β-Chitin-based wound dressing containing silver sulfurdiazine

YOUNG MOO LEE*, SEONG SOO KIM
School of Chemical Engineering, College of Engineering, Hanyang University, Seoul 133-791, Korea

MOON HYANG PARK, KANG WON SONG
Department of Pathology, College of Medicine, Hanyang University, Seoul 133-791, Korea

YONG KIEL SUNG
Department of Chemistry, College of Natural Sciences, Dongguk University, Seoul 100-715, Korea

IN KYU KANG
Department of Polymer Science, College of Engineering, Kyungpook National University, Taegu 702-701, Korea
E-mail: ymlee@email.hanyang.ac.kr

Physical and biological properties of some wound dressing materials based on β-chitin were studied. Water vapor transmission rates (WVTR), oxygen permeabilities and biodegradation kinetics were examined for film-type samples. WVTR of samples was in the range 2400–2800 g/m^2/day. However, oxygen permeabilities of the samples were relatively low. To improve oxygen permeabilities, porous sponge-type wound dressing materials were prepared. In addition, these sponge-type samples contained antimicrobial agents, silver sulfadiazine (AgSD), in order to prevent bacteria infection on a wound surface. Antimicrobacterial tests on agar plate were carried out to confirm the bactericidal capacity of present materials. These materials impregnating AgSD had the complete bactericidal capacity against *pseudomonas aeruginosa* up to 7 days. Finally, a wound healing effect of β–chitin-based semi-interpenetrating polymer networks was evaluated from the animal test using the wistar rat *in vivo*. Histological studies confirm the proliferation of fibroblasts in the wound bed and a distinct reduction in infectious cells.

1. Introduction

In the treatment of burn wounds or extensive skin loss, there has been a need to search for a novel method during the past decades. Various natural and synthetic polymers with good biocompatibility have been used in order to develop wound dressing materials. Many types of materials, including traditional absorbent or impregnated dressings, synthetic dressings such as semipermeable films, foam dressings, hydrogels, hydrocolloids and xerogels and biological dressings created wholly or in part from human or animal tissue, are currently applied to lesions characterized by skin loss [1]. The objective of the wound dressing material employed is to accelerate wound healing by preventing fluid loss and bacteria infection.

Generally, if severe burns or extensive skin loss have taken place, a large amount of fluid loss and bacteria infection leads to serious results. Therefore, among the general properties required for a successful burn wound covering, control of evaporative water loss and prevention of bacteria infection are the most important factors. As the wound suface contains relatively more water, it is necessary to evaporate water through the wound covering in order for the covering to adhere to the wound surface. In addition, exudates between the wound and covering result in infection. Acute inflammatory cells keep the wound from healing. Therefore, antimicrobial agent-impregnated materials are required and wound dressing materials developed in recent years meet these requirements [2, 3].

Recently, a wound dressing materials using poly-(amino acid) was successfully developed. Kuroyanagi *et al*. prepared poly(L-leucine) sponge (XEMEX Epicuel^R) impregnating antimicrobial agent [4]. This material effectively controlled evaporative water loss or body fluid and supressed bacteria infection. Biobrane^R by Hall Woodroof, Inc. is a composite of ultra thin silicone rubber and ultramicro nylon knit containing collagen [5]. It had flexibilty, elasticity and adherence. The permeation capacity of an antimicrobial agent through lesion is one of the critical features. It was reported that wound dressing materials using chitin facilitated wound healing

*Author to whom all correspondence should be addressed.

in clinical cases [6]. Beschitin W^R commercialized by Unitika in Japan is a non-woven fabric type of α-chitin.

For an ideal wound dressing, consequently, materials should have flexibility, durability, adherence, a capability of absorbing wound debris and to protect the lesion from dehydration. From the biological standpoint, wound dressing materials should have the absence of antigenicity, local and systemic toxicity. In the engineering respect, they should also be easy to handle and to apply, comfortable when in place and cost-effective.

In this study, the physicochemical properties of some β-chitin-based semi-interpenetrating polymer networks (semi-IPNs) hydrogels synthesized in our previous studies were investigated for wound dressing [7, 8]. The objective of the present investigation is to prepare a novel sponge-type wound dressing material impregnating antimicrobial agent. The ultimate goal of this study is to evaluate the bactericidal capacity and wound healing effect of these materials.

