Development of an artificial dermis preparation capable of silver sulfadiazine release

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Abstract: This article describes the antibacterial effects of an artificial dermis impregnated with silver sulfadiazine (Ag-SD) *in vitro* as well as *in vivo*. In the *in vitro* test, silver release from the artificial dermis impregnated with Ag-SD, by immersion in collagenase solution was controlled by the degradation of the collagen sponge.The artificial dermis impregnated with 3% or higher doses of Ag-SD completely suppressed the growth of *Pseudomonas aeruginosa* (Ps.) or *Staphylococcus aureus* (St.). The cytotoxicity test revealed that impregnation of 5% or higher doses of Ag-SD suppressed the growth of fibroblasts. However, when the artificial dermis impregnated with Ag-SD was implanted into full-thickness skin defects on the backs of guinea pigs, no tissue damage was histologically observed around the implanted site of the dermis. In the *in vivo* test, the artificial dermis impregnated with 10% Ag-SD, which was grafted on experimentally contaminated wounds in the backs of guinea pigs, macroscopically suppressed degradation of the collagen sponge, and significantly reduced the growth of both Ps. and St., compared with artificial dermis without Ag-SD. We conclude that collagen sponge impregnated with Ag-SD is a promising artificial dermis applicable to treat contaminated wounds. © 2001 John Wiley & Sons, Inc. J Biomed Mater Res 57: 346–356, 2001

Key words: artificial dermis; silver sulfadiazine; sustained release; full-thickness skin defect; antibacterial activity

INTRODUCTION

Since its development by Yannas and colleagues[1,2] in 1980, artificial dermis has been used for the treatment of full-thickness skin defects resulting from burns and injuries.[3–6] The artificial dermis (Pelnac®, Gunze Co., Kyoto, Japan) in the present study was composed of an outer silicone layer and an inner collagen sponge layer. For dermal regeneration, 2 or 3 weeks are normally required. During the period until secondary grafting, low resistance to infection is a clinical problem.[7,8] The outer silicone membrane of the artificial dermis can protect the wound from contamination by external bacteria. However, the silicone membrane is of no use in preventing infection. When bacterial contamination occurs, the inner collagen sponge is not only degraded by collagenase produced by bacteria, but also bacterial growth is promoted in the degraded inner collagen sponge. So an important issue is suppression of bacterial growth on the wound surface beneath the artificial dermis. To overcome this limitation, we focused on developing an artificial dermis impregnated with an antibacterial drug.

In this study, silver sulfadiazine (Ag-SD) was first selected as the antibacterial drug to be impregnated into artificial dermis, because it has a broad spectrum of antibacterial activity against *Pseudomonas aeruginosa* (Ps.), *Staphylococcus aureus* (St.), and other organisms. Ag-SD, a topical antibacterial agent, has been widely used for the treatment of burn wound infections.[9–15] Ag-SD is an organic complex of ionizable silver and sulfadiazine, and the silver ion acts as a bacteriostatic and bactericidal agent.[16–26]

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We prepared an artificial dermis impregnated with Ag-SD. The objective of this study was to investigate the *in vitro* Ag-SD release from the artificial dermis and its degradation in phosphate buffer solution (PBS) containing collagenase. We examined the antibacterial activity of artificial dermis impregnated with Ag-SD *in vitro* as well as *in vivo*.

MATERIALS AND METHODS

Experimental materials

Artificial dermis (Pelnac®; Gunze Co.), composed of an outer silicone layer and an inner collagen sponge layer with a pore size of 70–110 μm and pore volume fraction of 80–95%, was used as a control. A micronized powder of silver sulfadiazine (Ag-SD), 10 μm in average diameter, was pur-

