Hyaluronic acid and silver sulfadiazine-impregnated polyurethane foams for wound dressing application

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Five different kinds of PU foam wound dressings were prepared to investigate their wound healing capability. They include (i) PU + silver sulfadiazine (AgSD), (ii) PU + alginate (Al), (iii) PU + Al + AgSD, (iv) PU + hyaluronic acid (HA), and (v) PU + HA + AgSD. Physical properties and *in vitro* behaviors of AgSD release and fibroblast adhesion on those dressings were evaluated. From the drug release and fibroblast adhesion studies, it was observed that PU foam impregnated with both HA and AgSD shows good drug release behavior and low adhesion of the cells. Furthermore, the HA and AgSD-containing PU foam showed excellent wound healing effect without any inflammation or yellow cluster. The wound size decreased around 77% after 1 week application of that foam dressing onto a rat skin defect.

Introduction

Polyurethanes (PUs) have been studied for many different applications, including breast implant devices and implants in dentistry, urology and cardiology [1]. They have also been extensively studied in wound healing applications, particularly for occlusive and semiocclusive. Many types of wound dressings composed of synthetic, biological, and biosynthetic materials have been developed, and some of them have been applied successfully in the treatment of burns and pressure sores [2]. PU wound dressings, such as Tegaderm™ (3 M) and Op-Site® (Smith & Nephew), are suggested to be useful both preventing bacterial invasion and minimizing water loss from wound [3].

Recently, a new wound dressing, allogenic cultured dermal substitute composed of collagen sponge sheet with fibroblasts [4–6], was developed by Kuroyanagi *et al*. They reported that a cellular spongy collagen is able to function as a more suitable matrix for cultured dermal substitute compare with Biobrane® (Dow Hickam Pharmaceuticals) [4].

Hyaluronic acid (HA) is considered to be a useful biomaterial to promote wound healing, because HA promotes the formation both of early granulation tissue and of the smooth wound surface considered to be a proper wound bed for autografting [1, 7, 8]. HA may facilitate adhesion–disadhesion between the cell membrane and the matrix substratum during cell movement. Furthermore, HA can create additional space by hydrated molecular structure for facilitating the migration of more cells in wound bed [1]. Silver sulfadiazine (AgSD) is a useful antimicrobial agent to protect bacterial infection and external contamination. Kuroyanagi *et al*. reported [1, 9] the antimicrobial efficacy of AgSD-impregnated wound dressing. They found that the PU film wound

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dressing containing AgSD of 50 μg/cm^2 was shown effective for bacterial suppression.

In this study, we prepared five kinds of PU foam dressings; (i) PU + AgSD, (ii) PU + alginate (Al), (iii) PU + Al + AgSD, (iv) PU + HA, and (v) PU + HA + AgSD. In this study, the physical properties of those PU foam dressings, such as water contact angle, water absorption and mechanical properties, were evaluated. *In vitro* behaviors of AgSD release and fibroblast adhesion on those dressings were also studied. Finally, those wound dressings were evaluated by animal study using a rat model and histological appearance of wound.

Experimental

Materials

Toluene diisocyanate (TDI; 2.4 = 80%, 2.6 = 20%, Tokyo Chemical Industry, Japan) and Pluronic F-68 (BASF, USA) as a polymeric surfactant consisted with polyethylene glycol (PEG)-polypropylene glycol (PPG)-PEG, were used without further purification. KE-825 (PEG-PPG random copolymer, mol ratio 6 : 1, Korea Polyol Co.) was used after vacuum drying at 100 °C. AgSD (Aldrich Chemical Co., USA) as an antimicrobial agent, alginic acid sodium salt (Al; Acros Organics, USA) and hyaluronic acid sodium salt (HA; Korea Pacific Co.) as biocompatible natural polymers, and glycerin (Oriental Chemical Industries, Korea) were used without further purification.

Preparation of PU foams

To prepare PU foam wound dressing, polyol (KE-825) was slowly dropped into TDI at 70 °C under nitrogen atmosphere and reacted until remaining 7% NCO contents as determined by the di-n-butylamine. For the preparation of PU foams containing additives (AgSD, HA or Al), additives were introduced during foaming reaction with water, glycerin (20–25 wt %) and surfactant (F-68, 50–60 wt %). The amounts of AgSD was controled to release 50 μg/cm^2, and the contents of HA or Al was changed from 1 to 10 wt %.