2. Experimental

2.1. Materials

Silver sulfadiazine (AgSD) was supplied by Dong Wha Pharmaceutical Co., Ltd (Seoul, Korea). Ethyl alcohol and acetone were purchased from Duksan Pharmaceutical Co., Ltd. Lysozyme was a commercial product of Sigma Co. and used without further purification. Fine materials were selected among many samples to investigate the physicochemical and biological properties for wound dressing. They were PC 1–1, PC 1–2, PC 1–3, L–6–3, C–6–3. Table I lists the composition of the samples. See references [7] and [8] for detailed preparation of samples.

2.2. Preparation of sponge-type wound dressing materials impregnating AgSD

Blend solutions containing β-chitin and poly(ethylene glycol) macromer were cast onto a glass mold and irradiated using a 450 watt UV lamp (Ace Glass Co.), and placed above the mold at a height of 20 cm for 1 h until gelation occurred. At this stage, since AgSD is sensitive to UV irradiation, care must be taken to wait for about 2 h to prevent the degradation of AgSD. After irradiation, AgSD was added and partially gelled solutions were precipitated into an acetone bath for coagulation. Gels obtained were washed in an ethanol-water (50/50 w/w) bath. These gels were sufficiently swollen to reach an equilibrium and to remove uncrosslinked PEG segments in a deionized water bath. Finally, they were freeze-dried for 2 days after freezing at $-70\,^{\circ}C$.

2.3. Water vapor transmission rate (WVTR) measurement

The film was placed between two plastic chambers and the surface of the film was kept in contact with water. After weighing, a cell was put in a thermostatic oven at 37 °C and 51% relative humidity. During the initial test period, weight loss was measured every hour. As the changes in weight loss became constant, a test was carried out every 3 h up to 24 h. Effective membrane area was $7.065 \times 10^{-4}\,m^2$ and the thickness of the membranes between 45 to 80 μm.

2.4. *In vitro* biodegradation

Samples with a thickness of 45–80 μm were cut into small pieces (1 cm × 1 cm) and immersed in 1 mg/ml of phosphate buffer saline (PBS) lysozyme solution (pH 7.4) in vial. The vial was stored in a constant temperature bath maintaining 37 °C. After incubation, the film was repeatedly washed with ethanol and dried at 40 °C under reduced pressure (10^{-2} mm Hg). Weight loss of the film was measured and plotted against time.

2.5. Antibacterial test

A bactericidal capacity of sponge-type wound dressing materials impregnating 0.4 mg AgSD cm^{-2} was investigated on an agar plate. *Pseudomonas aeruginosa* (Ps. a.) was seeded for inoculation followed by incubation. An initial seeding density of Ps. a. calculated from the McFarland nephelometer method [9] was determined to be 1×10^7 Ps. a./cm^2. A piece (2 cm × 2 cm) of wound dressing material was placed on a bacteria-seeded agar plate and kept in an incubator at 37 °C for 1–7 days. After a given incubation time, the sample was removed and 1 cm^2 of agar beneath the sample was cut out followed by homogenization in a sterile saline solution of 10 ml. The resulting solution was repeatedly diluted to a 1/10 concentration. 1 ml of the dilute solution was inoculated on a new blood agar plate. Finally, bacterial colony formed beneath the wound dressing material was counted. A bacterial capacity of the present study was compared with that of the commercial products.

2.6. Animal test

A full thickness skin wound of 1 cm in diameter was prepared by excizing the dorsum of the wistar rat. The excized wound was covered with various wound dressing materials with or without AgSD. Then, a sterilized elastic band was employed to fix the materials. As a control, vaseline gauze was applied on a skin wound. At the fifth and twelfth postoperative day, the wistar rats were

TABLE I Sample designation and preparation

Sample*	Lactone in PEG macromer	PEG macromer (wt %)	β–chitin (wt %)
PC 1–1	–	50	50
PC 1–2	–	33	67
PC 1–3	–	25	75
L–6–3	D,L-lactide	25	75
L–6–3	ε–caprolactone	25	75

*Molecular weight of PEG = 6000.

sacrificed and dressings removed. A fixation in 10% formaldehyde was immediately carried out after macroscopic observation. A tissue of skin wound was biopsied followed by staining with hematoxylin-eosin. The resulting wound healing effect was histologically investigated.