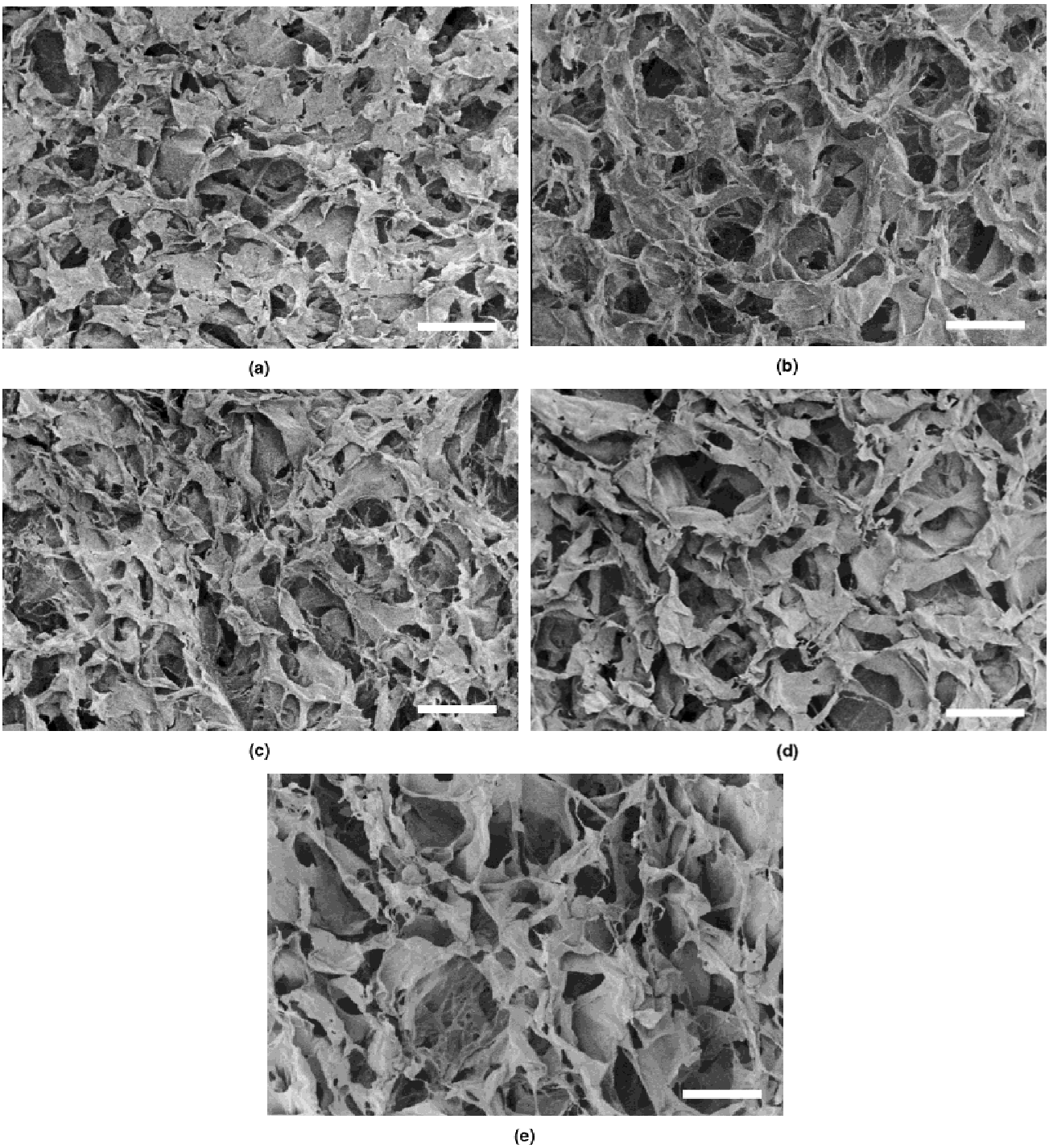

Figure 1. Scanning electron microscopic view of artificial dermis impregnated with Ag-SD at doses of 0% (a), 1% (b), 3% (c), 5% (d), and 10% (e). Original magnification: ×100. Bars correspond to 200 μm.

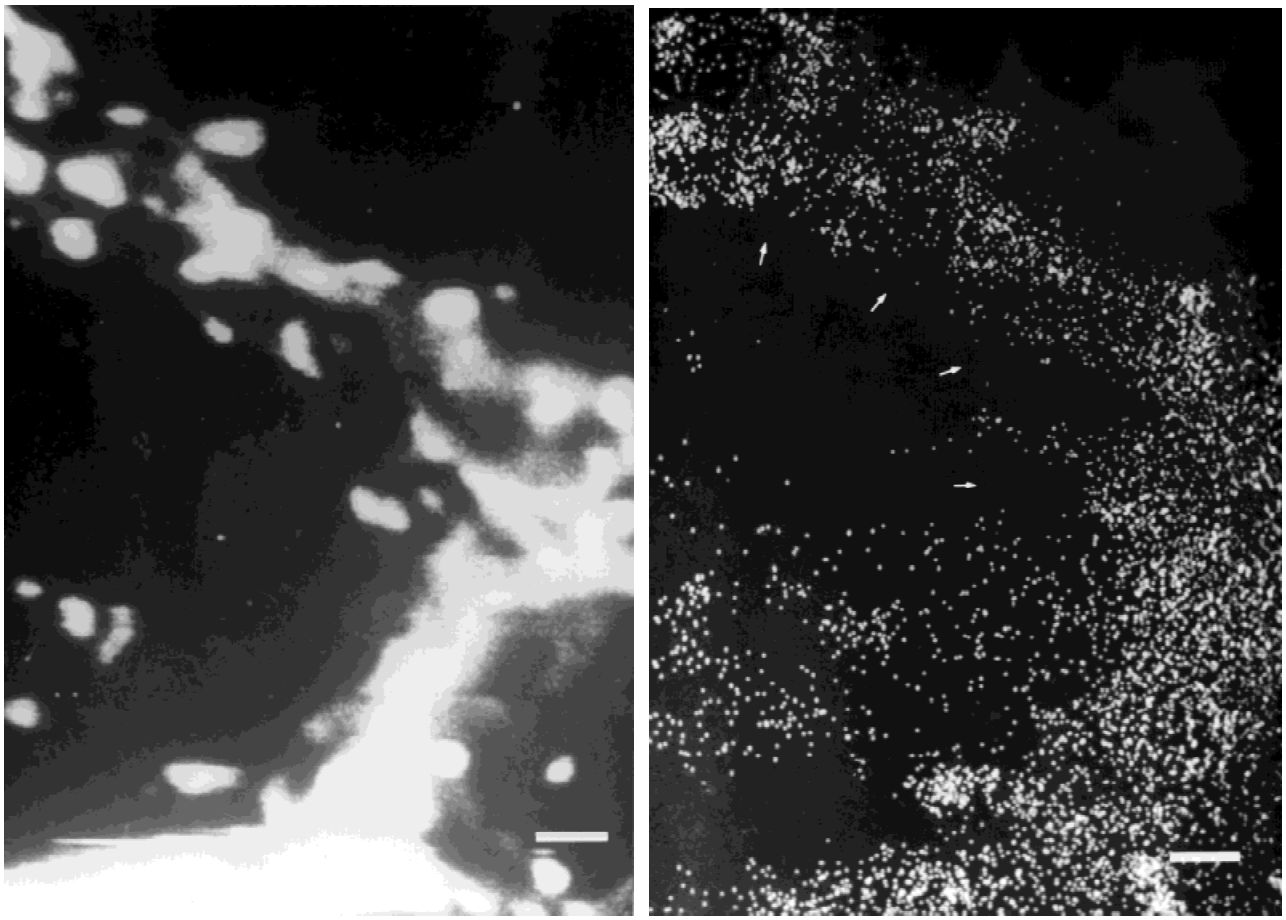

Figure 2. (a) Scanning electron microscopic view of artificial dermis impregnated with Ag-SD at a dose of 10%. (b) Electron probed microanalyzed view of the same sample. Original magnification: ×1600. Arrows indicate silver particles along collagen fiber of artificial dermis. Bars correspond to 50 μm.

chased from Lec Co., Slovenia. Type I-atelocollagen extracted from a pig tendon was purchased from Nitta Gelatin Co., Osaka, Japan, and type I-A collagenase was purchased from Sigma Co., St. Louis, MO.