Characterization of PU foam dressing

For the measurement of water absorption ratio, PU foams of squares with 1 cm^2 width and 0.4 mm thickness were immersed into deionized water at room temperature. The weight of absorbed foam was measured at desired times after removing excess water on the foam surface by kim wipes. Mechanical properties of the foams were tested by universal test machine (Instron, USA) using ASTM-1822-L method. To examine the hydrophilicity of the additive-containing PU, the water contact angles of the PU films were measured by an optical bench-type contact angle goniometer (Model 100-0, Rame-Hart, Inc., USA) using sessile drop method. Electron spectroscopy for chemical analysis (ESCA; ESCALAB MK II, V. G. Scientific Co., UK; Al K*a* radiation source at 1487 eV and 300 watt at the anode) and attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR; Magna-IR spectrometer 550, Nicolet, Japan) were also introduced to characterize the surface properties of PU films.

In vitro drug release and fibroblast adhesion tests

Release behavior of AgSD from PU foams with different additives was evaluated using Franz diffusion cells. The chamber held of 12 mL of phosphate buffered saline (PBS, pH 7.4) and was magnetically stirred. The area of PU foam exposed to each chamber was 1.8 cm^2. At predetermined time intervals, sampling was made and an equal volume of PBS was added to the chamber. Amount of AgSD released from the foams was measured by a high performance liquid chromatography (HPLC; Model Rainin, Dynamax, USA) with flow rate of 2 mL/min using acetonitrile/DI water/phosphoric acid as an eluent.

For the test of adhesion behavior of the PU foam surfaces, fibroblasts (NIH/3T3, KCLB 21658, Korea Cell Line Bank) were used as a model cell. The cells routinely cultured in tissue culture polystyrene (PS) flasks (Corning, USA) at 37 °C under 5% CO_2 atmosphere were harvested after treatment with 0.25% trypsin (Gibco Laboratories, USA). The PU films containing different additives were placed on 24 well PS plate (Corning) and equilibrated with prewarmed (37 °C) PBS (pH 7.4). After removing the PBS solution from the wells by pippetting, the fibroblasts were seeded onto the surfaces (seeding density, 4×10^4 cells/cm^2). The culture medium used was RPMI 1640 (Gibco laboratories, USA) containing 10% fetal bovine serum (FBS), 100 unit/mL penicillin and 100 mg/mL gentamycin. After incubation at 37 °C under 5% CO_2 atmosphere for 2 h and 1 day, the PU film surfaces were washed with PBS. The cells attached on the surfaces were examined by scanning electron microscopy (SEM; Model 2250N, Hitachi, Japan). For this, the cells attached on the surfaces were fixed with 2.5% glutaraldehyde (Gibco Laboratories) in PBS for 30 min at room temperature. After thorough washing with PBS, the cells on the surfaces were dehydrated in ethanol graded series (50%, 60%, 70%, 80%, 90%, and 100%) for 10 min each and allowed to dry on a clean bench at room temperature. The cell-attached surfaces were gold deposited in vacuum and examined by SEM. The cell density on the surfaces was estimated by counting the number of attached cells [9, 10].

Animal study

The skin defect (diameter 3 cm) was prepared by cutting dorsum surface of rat (7 weeks old) after shaving. The wound was covered with sterile PU foam dressing and fixed with elastic tape. Nine rats for each dressing were used to study wound healing behavior. Three rats were sacrificed every week after the application was started. Each wound surface was biopsied observed the wound surface, and then finally examined histologically after fixed in 10% formaldehyde and stained with hematoxylin-eosin for the sacrificed rats every week. The size of each wound was measured by taking photographs at every week after the application. The size reduction of

the wound was calculated by the ratio of the observed size to the original defect size.