3. Results and discussion

3.1. Water vapor transmission rate (WVTR)

Fig. 1 shows a transient weight loss of semi-IPN hydrogels. Considering the linearity of graphs, weight changes of samples stay at a constant value. Generally, the WVTR of a material is calculated according to the following equation:

$$\mathrm{WVTR} = (G/t)/A \qquad (1)$$

where, G is weight loss of the samples (g), t, test time (h), and A, effective membrane area (m^2). From Equation 1, WVTR of the present hydrogel wound dressing is calculated to be in the range of 2400–2900 $gm^{-2}day^{-1}$ (see Table II).

One of the important factors for wound dressing is an optimal WVTR. That is, an ideal wound dressing material should control the evaporative water loss from a wound surface. The WVTR of normal skin has been reported to be 200–500 $gm^{-2}day^{-1}$ [10–12]. If the WVTR of a material is lower than that of normal skin, tissue becomes dried and an exudate between wound and the covering results in an infection. Therefore, materials for wound dressing should have a higher WVTR value than normal skin. It is thought that the hydrogels prepared in this study can evaporate water from a wound surface without any dehydration.

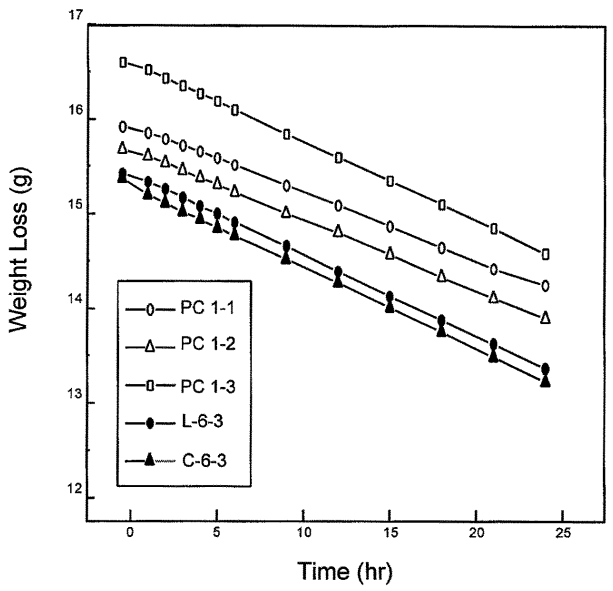


Figure 1 Transient weight loss of semi-IPN hydrogels in time.

On the other hand, the permeance or permeability of a material was calculated to estimate evaporative water loss [13]. Permeance of a material can be calculated from the WVTR.

$$\mathrm{Permeance} = \mathrm{WVTR}/P_w(\Delta RH) \qquad (2)$$

where, P_w is saturated water vapor pressure at test temperature, and ΔRH the difference of relative humidity between test cells in a thermostatic oven. Applying this formula, the permeance of hydrogels was determined to be in the range of 3.3×10^{-2} and $4.0 \times 10^{-2}\,g\,Pa^{-1}\,h^{-1}\,m^{-2}$. PC1–1 containing greater PEG showed a lower WVTR than that of PC1–2 and PC1–3. The water vapor permeance of human skin is known to be $4 \times 10^{-3}\,g\,Pa^{-1}\,h^{-1}\,m^{-2}$. Therefore, semi-IPN hydrogels tested have greater water vapor permeances than those of several commercially available wound dressing materials as well as normal skin.

In addition, permeability can also be employed to evaluate the specific properties of semi-IPN hydrogels.

$$\mathrm{Permeability} = \mathrm{permeance} \times \mathrm{thickness} \qquad (3)$$

According to Equation 3, permeability values of semi-IPN hydrogels were found to be $1.8 \sim 2.6 \times 10^{-5}\,g\,Pa^{-1}\,h^{-1}\,m^{-1}$. Table II lists the WVTR, permeance and permeability of semi-IPN hydrogels for wound dressing.