Preparation of artificial dermis impregnated with Ag-SD

Artificial dermis impregnated with Ag-SD was prepared through the mixture of silver sulfadiazine powder at 1, 3, 5, and 10% concentration with type I collagen in the process of Pelnac® production, as reported previously.[27] Briefly, hydrochloric acid solution containing 3% atelocollagen of pH 3.0 was stirred at 1800–2000 rpm for 60 min with a refrigerated homogenizer. Silver sulfadiazine powder was added dropwise to the stirred collagen solution to give weight ratios of silver sulfadiazine to collagen of 1, 3, 5, and 10%. After further stirring of the mixed solution for 5 min, the foaming solution was poured into a mold, rapidly frozen at −40°C, and freeze dried for 48 h to obtain a highly porous collagen sponge sheet. Then the sponge sheet was crosslinked in a vacuum at 105°C for 24 h and sterilized with ethylene oxide gas. A silicone membrane (Medical Adhesive Silicone Type A) was coated over the sheet. After the silicone was dried at room temperature for 24 h, the bilayer sheet was further crosslinked by immersion in 0.05 *M* acetic acid solution containing 0.2 wt % glutaraldehyde at 4°C for 24 h. The bilayer sheet was rinsed in phosphate buffer solution for 1 week to remove residual glutaraldehyde. Then the bilayer sheet was immersed in 15% ethanol, quickly frozen at −135°C, and refreeze dried for 48 h. The prepared artificial dermis impregnated with Ag-SD was composed of the inner collagen sponge adjusted to 3 mm in thickness and the outer silicone membrane adjusted to 25 μm in thickness. Artificial dermis (Pelnac®), without Ag-SD, was used as a control in this study.

The scanning electron microscopic appearance of the artificial dermis impregnated with different doses of Ag-SD is shown in Figure 1 (SEM model; JSM-5800LV, Nihon Denshi Co., Tokyo, Japan). Irrespective of the Ag-SD dose, all collagen sponges had morphologically similar structure. Furthermore, the distribution of silver contained in the artificial dermis impregnated with Ag-SD at a dose of 10% is shown in Figure 2, using an electron probed microanalyzer (EPMA-C1, Shimadzu Co., Kyoto, Japan). Silver particles were homogeneously dispersed along the collagen fibers of the artificial dermis.

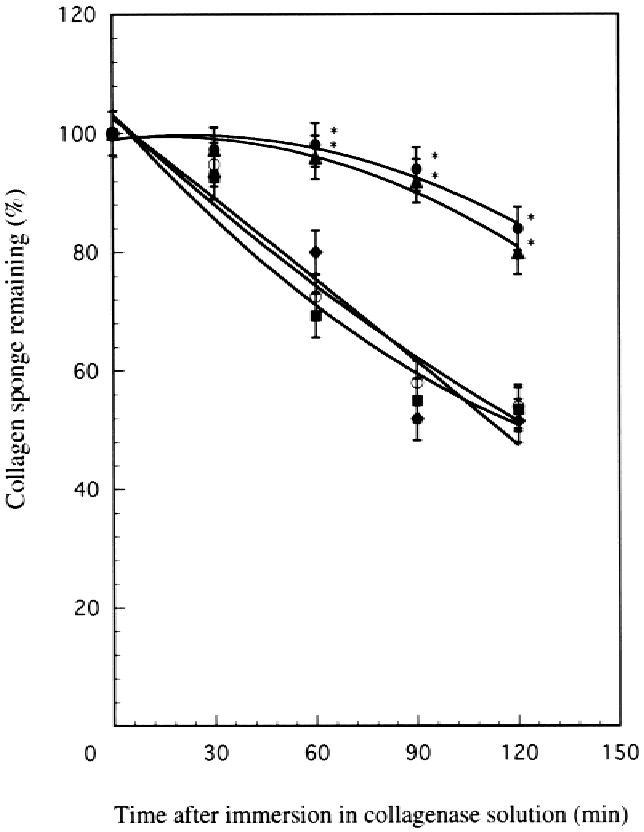

Figure 3. *In vitro* time profiles of degradation of collagen sponge impregnated with or without Ag-SD at doses of 0% (○), 1% (♦), 3% (■), 5% (▲), and 10% (●), in collagenase solution. Each point shows mean ± SD (n = 3). *$p < 0.05$, compared with artificial dermis without Ag-SD.

When evaluated on an atomic absorption spectrometer (AA-640-12 and GFA-2, Shimadzu, Co.), the amount of impregnated Ag-SD in the artificial dermis for fed-in doses of 1, 3, 5, and 10% was 7.5, 31.2, 75.2, and 148.0 μg Ag-SD/cm^2.

In vitro degradation of collagen sponge by immersion in collagenase solution

Pieces (1 × 1.5 cm) of the artificial dermis impregnated with different doses of Ag-SD were immersed in 5 mL PBS (pH 7.4) containing three units type I-A collagenase. After shaking at 37°C for 30, 60, 90, and 120 min, the pieces were freeze dried, and their remaining weight was calculated.

Silver release from artificial dermis impregnated with Ag-SD

After calculation of the remaining weight, the residual pieces of artificial dermis impregnated with Ag-SD were immersed in 5 mL 0.1 *N* sodium hydroxide at 100°C for 1 h in a shaking incubator in order to be hydrolyzed. The silver concentration of the pieces was determined by atomic absorption spectrometry, and expressed as the total amount of silver released.

Assessment of antibacterial activity of artificial dermis impregnated with Ag-SD

A piece (1.5 cm in diameter) of artificial dermis impregnated with different doses of Ag-SD was placed at the center of a simple agar plate (9 cm in diameter) inoculated with 4 × 10^6 cfu /cm^2 Ps. (IFO 3452) or St. (IFO 12732), and 400 μL PBS was added to the piece. Then the plate was incubated at 37°C in 5% CO_2 for 3 days to observe the inhibition of bacterial invasion on the agar. After culturing, the size of the bacterial inhibitory zone on the agar was measured.

After bacterial inhibition test, both a piece of the artificial dermis impregnated with different doses of Ag-SD and the agar beneath the artificial dermis were punched out at the center of the plate (1 cm in diameter), and immediately homogenized in 5 mL saline solution with a Vortex mixer (Scientific Industries, Inc., Bohemia, NY) for 30 s. The supernatant of the resulting solution was serially diluted to a 1/10 concentration, and 0.1 mL of the diluted solution was inoculated on a new agar plate. After 24 h incubation at 37°C, the bacterial colonies were counted, and expressed as the number of bacteria (cfu/cm^2).

