Macromolecular Research, Vol. 11, No. 5, pp 368-374 (2003)

Artificial Dermis Composed of Gelatin, Hyaluronic Acid and (1→3),(1→6)-β-Glucan

Sang Bong Lee, Hyun Wook Jeon, Young Woo Lee, Seong Kwan Cho, and Young Moo Lee*

School of Chemical Engineering, College of Engineering, Hanyang University, Seoul 133-791, Korea

Kang Won Song and Moon Hyang Park

Department of Pathology, College of Medicine, Hanyang University, Seoul 133-791, Korea

Sung Hwa Hong

Korea FDA Biologics Evaluation Department, Blood Products Division, Nokburn-Dong 5, Eunpyung-Ku, Seoul 122-070, Korea

Received July 14, 2003; Revised Sept. 2, 2003

Abstract: Porous scaffolds composed of gelatin and polysaccharides such as hyaluronic acid and β-glucan were prepared by using the freeze-drying method after cross-linking with 1-ethyl-(3-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC). The scaffold had an inter-connected pore structure with the sufficient pore size for use as a support for the growth of fibroblasts. Results for the contact angle and cell attachment confirmed that high gelatin content in a mixture was suitable for cellular attachment and distribution in two- or three-dimensional fibroblast cultures. However, the addition of polysaccharides aroused the synergistic effects of morphologic and mechanical property of gelatin-based scaffolds. To prepare the artificial dermis for the wound dressing to mimic the normal human dermal skin, fibroblasts were isolated from a childs foreskin, and cultured in gelatin-based scaffolds. An *in vivo* study showed that the artificial dermis containing the fibroblasts enhanced the wound healing rate and re-epithelialization of a full-thickness skin defect rather than the acellular scaffold after one week.

Keywords: gelatin, β-glucan, hyaluronic acid, scaffold, skin.

Introduction

In tissue engineering, scaffolds, an artificial extracellular matrix (ECM), should be easy to be synthesized, structured to hold cells close enough together to be able to organize themselves, and preferably, biodegradable so that the polymer would eventually disappear, so reducing the risk in long-term complications. Moreover, the temporary scaffold performs as a template to promote cell adhesion and maintenance of differentiated function without inhibiting proliferation and to derive the formation of new tissue.[1-3]

We have already reported the preparation of gelatin-based sponges containing only one component of polysaccharide such as alginate, hyaluronic acid or β-glucan for wound dressing materials,[4-7] finding out the potential application as components of artificial skin or tissue transplantation to promote epitherialization and granulation tissue formation in the wound. Based on our previous studies, in this work, two kinds of polysaccharides were mixed together with gelatin to synergistically supplement the physico-chemical and biological properties.

Gelatin, the denatured type of collagen is a natural polymer extracted from collagen and can be used as a scaffold for tissue engineering and has been widely used in medicine as a wound dressing, and as an adhesive and absorbent pad for surgical use.[8,9] In particular, collagen and degradation products of collagen may be often used for the attractants of fibroblasts *in vivo* during wound repair, facture healing and embryogenesis.[10,11]

Hyaluronic acid (HA), a component of the extracellular matrix of some tissue such as cockscomb and vitreous humour, consists of 2-acetamide-2-deoxy-α-D-glucose and β-D-glucuronic acid residues linked by alternate (1-3) and (1-4) glycoside bonding (see Figure 1(a)) and has the high capacity of lubrication, water-sorption, and water retention, and influences several cellular functions such as migration, adhesion, and proliferation.[12,13] Recent biomedical applications of HA included scaffolds for wound healing and tissue engineering, as well as ophthalmic surgery, arthritis treatment, and component of implant materials.[14-16]