3.2. Biodegradability

Fig. 2 illustrates the biodegradation profile of semi-IPN hydrogels at pH 7.4 in phosphate buffer saline (PBS) solution. Most of the hydrogels made of β-chitin degraded in PBS lysozyme solution within one week, resulting from enhanced susceptibility to lysozyme of semi-IPN hydrogels based on β-chitin compared to α-chitin. As the β-chitin content in semi-IPN hydrogels increases, the weight loss also increases. This result indicates that the lower crosslinking degree causes the higher solubility in PBS lysozyme solution. Moreover, biodegradability of L–6–3 and C–6–3 containing D,L-lactide or ε-caprolactone was markedly enhanced because of an ester linkage introduced in a crosslinked network.

3.3. Evaluation of antibacterial activity

An ideal wound dressing material should prevent the bacteria infection (*Pseudomonas aeruginosa*). However, most of the commercially available wound dressing materials developed so far did not contain the antimicrobial agent [5, 14, 15]. Recently, Kuroyanagi *et al.*

TABLE II Water vapor permeabilities of wound dressing materials

Sample	WVTR ($gm^{-2}day^{-1}$)	Permeance $\times 10^2$ ($gPa^{-1}h^{-1}m^{-2}$)	Permeability $\times 10^5$ ($gPa^{-1}h^{-1}m^{-1}$)
PC 1–1	2 410	3.3	2.6
PC 1–2	2 533	3.4	2.4
PC 1–3	2 846	3.9	1.6
L–6–3	2 915	4.0	2.0
C–6–3	2 930	4.0	1.8

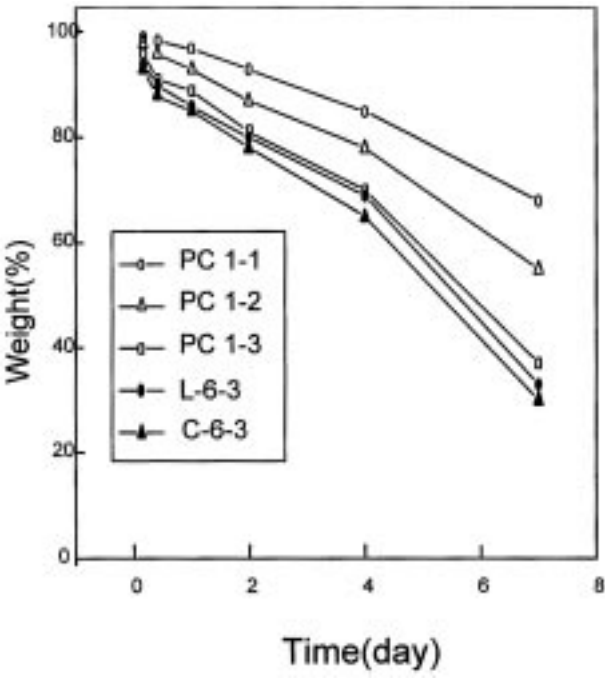


Figure 2 Biodegradation profiles of semi-IPN hydrogels in PBS lysozyme solution.

prepared a synthetic wound dressing material capable of releasing antimicrobial agent and reported their bactericidal capacity [4].

In the present work, novel sponge-type wound dressing materials impregnating antimicrobial agent, AgSD, were prepared to supress bacteria invasion. Fig. 3 shows the results of the antibacterial test using *Pseudomonas aeruginosa*. In the case of commercial product without antimicrobial agent, bacteria grew very easily to an order of 10^9–10^{10}. Poly(L-leucine), however, showed a complete supression of bacterial growth. The present wound dressing materials without AgSD also revealed bacterial growth. Suprisingly, bacteria were not found for AgSD-impregnated wound dressing materials up to 7 days. This result clearly demonstrates that the novel β-chitin-based sponge-type wound dressing materials impregnating AgSD can effectively prevent bacterial infection.