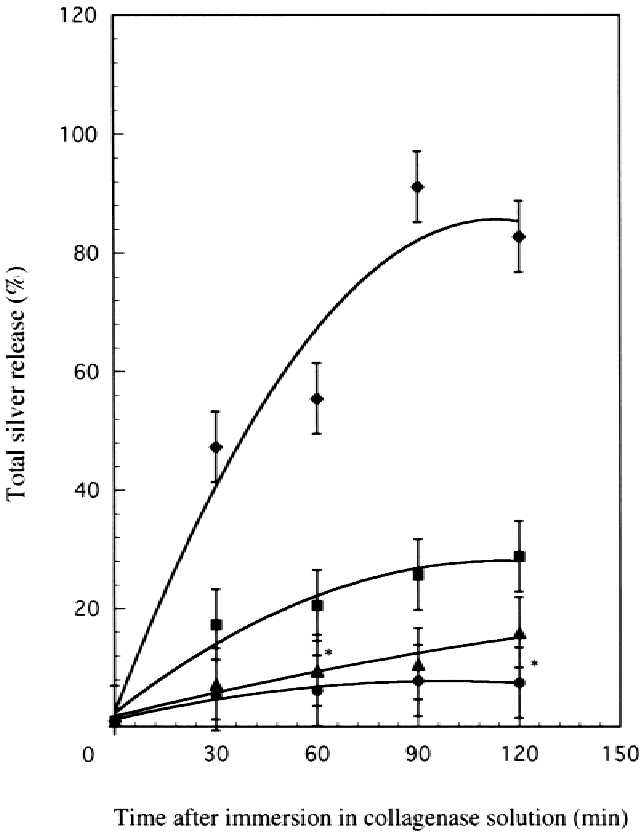

Figure 4. *In vitro* time profiles of silver release from artificial dermis impregnated with Ag-SD at doses of 1% (♦), 3% (■), 5% (▲), and 10% (●), in collagenase solution. Each point shows mean ± SD (n =3). *$p < 0.05$, compared with artificial dermis with 1% Ag-SD.

TABLE I
Size of Bacterial Inhibitory Zone Against Ps. or St.

Bacterial Strain	Control	1%	3%	5%	10%
Ps.	0	1.24 ± 0.244	1.45 ± 0.200	1.47 ± 0.493	2.28 ± 0.252*
St.	0	0.67 ± 0.348	1.14 ± 0.417	1.32 ± 0.444	1.54 ± 0.327*

mm

Ps.: *Pseudomonas aeruginosa* IFO3452.
St.: *Staphylcoccus aureus* IFO12732.
Data shows mean ± SD ($n = 3$).
*$p < 0.05$, compared with control.

In vitro cytotoxicity test of artificial dermis impregnated with Ag-SD

Fibroblasts were serially subcultured in plastic flasks with Dulbecco's Modified Eagle Medium (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum (Cansera International, Inc., Canada). The artificial dermis impregnated with different doses of Ag-SD (16 mm in diameter) was placed on a 24-well tissue culture plate, and then 1×10^6 cells/well cultured human fibroblasts in 500 μL culture medium were added on the artificial dermis. These plates were incubated at 37°C in 5% CO_2, and the medium was renewed every other day. After incubation for 3, 7, 14, 21, and 28 days, the number of surviving fibroblasts was counted. The cellular response was evaluated by MTT assay,[28,29] and expressed as survival ratio: the number of surviving fibroblasts of artificial dermis impregnated with Ag-SD at each dose against that of artificial dermis without Ag-SD for the same incubation period. Fibroblasts used in this study were purchased from Kurabo Co., Osaka, Japan. All subjects enrolled in this research have responded to an Informed Consent that has been approved by my Institutional Committee on Human Research and that this protocol has been found acceptable by them.

Implantation of artificial dermis on the back of guinea pigs

Furthermore, tissue damage was examined in animal studies. NIH guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1985) have been observed. Male 6-week-old guinea pigs (Shimizu Laboratory Animal Supply Co., Ltd., Kyoto, Japan) were anesthetized by intraperitoneal injection of a mixture (1 : 1) of nembutal and atropine sulfate (3.5 mg/0.07 mL/guinea pig). After shaving and depilation, two full-thickness skin defects (1.5 × 1.5 cm) were made, preserving the panniculus carnosus, on the back of each guinea pig. Hemostasis was achieved by direct pressure with sterile gauze. The materials impregnated with different doses of Ag-SD were applied to full-thickness skin defects. All the wounds were covered with gauze, and the edges of the gauze were sutured to the skin using 4-0 nylon monofilament. The guinea pigs were killed 1 or 2 weeks after implantation, and biopsy specimens were taken and fixed in 10% neutral-buffered formalin solution, embedded in paraffin wax, sectioned in two selected areas of each piece of explanted artificial dermis (3 μm thickness), and then stained with hematoxylin and eosin. These sections were viewed microscopically to evaluate cellular damage.

Assessment of *in vivo* antibacterial effect of artificial dermis impregnated with Ag-SD

Two full-thickness skin defects on the back of each guinea pig were made as described above. Each defect was inoculated with 1×10^4 cfu/cm^2 Ps. or 1×10^9 cfu/cm^2 St. by pipetting 50 μL of the bacterial suspension onto the panniculus carnosus. Care was taken to prevent runoff from the wound surface. Then the artificial dermis impregnated with Ag-SD at a dose of 10% and the control without Ag-SD were immediately implanted into the skin defects and secured with gauze.

To evaluate the antibacterial efficacy of artificial dermis impregnated with Ag-SD at a dose of 10%, we employed a quantitative method using mechanical tissue homogenization and serial dilution.[30,31] One or 3 days after implantation, artificial dermis and the underlying muscle tissues were excised, and immediately homogenized in 10 mL saline solution with a Vortex mixer for 1 min. The supernatant of the resulting solution was serially diluted, and 0.1 mL of the diluted solution was inoculated on an agar plate. After

TABLE II
Quantitation of Residual Bacterial Content in Agar Plates Coated With Artificial Dermis Impregnated With or Without Ag-SD

Bacterial Strain	Control	1%	3%	5%	10%
Ps. (4.0×10^6)	3.4 ± 2.1 ($\times 10^7$)	8.8 ± 8.9 ($\times 10^3$)	N.D.	N.D.	N.D.
St. (4.0×10^6)	8.7 ± 8.9 ($\times 10^7$)	3.9 ± 3.4 ($\times 10^4$)	N.D.	N.D.	N.D.

cfu/cm^2

Ps.: *Pseudomonas aeruginosa* IFO3452.
St.: *Staphylcoccus aureus* IFO12732.
Data shows mean ± SD ($n = 3$).
The lower limit of measurement is 2×10^1 cfu/cm^2.

24 h incubation at 37°C, the bacterial colonies were counted, and expressed as the number of bacteria (cfu/cm^2).

In this study, each experiment was performed in triplicate for *in vitro* tests, and six times for *in vivo* tests.

Statistical analysis

All the data were analyzed by Fisher's protected least significant difference (Fisher's PLSD) and expressed as mean ± standard deviation (SD). A value of $p < 0.05$ was accepted as statistically significant.