The other component of the scaffold, extracellular (1→3),

*e-mail : ymlee@hanyang.ac.kr

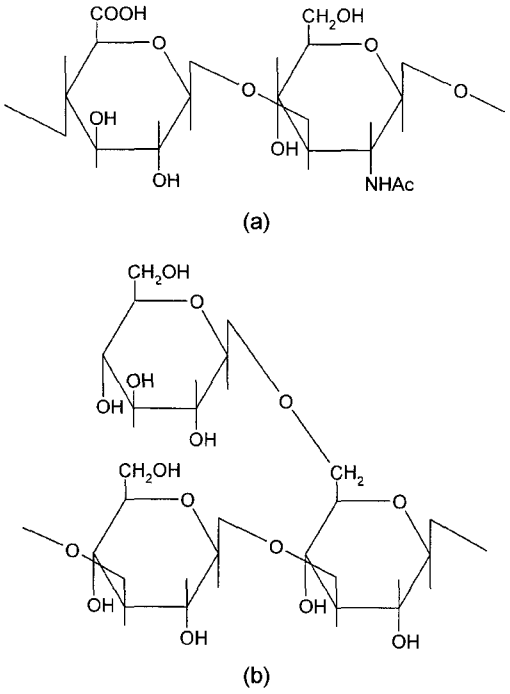

Figure 1. Molecular structures of (a) hyaluronic acid and (b) (1 → 3), (1 → 6)-β-glucan.

(1 → 6)-β-glucan purified by the liquid culture of *Schizophyllum commune,* is a natural complex carbohydrate that works to boost the immune response by activating macrophage cells. It consists of β-(1 → 3) linked-D-glucose residues with one β-(1 → 6) linked D-glucosyl group for every three glucose residues (see Figure 1(b)). β-Glucan is effective against allogeneic, syngeneic and autochthonous tumors and also shows antibacterial, antiviral and anticoagulatory effects and exhibits the wound healing activity.[17]

The objective of the present study is to prepare a porous scaffold composed of gelatin, HA and β-glucan with various mixing ratios, and to investigate their cell attachment and proliferation, surface property such as contact angle and morphological characteristics. Furthermore, the ultimate goal of this study is to culture the fibroblasts isolated from a childs foreskin into the prepared scaffolds to mimic the dermal skin and to evaluate their efficiency for wound healing.

Experimental

Materials. Gelatin was derived from lime-cured tissue, and was purchased from the Sigma Chemical Co. (St. Louis, MO). The (1 → 3),(1 → 6)-β-glucan (Molecular weight by GPC = 2.35 × 10^6) and HA (sodium form, M_v = 1.7 × 10^6) were kindly supplied by the Biochemistry Division of the Pacific Chemical Co., Ltd. (Ansan, Korea). 1-Ethyl-(3-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was also purchased from Sigma Chemical Co. Normal human fibroblasts were aseptically isolated from a foreskin donated by the Urology Department of Hanyang University Hospital in Seoul, Korea, as previous methods.[18-20] The fibroblasts culture medium was composed of Dulbecco's Modified Eagles Medium (DMEM, Gibco BRL, Rockville Maryland, USA) and 10% fetal bovine serum (FBS, Gibco BRL). Dulbeccos phosphate buffered saline (PBS), trypsin-EDTA (0.5% trypsin 5.3 mM EDTA 4 Na) and antibiotic agent, penicillin-streptomycin (100 U/mL), were purchased from Gibco BRL. Trypan blue (0.4% 100 mL) T-8154, and sodium bicarbonate were purchased from the Sigma Chemical Co. The water was distilled and deionized using the Milli-Q System (Waters, Millipore, USA). All other chemicals used were of reagent grade, and were used without any further purification.

Preparation of Gelatin-based Scaffolds. Gelatin, HA and β-glucan with a concentration of 0.7 wt%, respectively were dissolved in deionized water at 50 °C, with the mixture assuming several compositions, as listed in Table I. The solutions were poured into a polystyrene petri dish, frozen at -70 °C and then lyophilized for 24 h. The formed matrixes were cross-linked by immersing them into 20 mL of an acetone: water mixture (9 : 1 by volume) containing 0.35% EDC, and then they were slowly shaken at room temperature for one day. The cross-linked scaffolds were rinsed with deionized water three times to remove any residual chemicals. The rinsed scaffolds were then frozen, lyophilized, and sterilized with ethylene oxide gas.

The morphologies of scaffolds were observed by scanning electron microscopy (SEM, JEOL-6340F electron microscope, Kyoto, Japan) using an operating voltage of 5 kV. Then, the microstructures such as porosity and average pore size of the scaffolds were measured using image analyzer (Bum-Mi Universe Co., Ltd., Seoul, Korea).