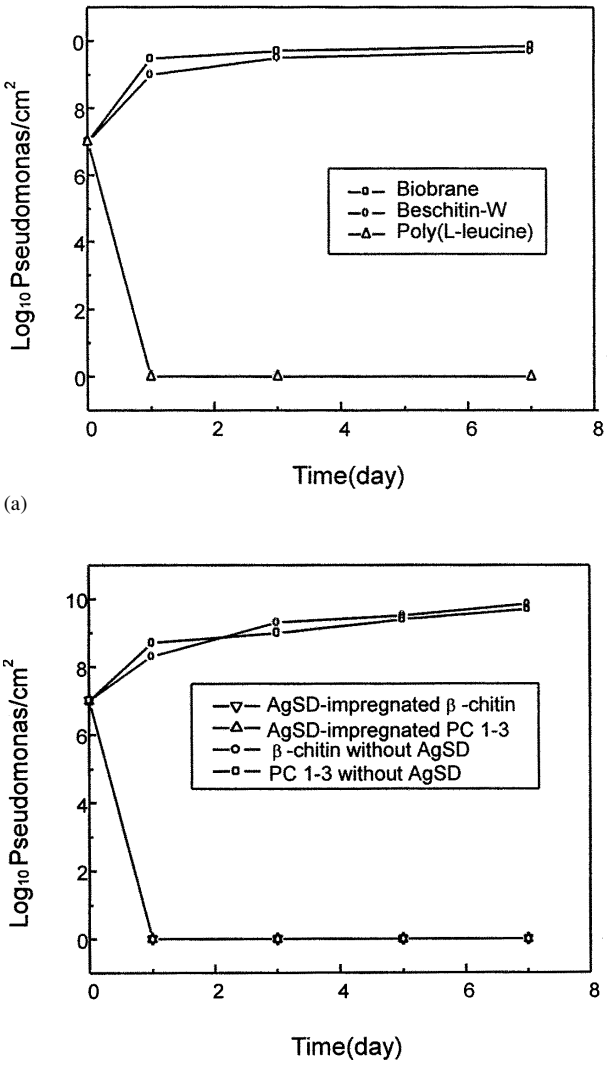


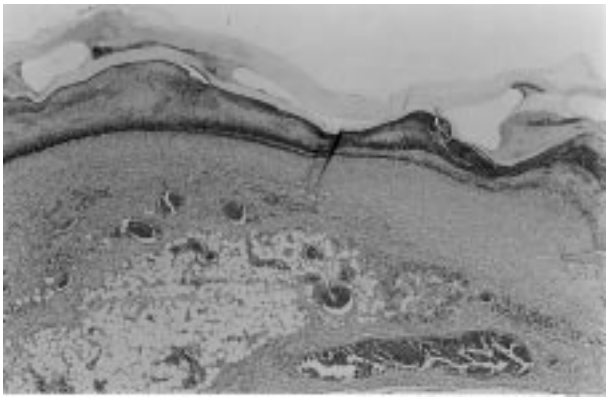
Figure 3 Evaluation of antibacterial capacity using *Pseudomonas aeruginosa*. (a) Commercial product; (b) prepared in this study.

3.4. Histological studies

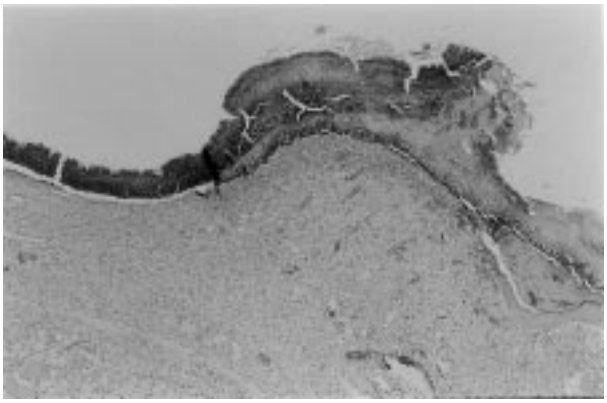
Fig. 4 exhibits the histological results of wound dressing materials. Histologic cross-sections of PC 1–3 without AgSD after 5 days of covering on the wound are shown in Fig. 4(a). The skin defect is evoked by a thick zone of acute inflammatory exudate. An abundant granulation tissue grows in from the margin. Note the neovascularized and dilated blood vessels in the subcutis and dermis. In the case of PC 1–3 with AgSD (Fig. 4b), the wound is covered by fibrinous acute inflammatory exudate. The dermis shows an early granulation tissue, numerous blood vessels and fibroblast cells. Fig. 4(c–d) illustrate the histologic cross-sections of PC 1–3 after 12 days. In Fig. 4(c), without AgSD a skin defect still persists and is covered with a neutrophilic exudate. The dermis shows a granulation tissue rich in neutrophils and macrophages. In Fig. 4(d), with AgSD the dermis is covered with a newly formed thin epidermis. The skin appendages are lacking. The dermis shows a healing fibrous scar with mononuclear cells.