RESULTS

In vitro degradation of collagen sponge by immersion in collagenase solution

Figure 3 shows the profiles of the degradation of the artificial dermis impregnated with or without Ag-SD immersed in collagenase solution. It was shown that the collagen sponge of artificial dermis was degraded in a time-dependent manner. Collagen sponges impregnated with Ag-SD at doses of 1 and 3% were degraded with the same time profile as that without Ag-SD. On the other hand, at Ag-SD doses of 5 and 10%, degradation of the collagen sponge was significantly suppressed compared with that of control.

Silver release from artificial dermis impregnated with Ag-SD

Figure 4 shows the *in vitro* profiles of silver release from artificial dermis impregnated with different doses of Ag-SD. The silver release ratio decreased in inverse proportion to the dose of Ag-SD impregnated into artificial dermis.

Antibacterial activity of artificial dermis impregnated with Ag-SD

Table I shows the size of the bacterial inhibitory zone against Ps. or St. on the agar plates. The artificial dermis impregnated with Ag-SD at doses of 1% or higher showed antibacterial efficacy against both Ps. and St., and the size of the inhibitory zone increased in a dose-dependent manner.

The results of quantitative analysis are shown in Table II. The artificial dermis impregnated with Ag-SD at a dose of 1% reduced bacterial growth, compared with control, while no bacteria were detected at doses of 3% or higher for both Ps. and St.

Overall, in the *in vitro* bactericidal tests, artificial dermis impregnated with Ag-SD at doses of 3% or higher was effective in suppressing bacterial growth.

In vitro cytotoxicity test of artificial dermis impregnated with Ag-SD

Figure 5 shows the effect of artificial dermis impregnated with Ag-SD on the *in vitro* proliferation of human fibroblasts. Artificial dermis impregnated with Ag-SD at a dose of 3% or lower did not suppress the growth of cultured fibroblasts, which was similar to the case of the control without Ag-SD. However, when the Ag-SD dose was 5% or higher, the number of fibroblasts grown was smaller than that of the control without Ag-SD. However, at a dose of 5%, the surviving cells were increased 14 days after incubation. At a dose of 10%, they were increased 21 days after incubation. Surviving fibroblasts amounted to 80% at a dose of 5%, and 20% at a dose of 10% after 28 days of incubation.

Histological evaluation of tissue damage *in vivo*

One week after implantation of artificial dermis impregnated with Ag-SD, fibroblasts had infiltrated into the upper layer of the collagen sponge at Ag-SD doses of 5% or lower, and into the middle layer at a dose of 10%. No tissue damage was observed histologically at a dose of 10%. However, 2 weeks after implantation, infiltration of fibroblasts was observed in the uppermost layer of collagen sponge at all doses of Ag-SD, and preparation of a good wound bed for secondary

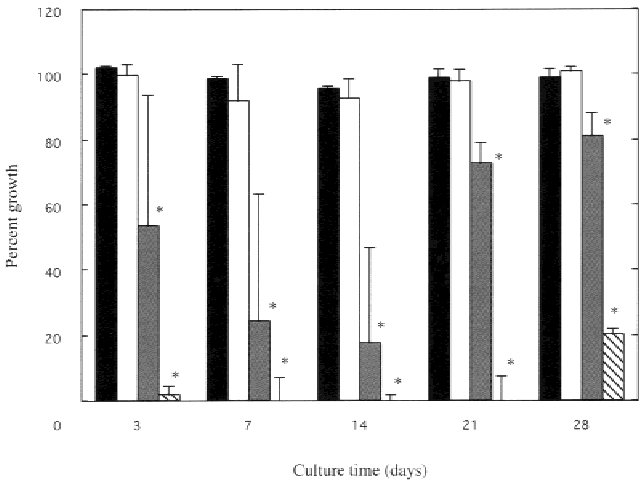

Figure 5. Time course of human fibroblasts grown following plating onto artificial dermis impregnated with Ag-SD at doses of 1% (filled columns), 3% (dotted columns), 5% (heaily dotted columns), and 10% (diagonally striped columns). Percent growth was expressed as the survival ratio of the number of surviving fibroblasts of artificial dermis impregnated with Ag-SD at each dose against that of artificial dermis without Ag-SD at the same incubation time. Each bar shows mean ± SD ($n = 3$). *$p < 0.05$, compared with artificial dermis without Ag-SD.