A universal testing machine (UTM, INSTRON No.4465, NY, USA) was used to determine the deformation of scaffolds by compressing the sample (2.0 × 2.0 × 0.2 cm) with the indenter at a constant deformation rate of 1.0 mm/min. The values were given as the average of four speciments. The standard deviation from the mean was within ±5%.

Cell Attachment and Proliferation. To coat the materials used to prepare the scaffold onto the well plates, gelatin, β-glucan and HA solutions were mixed as listed in composition of Table I. Then, EDC was added to cross-link the mixture. After homogeneous mixing, 300 μL aliquots were dispersed into a six-well tissue culture dish (Multiwell™, Becton Dickinson, USA). The wells were dried overnight at 50 °C in a vacuum oven, and dried for 12 h at 50 °C after washed three times with deionized water. The contact angles were measured on plates that were either uncoated or coated with the mixture using a Kyowa CA-X interfacer (Kyoto, Japan). In addition, to measure the cell attachment and proliferation, a fibroblast suspension (cell density = 1 × 10^5 cells/well) was placed in each coated well. The uncoated wells were

also seeded to act as controls. All the wells were incubated in an atmosphere of 5% (v/v) CO_2 in air at 37 °C. The number of viable and non-viable cells was countered until 48 h after incubation, using an inverted optical microscope (Nikon Co., TMS-F, Tokyo, Japan) with a hemacytometer attachment (Marienfeld, Germany).

Preparation of Artificial Dermis. Cultured fibroblast cells were suspended in 100 μL of DMEM containing 10% FBS. The cell concentration was adjusted to 4×10^6 cells/scaffold, and then spread on each scaffold (diameter = 1.35 cm, thickness = 0.2 cm) and incubated for 30 min. Fresh medium was added to each culture dish, and this was then incubated at 37 °C in 5% CO_2 atmosphere incubator. The medium was changed every other day. For the staining with hematoxylin-eosin (H&E), the samples were embedded in paraffin, examined with a microscope and photographed.

***In vivo* Wound Healing of Artificial Dermis.** Under intraperitoneal anesthesia with Nembutal, a full-thickness wound of 10 mm in diameter was experimentally prepared on the back of athymic mice (BALB/c Slc-nu, four weeks-old, Japan SLC, Inc., Hamamatsu, Japan). After disinfection of the wound with povidone-iodide, the cellular artificial dermis (Gel100 and GHg721) and acellular scaffold (Gel100) were grafted onto an excised wound ($n = 2$). Tegaderm (3M Health Care, St Paul, MN, USA), was applied over the artificial dermis to prevent the cellular artificial dermis from drying out, and a 6-0 nylon suture (Ethicon, INC., a Johnson & Johnson Company, Somerville, NJ, USA) was used to sew the Tegaderm. The dressed wound was bandaged with adhesive plaster (FIX ROLL®, Young Chemical Co., LTD., Busan, Korea). The mice were scarified after a post-operative period of one week. The wound tissues were embedded in paraffin for staining with H&E.

Results and Discussion

Contact Angle of the Mixtures. To investigate the affinity between the scaffold and the cells, it is important to observe the contact angle, the charge on the materials surface, the existence of a connecting sequence such as arginine-glycine-aspartic acid (RGD) groups, and a surface texture, because cell attachment and proliferation mainly depend on the physical and chemical state on the surface of the materials contacting with the cells.[21] Thus, in this study, cell interactions with each material itself were firstly studied in a two-dimensional culture by measuring the contact angle and by counting the number of attached cells as a function of time, before three-dimensional culture of cells was conducted.

Figure 2 shows the contact angle of the surface of the materials used in the preparation of the scaffold. Comparison of the three materials, their mixtures and polystyrene (PS) petri dish clarifies that the gelatin shows low hydrophilic behavior, whereas β-glucan and HA exhibit relatively high hydrophilic behavior on the PS petri dish of the cell culture.

