J. Polym. Sci.: Polym. Chem. Ed.*, **33**, 2285, 1995

4. S. S. Kim, Y. M. Lee and C. S. Cho, *Polymer*, **36**, 4497, 1995

5. Y. M. Lee and S. S. Kim, *Polymer*, ***in press***

6. M. J. Tavis *et al. Burns*, **7**, 123, 1980

7. M. Chvapil *et al. J. Biomedcal. Mater. Res.*, **16**, 245, 1982

8. Y. Kuroyanagi *et al. J. Appl. Biomater.*, **3**, 153, 1992

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Table 1. Mechanical properties of semi-IPN hydrogels

Sample	EWC (%)	Tensile strength(Mpa) Dry	Wet
PC1-1	60	21.8	1.35
PC1-2	73	33.1	2.12
PC1-3	81	35.0	2.41

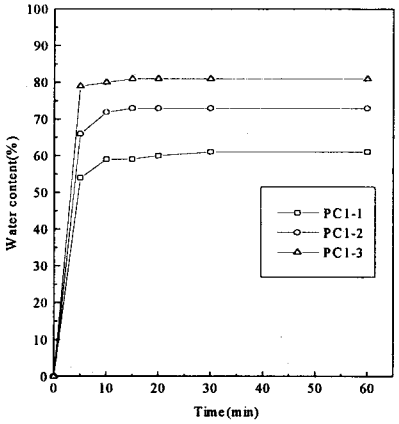


Fig 1. Swelling kinetics of semi-IPN hydrogels

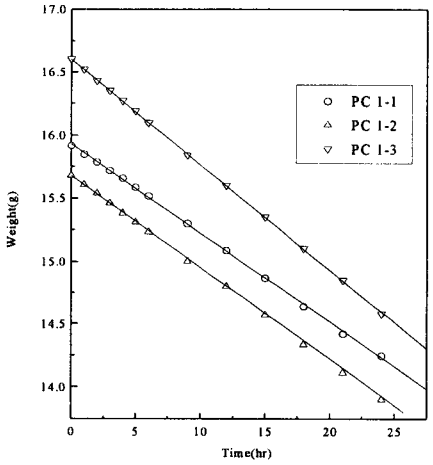
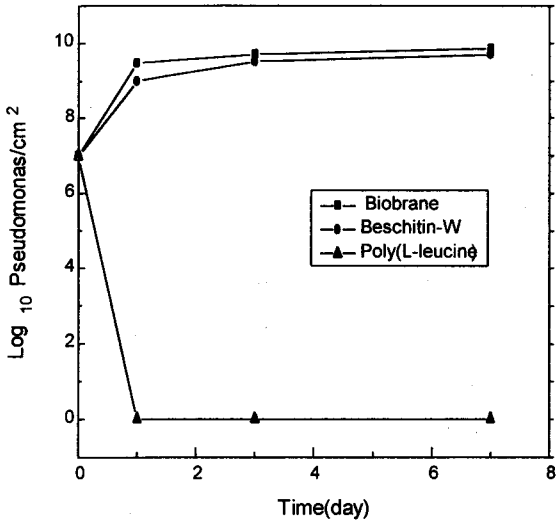


Fig 2. WVTR of semi-IPN hydrogels

Commercialized product



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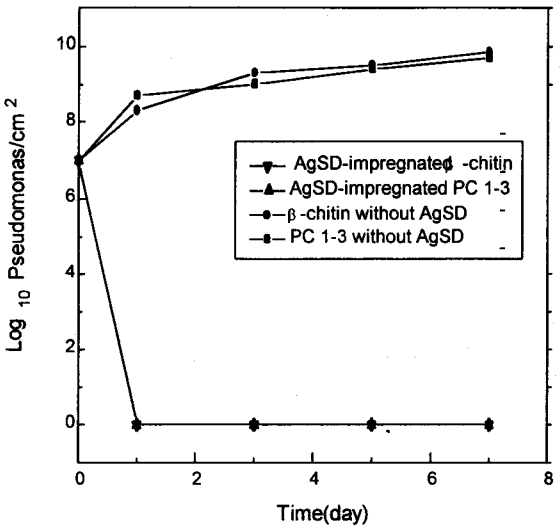

Fig 3. Antibacterial test results of some wound dressing materials