Histological results of C–6–3 after 5 days are shown in Fig. 4(e–f). In Fig. 4(e) for C–6–3 without AgSD, the surface is covered with thick neutrophilic exudates and bloody fibrinous materials. A thick granulation tissue is noted in the dermis. In Fig. 4(f) for C–6–3 with AgSD, the skin defect is covered with necrotic acute inflammatory exudate. The underlying granulation tissue invades the skin defect. Fig. 4(g–h) exhibits the results of histological studies for C–6–3 after 12 days. As shown in Fig. 4(g) for C–6–3 without AgSD, at the 12th postoperative day the wound is completely re-epithelialized. The skin appendages are destroyed. The underlying granuation tissue reveals largely disappeared leukocytic infiltration, edema and increased vascularity. In Fig. 4(h) for C–6–3 with AgSD, the epidermis is almost completely regenerated and shows a small central defect. There is a proliferation of fibroblasts and an increased accumulation of collagen.

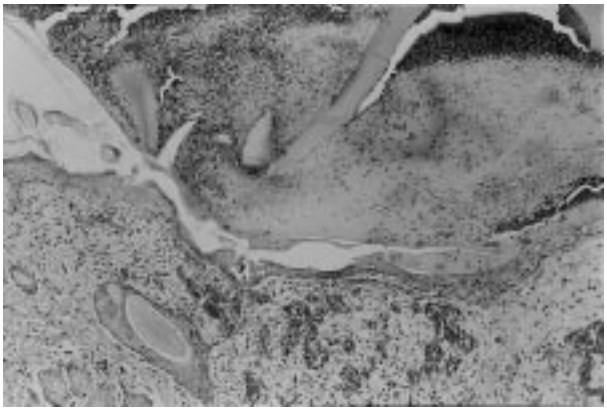
Histological studies for commercial wound dressing materials were also carried out to investigate the wound healing effect compared with materials prepared in this



(a)



(e)

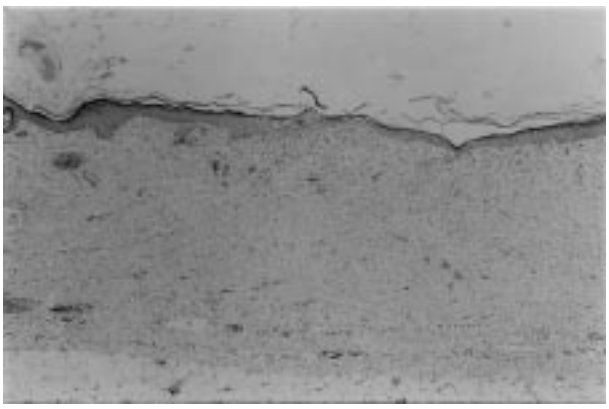


(b)

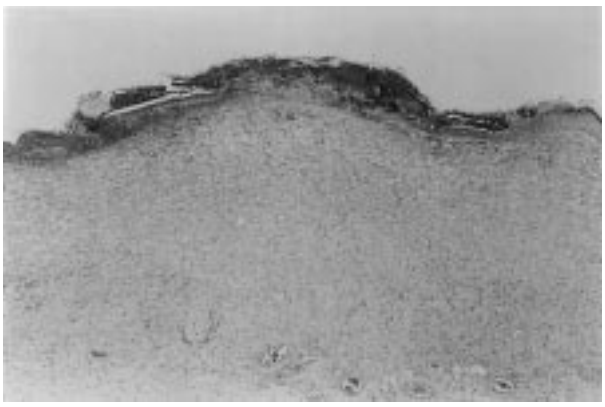
(f)

(c)

(g)



(d)



(h)

Figure 4 Histologic cross-sections of rat skin covered with (a) PC 1–3 without AgSD for 5 days, (b) PC 1–3 with AgSD for 5 days, (c) PC 1–3 without AgSD for 12 days, (d) PC 1–3 with AgSD for 12 days, (e) C–6–3 without AgSD for 5 days, (f) C–6–3 with AgSD for 5 days, (g) C–6–3 without AgSD for 12 days and (h) C–6–3 with AgSD for 12 days. $\times 40$.