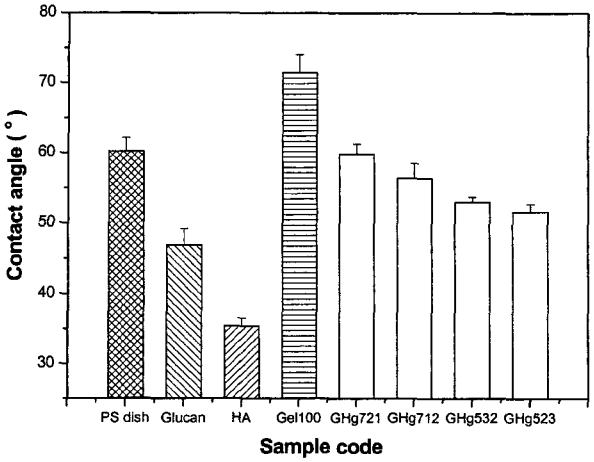

Figure 2. Contact angles measured on plates either uncoated or coated with the mixture composed of gelatin, HA and β-glucan.

The contact angle of the mixture decreases with the increase of the content of β-glucan and HA, owing to the hydrophilic property of glucan and HA. Among the polysaccharides, the contact angle of HA is relatively lower than that of glucan.

In particular, the mixtures containing 70 wt% gelatin exhibited a similar contact angle with the PS petri dish about 60°. For a given contact angle, it is known that a moderate hydrophilic state on the surface is desirable for cell proliferation rather than an extreme hydrophilic or hydrophobic state.[21] Tamada and Ikada reported that water contact angles of material surfaces strongly influence cell attachment behavior, and show maximum cell attachment around 70° of contact angle.[22] The results from contact angle reveal that a suitable contact angle to attach the cell on the matrix could be controlled by mixing the gelatin with the appropriate ratios of HA and β-glucan.

Cellular Attachment and Proliferation. Figure 3 shows the degrees of attachment and proliferation of the fibroblasts on a gelatin/HA/β-glucan-coated well plate. The number of attached cells in the early stage was similar to that of the PS petri dish. However, after 24 h, the number of proliferated cells significantly increased in the Gel100 and those of GHg 721 and GHg712 were similar to the PS, whereas GHg532 and GHg523 with 50% gelatin contents showed the relatively low proliferation rates. Thus, it was found that the cell attachment and proliferation increased with gelatin content in the mixtures containing the gelatin and polysaccharides.

The improved cell attachment in the high gelatin content may be due to the charge and the existence of specific amino residues in the gelatin sequences. Gelatin contains basic residues, such as lysine and arginine, which have a positive charge and the specific cell adhesion sites such as RGD groups.[21] Cells adhere much more strongly to substrates with basic groups than to substrates with acidic or neutral groups, because cells have negative charges on their surfaces.[23] In addition, the RGD group actively induces cellular adhesion

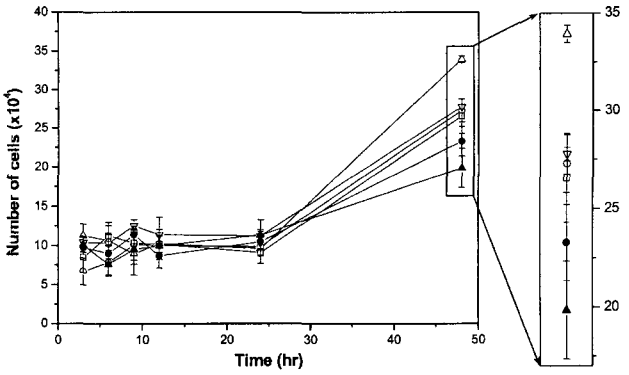

Figure 3. Attachment degree of fibroblast on gelatin, HA and β-glucan coated well-plates. PS tissue culture dish(○), Gel100 (△), GHg721(□), GHg712(▽), GHg532(●), and GHg523(▲).