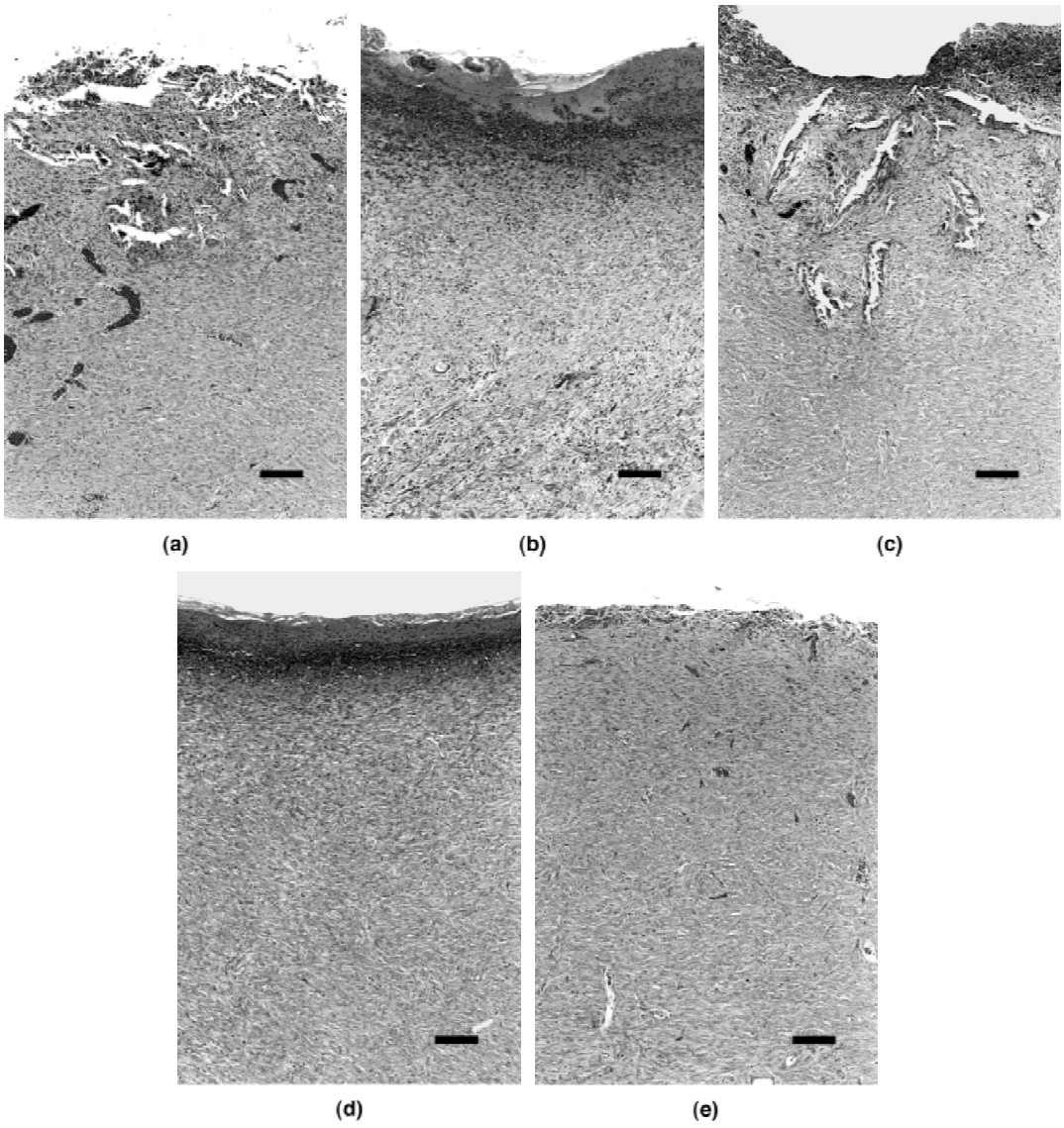

Figure 6. Histological sections of artificial dermis impregnated with Ag-SD at doses of 0% (a), 1% (b), 3% (c), 5% (d), and 10% (e) 2 weeks after implantation. Hematoxylin and eosin, original magnification ×100. Bars correspond to 100 μm.

grafting was obtained even at a dose of 10% (Fig. 6). These findings indicate that artificial dermis impregnated with Ag-SD did not bring about histologically serious damage *in vivo*, irrespective of the Ag-SD dose.

Preliminary *in vivo* tests

Macroscopic tissue appearance

Figures 7 and 8 show the tissue appearance 1 and 3 days after implantation of the artificial dermis with or without Ag-SD impregnation into skin wounds contaminated with Ps. or St. Collagen sponge without Ag-SD started to be degraded at the contaminated wound sites 1 day after implantation, followed by degradation for a further 3 days. Especially, collagen sponge without Ag-SD containing Ps. was completely degraded 1 day after implantation. On the contrary, degradation of collagen sponge with Ag-SD was suppressed, compared with that without Ag-SD, and collagen sponge with Ag-SD, even contaminated with Ps. still remained 3 days after implantation.

Bacteriologic examination

Table III shows the antibacteriologic activity of artificial dermis impregnated with Ag-SD. For artificial dermis without Ag-SD, Ps. count was 2.04×10^{10} and 2.14×10^{9} cfu/cm^2 1 and 3 days after the start of the test. On the other hand, Ps. count was 1.93×10^{6} and 1.49×10^{6} cfu/cm^2 after 1 and 3 days for artificial dermis with Ag-SD. A significant difference between artificial dermis with and without Ag-SD was found 3