study. Fig. 5 shows the histologic sections after 12 days of some commercial wound dressing materials. In the case of Biobrane^{R} (Fig. 5a), the skin defect is covered with neutrophilic exudates and replaced by exuberant granulation tissue. For Beschitin^{R} shown in Fig. 5b, the epidermis recovered its normal thickness and produced a mature epidermal architecture with surface keratinization. The skin appendages are not completely regenerated. The dermis reveals a healing scar showing deposition of connective tissue matrix and only scattered vascular channels. In Fig. 5(c), XEMEX Epicuel^{R} is histologically studied. The wound is covered with acute inflammatory exudate admixed with red blood cells and proteinaceous materials. The epidermis is partly re-epithelialized at the margin. The underlying granulation tissue is noted. There is a focal foreign body reaction at the margin of a deep dermis.

As a control, a conventional vaseline gauze was employed for wound dressing. Figs. 6a and b show the histologic sections covered with a conventional vaseline gauze after 5 and 12 days, respectively. In Fig. 6a, the skin defect is covered with blood and fibrinous exudate rich in neutrophils and macrophages. The granulation tissue progressively invaded the skin defect. After 12 days, the skin defect is covered by an intact epidermis. An abundant granulation tissue grows from the margin.

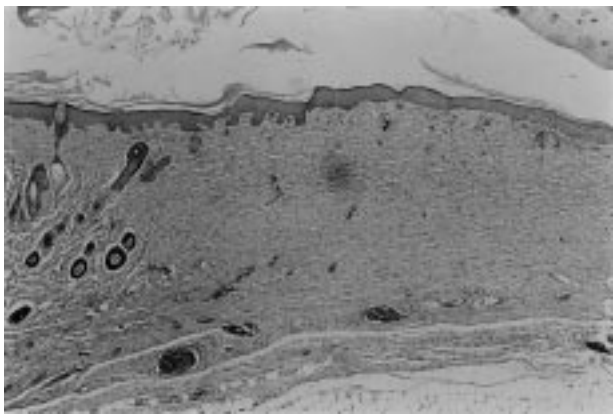
4. Conclusions

WVTR of the wound dressing materials was calculated to be between 2400–2900 gm^{-2} day^{-1}. It was thought that the semi-IPN hydrogels prepared in this study could evaporate water from a wound surface without dehydration. Most of the hydrogels based on β-chitin degraded within one week in PBS lysozyme solution. A biodegradability of L–6–3 and C–6–3 containing D,L-lactide or ε-carprolactone was markedly enhanced because of an ester linkage introduced in a crosslinked network. Novel sponge-type wound dressing materials impregnating AgSD were prepared to improve oxygen permeability and to prevent bacteria infection. The wound dressing materials impregnating AgSD had complete bactericidal capacity against *Pseudomonas aeruginosa*. Histological studies of the novel sponge-type materials containing AgSD confirm a proliferation of fibroblast in the wound bed of a wistar rat after 12 days and a reduction of infectious cells. Based on the histological results, the present products showed a comparable performance with the commercially available wound dressing materials.

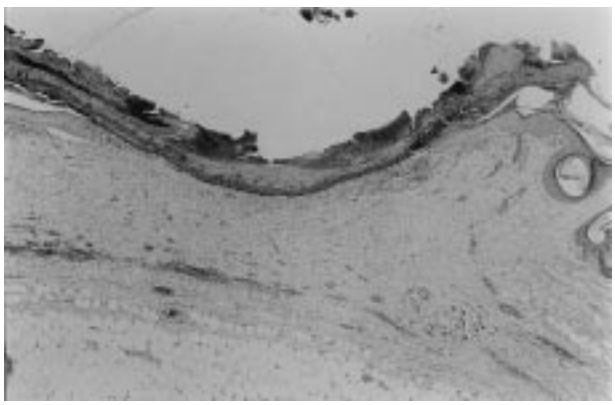
Acknowledgments

This work was supported by the 1997 Korean Ministry of Education Research Fund for Advanced Materials. S. S. Kim is grateful to the Graduate School of Advanced Materials and Chemical Engineering at Hanyang University for a fellowship. Donation of AgSD by Dr J. H. Chung at Dong Wha Pharmaceutical Co., Ltd (Seoul, Korea) is appreciated.