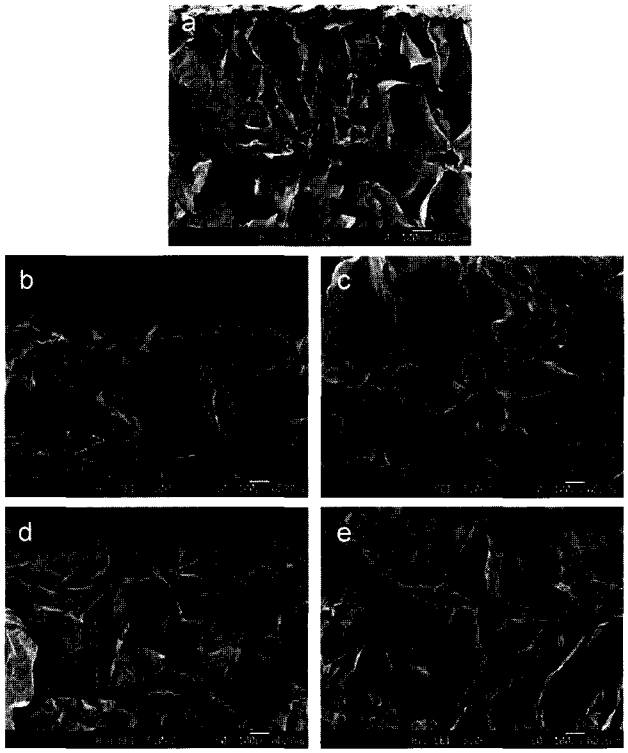

Figure 4. Cross-sectional scanning electron microscopy images of (a) Gel100, (b) GHg721, (c) GHg712, (d) GHg532, and (e) GHg523 (× 80).

by binding to integrin receptors, and this interaction has been shown to play an important role in cell growth and differentiation and in overall regulation of cell functions.[24]

If only the cell attachment function may be considered, then β-glucan and HA are not suitable for manufacturing the scaffold, because polysaccharides, such as β-glucan and HA, have been reported to be poor substrates for cell attachment.[25] However, it is expected that the addition of β-glucan and HA may have advantages for the mechanical properties, for the morphologies, and for its effect on certain bio-active wound healing properties.

Morphology and Mechanical Properties of Scaffolds. The porosity, mean pore size and orientation of pores have influenced on the migration and distribution of the cells in the scaffolds containing open-pore structures.[5] The highly porous structure of the scaffolds assists cell penetration as well as polymer degradation. The rate of degradation is affected by the morphology of the scaffold, because large surface areas promote the diffusion of water molecules into the bulk of the polymers when they are placed in an aqueous environment.

Figure 4 shows the cross-sectional morphologies of gelatin /HA/β-glucan scaffolds with various weight ratios. Gelatin-based scaffolds appear in the inter-connected network pore configuration characterized by its membrane-like structure with an average pore size of about 90~150 μm, listed in Table I. This pore size facilitated cellular infiltration into the pores and channels of the scaffold.

From Table I, the average pore size and porosity of the scaffolds decreased with increasing HA and β-glucan content, owing to the high molecular weight and viscosity of the HA and β-glucan solution. Moreover, the higher HA content in the scaffold containing the same gelatin content helps to

Table I. Composition, Physical and Morphological Characteristics of Scaffolds

Sample Code	Weight Ratio[a] (wt%)			Porosity[b] (%)	Average Pore Size[c] (μm)	Water Uptake[d] (g H_2O/ g sponge)
	Gelatin	β-glucan	Hyaluronic acid			
Gel100	100	0	0	70.1 ± 0.2	152 ± 2.4	70 ± 2
GHg721	70	20	10	68.6 ± 0.5	130 ± 3.2	72 ± 3
GHg712	70	10	20	65.6 ± 0.6	121 ± 6.5	75 ± 6
GHg532	50	30	20	58.4 ± 0.3	97 ± 7.5	78 ± 4
GHg523	50	20	30	55.3 ± 0.2	89 ± 6.4	80 ± 5

[a]Concentration of gelatin, HA and β-glucan solutions: 0.7 (w/v) %.
[b]Porosity was obtained from area analysis between the pore zone and matrix zone by the Image Analyzer Program.
[c]Average pore size was calculated by measuring the size of 30 pores by the Image Analyzer tools.
[d]Water uptake ability was calculated from the weight difference between wet and dry of the scaffolds.

improve the pore size and porosity. Water uptake of scaffolds increased with HA and β-glucan content, although the pore size and porosity were decreased, owing to the naturally hydrophilic properties of HA and β-glucan.