Results and discussion

Characterization of PU foam wound dressing

Surfactant was used to stabilize the rising foam by reducing stress concentrations in thin cell walls and to promote nucleation of bubbles during mixing. The cell size of the foam decreased with the increasing amount of surfactant and decreasing amount of water. The optimum amount of 10 wt % aqueous surfactant was 60 wt % to prepolymer.

Water absorption content was measured for the following PU foams, (i) PU + AgSD, (ii) PU + Al, (iii) PU + Al + AgSD, (iv) PU + HA, and (v) PU + HA + AgSD, to study the ability of absorption of exudates. PU foam (control) was absorbed water over seven times compared to the original due to the hydrophilic polyol (Fig. 1). The PU foams containing HA and Al show high water absorption compared to the foam containing AgSD due to hydrogel properties of HA and Al. PU foam containing AgSD and HA was swollen over eight times within 10 h. The water absorption was almost constant after 10 h for all PU foams [11, 12].

Mechanical properties were measured for the six kinds of PU foams. The additives such as HA, AgSD and Al affected the mechanical properties of the foams. The strain and stress decreased and the modulus increased for the PU foams containing additives. PU foams impregnated with additives were harder than the control one.

The pore size of PU foams was designed to be around 100 μm to control absorption ratio and water permeability. All the prepared foams, density 0.234 to 0.26 g/cm^3, had open cells ranged from 50 to 200 μm. Pore size was controlled by a surfactant (F-68) aqueous solution and foam's density. At increasing 10 wt % F-68 aqueous solutions, the pore size increased. However, AgSD, Al and HA did not have an effect on pore size when hydrogel materials were impregnated 5 wt % into PU foam wound dressing. Mechanical properties (stress, strain, and modulus) of all the foams were almost same as just control PU foam. Modulus of those foams was constant even when sorbitol and surfactant additions were changed.

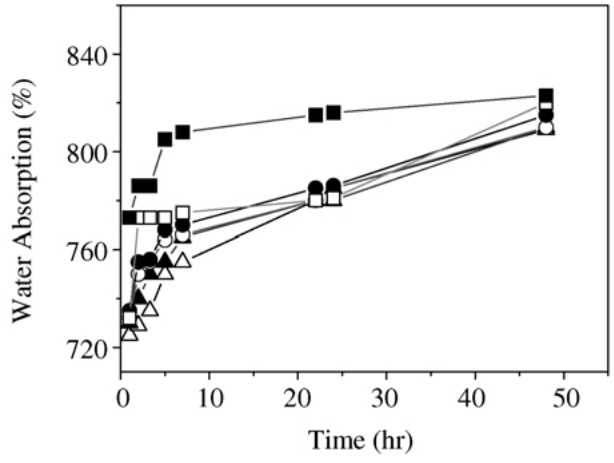

Figure 1 Water absorption behavior of PU foams (△, Control PU; ▲, sample 1 (PU + AgSD) ○, sample 2 (PU + Al); ●, sample 3 (PU + Al + AgSD); □, sample 4 (PU + HA); ■, sample 5 (PU + HA + AgSD)).

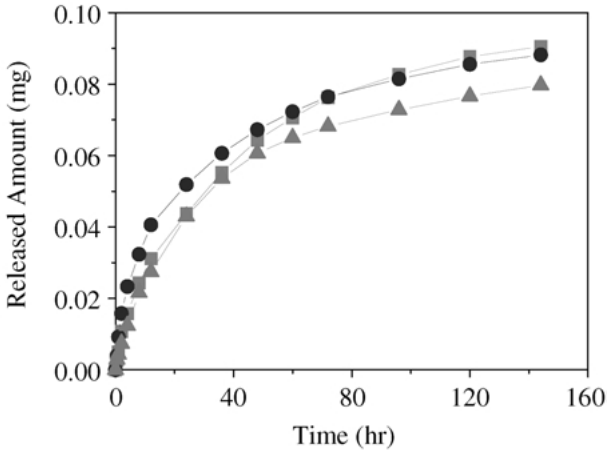

Figure 2 AgSD release behavior from PU foams with different additives (▲, sample 1 (PU + AgSD); ●, sample 3 (PU + Al + AgSD); ■, sample 5 (PU + HA + AgSD)).