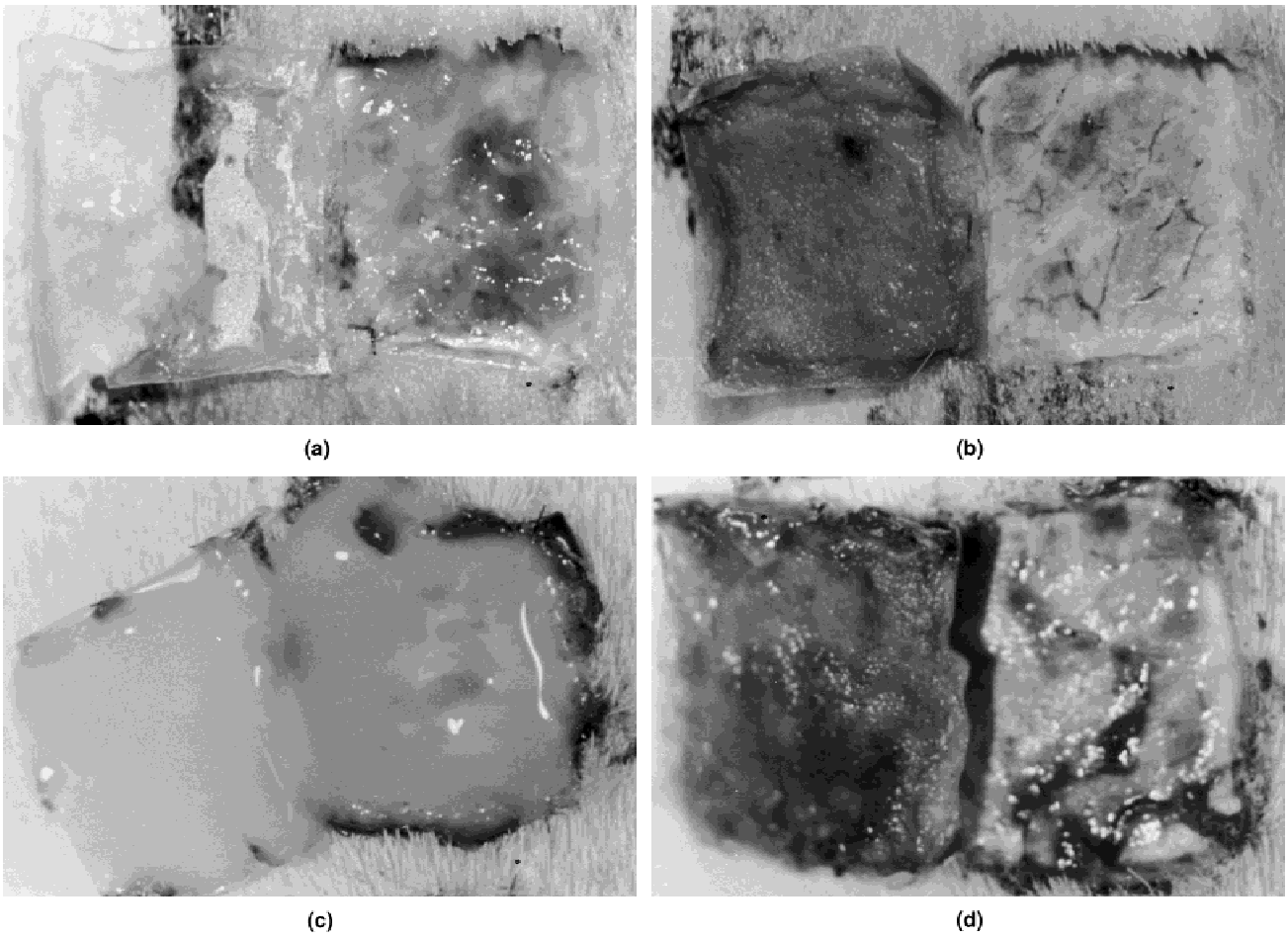

Figure 7. Tissue appearance of wound surface and residual collagen sponge, 1 day after implantation of artificial dermis impregnated with Ag-SD at doses of 0% (a) and 10% (b), and 3 days after implantation with 0% (c) and 10% (d) Ag-SD onto experimentally contaminated wounds with Ps.

days after implantation. For artificial dermis without Ag-SD, St. count was 4.49×10^4 and 7.30×10^3 cfu/cm^2 1 and 3 days after the start of the test. On the other hand, St. count was 6.97×10^2 and 1.07×10^2 cfu/cm^2 after 1 and 3 days in artificial dermis with Ag-SD. A significant difference between artificial dermis with and without Ag-SD was detected on both the sampling days. The artificial dermis impregnated with Ag-SD at a dose of 10% significantly reduced bacterial growth of both Ps. and St. to a significantly greater extent compared with that without Ag-SD.

DISCUSSION

Various wound dressings have been developed for wound repair. However, they cannot be used if the wound surface is already contaminated or infected. Recently, many wound dressings containing antibacterial drugs have been developed.[7,8,32–34] As the drugs are impregnated into the wound dressings, it is ideal to have a broad spectrum of antibacterial activity and less bacterial resistance.

Since Ag-SD was first investigated by Fox[35] in 1968, several reports have described *in vitro* assays of its antibacterial activity and clinical studies. Ag-SD is bactericidal for a wide range of Gram-negative and Gram-positive pathogens. In the management of severe burns, decubitus ulcers and chronic dermal ulcers with infection, Ag-SD cream has been applied to the wound surface. Especially, Ag-SD suppresses the growth of Ps. and St., which are clinically problematic bacteria in burn wounds. To enhance the *in vivo* pharmacological efficacy, several wound dressings impregnated with Ag-SD, released in a sustained manner, have been developed. Various materials have been used for these dressings, such as porcine skin,[36–38] polymer gel composed of poly-2-hydroxyethylmethacrylate and poly(ethyleneglycol),[39] polymeric sheet composed of polyethyleneglycol, hydroxyethyl methacrylate, and dimethyl sulfoxide,[40,41] poly(L-leucine),[42,43] and polyelectrolyte complex composed of chitosan and sodium alginate.[44] However, it is difficult to apply