(a)

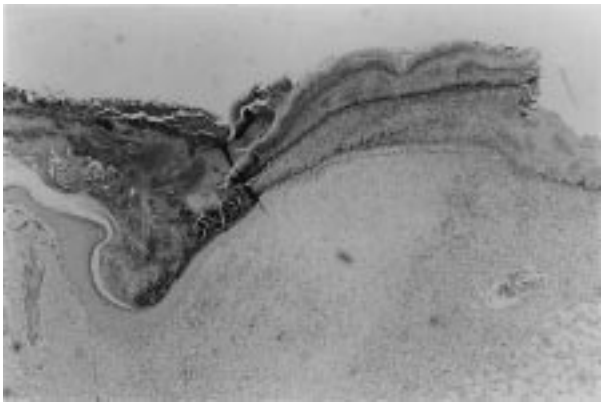


(b)

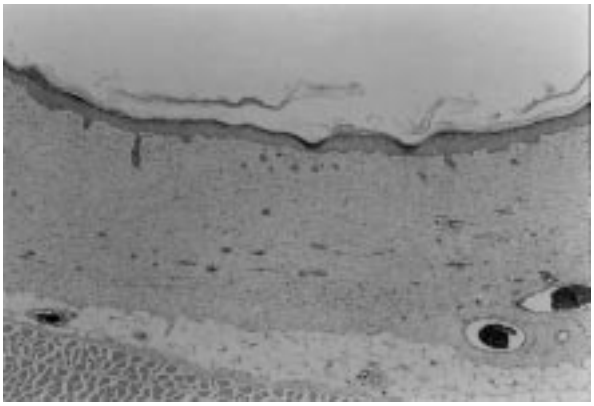


(c)

Figure 5 Histologic cross-sections of rat skin covered with (a) Biobrane®, (b) Beschitin® and (c) XEMEX Epicuel® for 12 days. ×40.



(a)



(a)

Figure 6 Histologic cross-sections of rat skin covered with vaseline gauze (a) after 5 days and (b) after 12 days. × 40.

References

1. A. M. GATTI, P. PINCHIORRI and E. MONARI, *J. Mater. Sci.: Mater. Med.* **5** (1994) 190.
2. Y. KUROYANAGI, E. KIM and N. SHIOYA, *J. Burn. Care Rehabilitation* **12** (1991) 106.
3. E. KIM, N. SHIOYA and Y. KUROYANAGI, *J. Jpn. Plast. Reconstr. Surg.* **9** (1989) 676.
4. Y. KUROYANAGI, Y. KOGANEI and N. SHIOYA, *ibid.* **7** (1987) 526.
5. E. A. WOODROOF, *Burn Wound Coverings* **2** (1984) 1.
6. J. F. PRUDDEN and L. L. BALASSA, *Proceedings of the International Conference on Chitin and Chitosan* (1977) 296.
7. S. S. KIM, Y. M. LEE and C. S. CHO, *Polymer* **36** (1995) 4497.
8. Y. M. LEE and S. S. KIM, *ibid.* **38** (1997) 2415.
9. E. H. LENNETE, A. BALOWS, W. J. HAUSLER JR and H. J. SHADOMY, ''Manual of Clinical Microbiology'', 4th Edn (1985) 1095.
10. A. D. SCHWOPE, D. L. WISE, K. W. SELL, D. P. DRESSLER and W. A. SKORNICK, *J. Biomed. Mater. Res.* **11** (1977) 489.
11. L. O. LAKE and S. O. LILJEDAHL, *Scand. J. Plast. Reconstr. Surg.* **5** (1971) 17.
12. G. B. PARK, J. M. COURTNEY, A. MCNAIR and J. D. S. GAYLOR, *Eng. Med.* **7** (1978) 11.
13. L. R. CARDONA, Y. D. SANZGIRI, L. M. BENEDETTI, V. J. STELLA and E. M. TOPP, *Biomateials* **17** (1996) 1639.
14. A. UTSUO and K. MATSUTOMO, *Kagaku Kogyo* **25** (1974) 1245.
15. D. E. SEYMOUR, N. M. DA COSTA, M. E. HODGSON and J. DOW, British Patent 1 280 631

Received 13 November 1998
and accepted 20 April 1999