To study surface properties of the additive-containing PUs, PU films were investigated by FT-IR and ESCA. In FT-IR spectra of PU films with different additives, no difference was observed. In ESCA, very small amount of Ag and Na was detected on the surface of samples 1–5 at the binding energy of 367 eV and 1072 eV, respectively. Also, oxygen (531 eV) and nitrogen (399 eV) peaks in the samples 1–5 increased compared to control PU. These results indicate that AgSD, HA, and Al in samples 1–5 were distributed on the surface.

Release behavior of AgSD from PU foams

Release behavior of AgSD from PU foam wound dressings which contain hydrogel moiety such as Al or HA was compared with that without hydrogel moiety. Fig. 2 shows the release pattern of AgSD from PU foams with different additives. The released amount of AgSD in PU foam with AgSD (sample 1) was almost constant during the 150 h. The release pattern of Al-containing PU foam (sample 3) was almost same as the sample 1, however, the amount of released drug was larger. For HA-containing PU foam (sample 5), the initial released amount was almost same as the sample 1, however, the amount increased as time increased. The increased release of AgSD from the foams with hydrogel moiety (HA or Al) may be due to the swelling of the foams. Theoretical values of the impregnated AgSD are almost same as the experimental values.

Fibroblast adhesion behavior on PU surfaces

It is important to considering the adhesion behavior of cells on wound dressing since it can be an indicator of the feasibility of dressing to detach without any pains from wound. Fig. 3 shows the results of fibroblast cell adhesion on the different additive-containing PU film surfaces. Initial seeding density of fibroblasts was

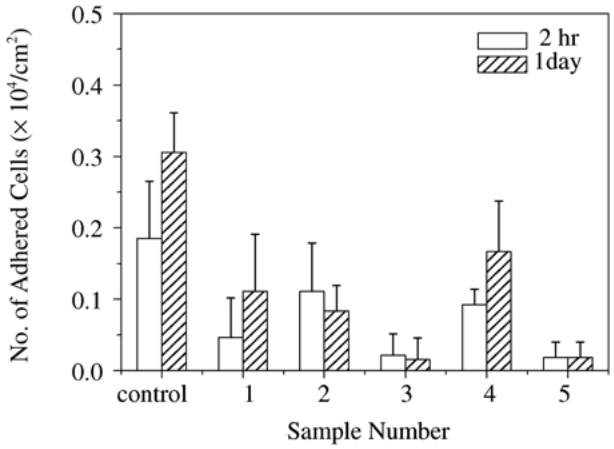

Figure 3 Fibroblast adhesion behavior on PU foams with different additives (control PU; sample 1, PU + AgSD; sample 2, PU + Al; sample 3, PU + Al + AgSD; sample 4, PU + HA; sample 5, PU + HA + AgSD).

$4 \times 10^4/\text{cm}^2$. After 2 h and 1 day culture, the number of adhered cells on the additive-containing PU surfaces decreased dramatically compared to the control PU surface. According to the literature [1], HA may facilitate adhesion–disadhesion between the cell membrane and the matrix substratum during cell movement. It seems that AgSD plays some roles as a cell adhesion barrier. The adhered cells on the surfaces of PU without AgSD (samples 2 and 4) were larger than those of PU with AgSD (samples 1, 3 and 5).

Animal study

Wound healing effect of PU foams was investigated by *in vivo* animal study. Animal study was performed with rats by observing wound size and regenerating tissue. PU foams impregnated with Al (sample 3) or HA and AgSD (sample 5) showed excellent wound healing effect compared to control PU foam, because hydrogel moiety promotes the release of AgSD (samples 3 and 5). Especially, HA was shown to promote the formation of early granulation tissue and the smooth wound surface. In addition, HA may facilitate adhesion–disadhesion between the cell membrane and the matrix substratum during cell movement. As shown in Fig. 4, PU foam incorporating HA and AgSD (sample 5) had the best healing effect than others. In deep wound on dorsum, 4.5 cm size, the wound size was decreased around 77% within 1 week after skin defect. Also there was no inflammation, no yellow crust, and no dressing deformation (Fig. 5). The progress of granulous tissue formation of sample 5 was fastest in all case.