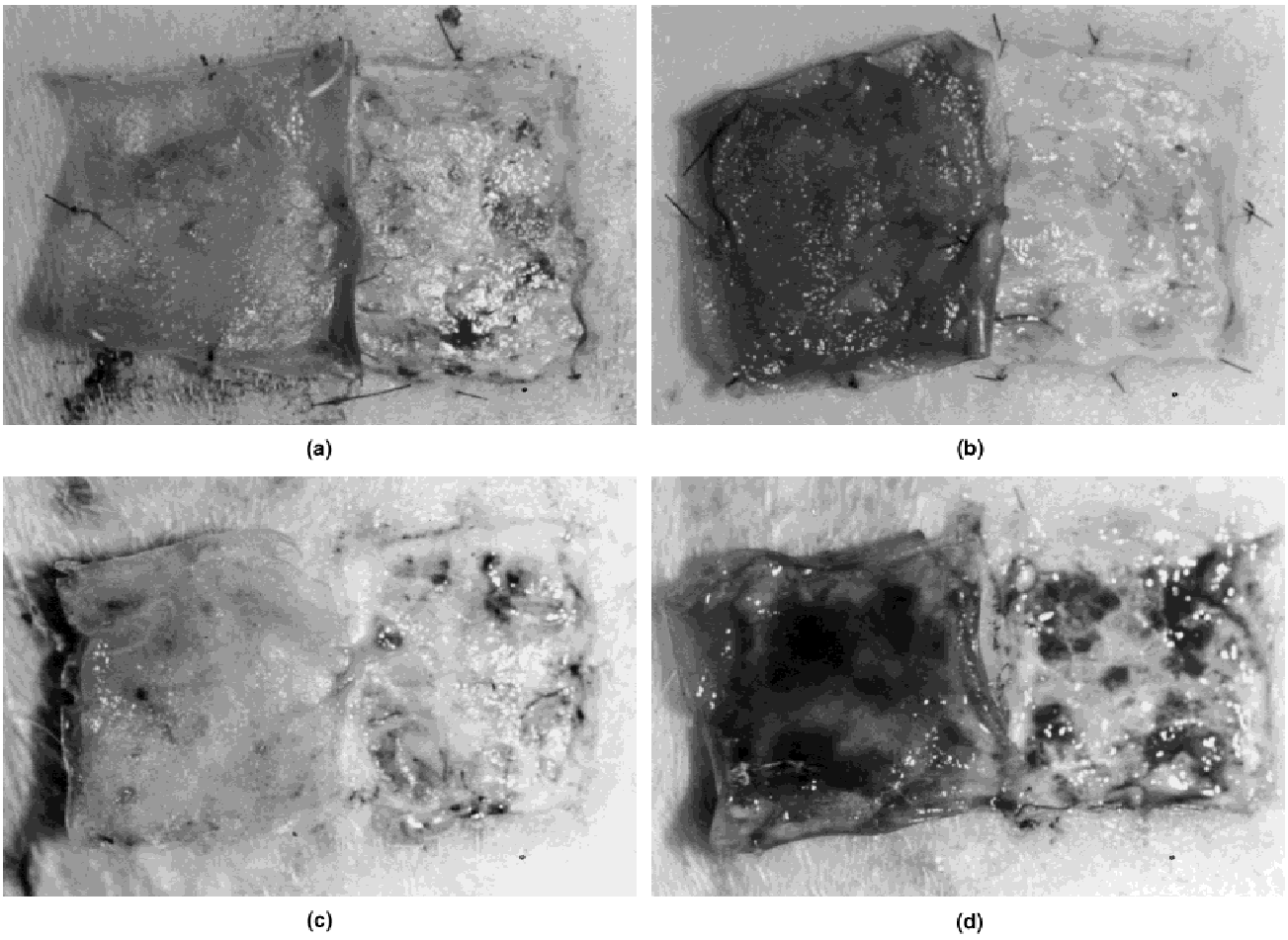

Figure 8. Tissue appearance of wound surface and residual collagen sponge, 1 day after implantation of artificial dermis impregnated with Ag-SD at doses of 0% (a) and 10% (b), and 3 days after implantation with 0% (c) and 10% (d) Ag-SD onto experimentally contaminated wounds with St.

these wound dressings to full-thickness skin defects because they are not converted into regenerated connective tissue similar to true dermis (dermis-like tissue).

Artificial dermis has been used for the treatment of full-thickness skin defects, but it is not always possible to obtain a good result with the presently available artificial dermis in the management of contaminated or infected wounds. One of the requirements for artificial dermis that can be safely applied to contaminated wounds, such as extensive deep burns, is resistance to infection. Therefore, we prepared collagen sponge three-dimensionally impregnated with Ag-SD, which accelerates regeneration of skin dermis as well as possesses antibacterial activity.

The relationship between degradation of the collagen sponge and silver release from the artificial dermis impregnated with Ag-SD was examined in an *in vitro* system with collagenase solution. Irrespective of the Ag-SD dose, the collagen sponge was degraded by collagenase solution in a time-dependent manner, but at doses of 5 and 10%, suppression of the degradation of collagen sponge was observed (Fig. 3). Because it is reported that the enzymatic activity is inhibited by heavy metals such as silver,[45] it is possible that the increase of silver released from the artificial dermis inhibited collagenase activity. This delayed degradation of collagen sponge would be favorable in terms of fibroblast and capillary infiltration into the sponge pores. As shown in Figure 4, silver was released more slowly from the artificial dermis with an increase in Ag-SD dose. This can be explained from the viewpoint of sponge degradation. Because it is likely that Ag^+ molecules bind to the collagen, they will not be released from the sponge unless the sponge is degraded to generate water-soluble collagen fragments. As a result, slower degradation of collagen sponge would result in a lower amount of Ag-SD release. The present artificial dermis homogeneously impregnated with Ag^+ could achieve controlled release of Ag-SD, leading to an effective bactericidal action.

The *in vitro* assay revealed that artificial dermis impregnated with Ag-SD had strong bactericidal activity. However, it is reported that Ag-SD contributes to

TABLE III
Quantitation of Bacterial Content in Experimentally Contaminated Wounds on the Backs of Guinea Pigs 1 or 3 Days After Implantation of Artifical Dermis

Ps. aeruginosa	1 Day	3 Days	*St. aureus*	1 Day	3 Days
Control	2.04×10^{10}	2.14×10^{9}	Control	4.49×10^{4}	7.30×10^{3}
10%	1.93×10^{6}	1.49×10^{6}*	10%	6.97×10^{2}*	1.07×10^{2}*
		cfu/cm^2			cfu/cm^2

Data shows mean ($n = 6$).
*$p < 0.05$, compared with control.

cellular damage if the silver remains on the wound surface.[16] Thus, cellular damage by silver released from the artificial dermis impregnated with Ag-SD was evaluated. The *in vitro* assay showed cytotoxicity of the artificial dermis at Ag-SD doses of 5 and 10% (Fig. 5), whereas no tissue damage was observed histologically around the artificial dermis implanted into full-thickness skin defects on the back of guinea pigs (Fig. 6). In artificial dermis impregnated with Ag-SD at a dose of 10%, delayed infiltration of fibroblasts was observed 1 week after implantation. However, dermis-like tissue was obtained at the implanted site 2 weeks later. In both the *in vitro* and *in vivo* tests, it seemed that the proliferation of fibroblasts was accelerated after Ag-SD release was over. These findings indicate that the Ag-SD released caused cellular damage to fibroblasts, although the extent was not severe enough to stop their growth.

In the *in vivo* test, the artificial dermis impregnated with Ag-SD at a dose of 10% suppressed the degradation of collagen sponge, in marked contrast to that without Ag-SD (Figs. 7 and 8). This phenomenon seems to be advantageous from the viewpoint of tissue regeneration, because the collagen sponge functions as a good matrix for cell infiltration and proliferation. Table III demonstrated that the artificial dermis impregnated with Ag-SD at a dose of 10% significantly suppressed bacterial growth, in contrast to that without Ag-SD, but it did not reduce the growth of Ps. more markedly than that of St. This may depend on the virulence of these bacteria, or, it seems that the collagenase activity of Ps. was higher than that of St., and the accelerated degradation of collagen sponge may have further promoted bacterial growth. Suppression of bacterial growth for 3 to 5 days after implantation of artificial dermis seemed to enhance resistance to infection, because new capillaries started to infiltrate into the collagen sponge. Therefore, the sustained Ag-SD release during this period is most effective. Kuroyanagi et al. reported that 0.4 mg Ag-SD/cm^2 impregnation into poly-L-leucine suppressed bacterial growth in infected wounds inoculated with 1×10^3 Ps. However, 3 days after implantation, bacteria increased. Kim et al. reported that 50 μg Ag-SD/cm^2 impregnation into polyelectrolyte complex sponge suppressed bacterial proliferation *in vitro*. It seems that the Ag-SD doses used in each study were different, because of differences in the impregnation method into various matrixes and in optimal doses for the materials.

In this study, the optimal dose of Ag-SD impregnated into the artificial dermis so as to suppress bacterial growth and to minimize cytotoxicity by sustained silver release for a short time period was considerated to be 10% (148 μg).

CONCLUSIONS

Artificial dermis was able to be made infection-resistant through treatment with Ag-SD. Impregnation of Ag-SD into artificial dermis was effective for both suppressing bacterial growth and assisting in the preparation of the wound bed for secondary grafting even on contaminated wounds. The results of this study indicate that artificial dermis impregnated with Ag-SD was especially useful in treating contaminated wounds or when unsuspected infection was present in the wound.

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