Conclusions

In the study, PU foam containing HA and AgSD moieties were successfully prepared for wound dressing application. The PU foam containing HA and AgSD showed excellent wound healing effect. AgSD as an antimicrobial agent protects from external contamination and has sufficient bactericidal effect to inhibit infection on wound area. In addition, HA facilitates migration of more cells into wound area, not to mention facilitated

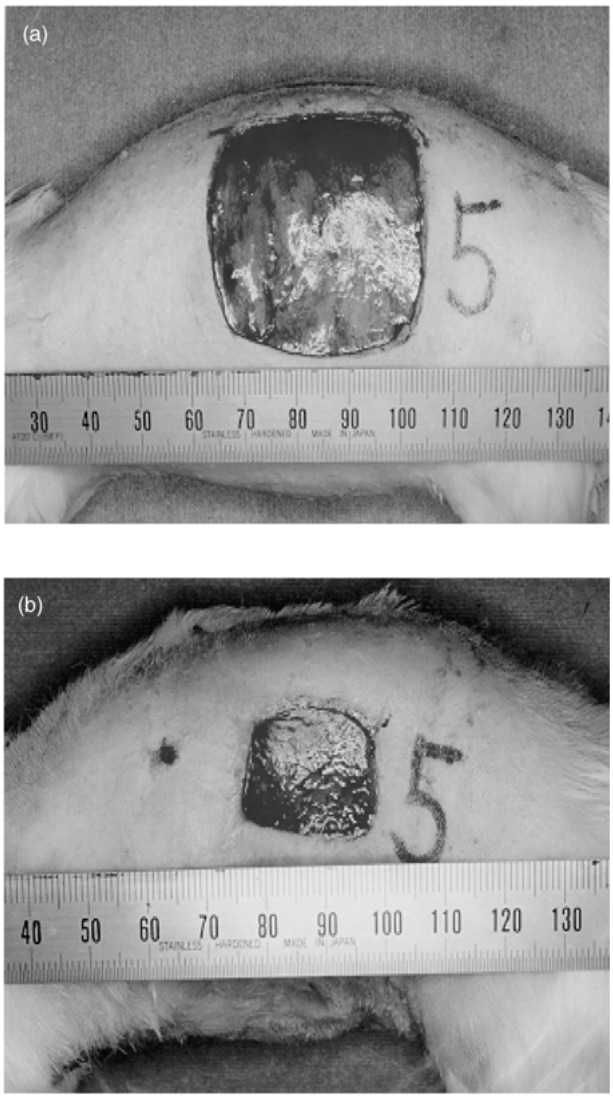

Figure 4 Wound surfaces covered with PU foam (sample 5, PU + HA + AgSD) (a) after skin defect; (b) 1 week after application.

AgSD release by diffusion mechanism. Another advantage of HA is to promote the early granulation tissue formation and smooth wound surface formation. From *in vivo* test, we confirmed that HA and AgSD-impregnated PU foam show excellent wound healing effect and that wound dressing did not show any inflammation or yellow cluster, and that the wound size decreased around 77%.

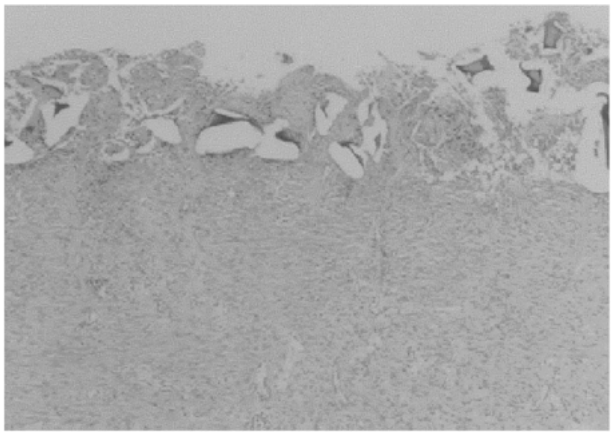

Figure 5 Histological appearance of wound (1 week after application of PU foam (sample 5, PU + HA + AgSD).

Acknowledgment

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