Note that the gelatin-based scaffolds prepared in this study did not form a dense surface layer. This seems to be from the low concentration of gelatin (0.7 wt%), but also from the addition of β-glucan and HA. In our previous studies, gelatin-based scaffolds had a dense surface that prevented cells from penetrating them into the inner areas when the cell suspension was seeded on the scaffold.[4-7] The dense layer may contribute to the uni-axial orientation of the pore channels in the upper part of the scaffold, because the upper part of the scaffold was directly exposed to the vacuum during the lyophilization process, and had a different structure to the lower region in contact with the mold surface. A dense surface in the wound dressing materials is suitable for protecting from the evaporation of exudates from the wound. In contrast, a film-like surface has a detrimental effect on cell migration in the cell seeding on the surface of a scaffold.

Figure 5 shows the compressive curves of gelatin-based scaffolds. The compression testing was conducted to evaluate the mechanical strength of scaffolds with different ratios of gelatin and polysaccharides. The initial modulus or slope of load vs. strain curve of scaffolds increased with polysaccharides content of scaffold, due to relatively much higher molecular weight and molecular structure with rigid hexagonal chain of polysaccharides.

Artificial Skin Composed of Dermis. From Figure 6, the fibroblasts show a good affinity to, and distribution on Gel100 rather than on the other compositions composed of gelatin, HA and β-glucan during the short culture periods (two weeks). The cells and secreted collagen were spread onto the scaffold surface in the gelatin alone, whereas cell attachment was inhibited, and cells became aggregated when β-glucan and HA were added.

An eight-week long-term culture study revealed that seeded fibroblasts proliferated and were well distributed in all samples (see Figure 7). This means that some elements

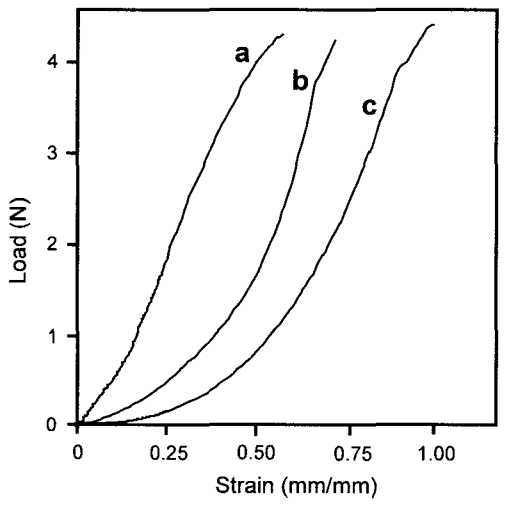

Figure 5. Compressive curves of GHg scaffolds at the constant deformation of 1.0 mm/min. (a) GHg721, (b) GHg532, and (c) Gel100.

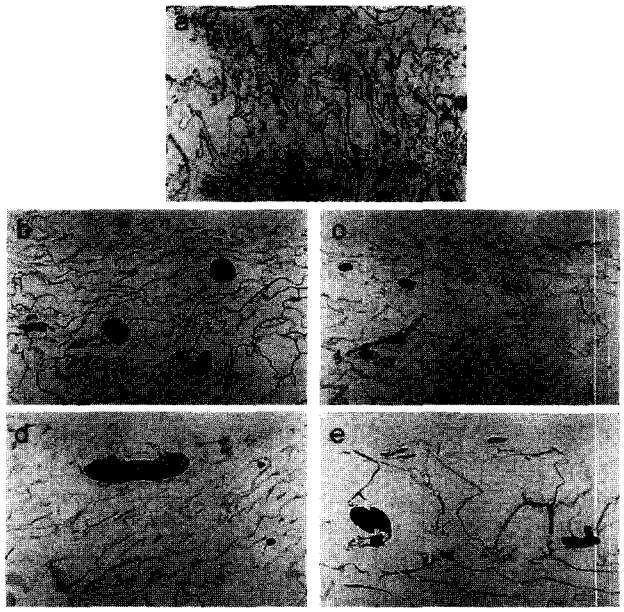

Figure 6. Histological staining with H&E of GHg scaffolds cultured with fibroblasts for two weeks. (a) Gel100, (b) GHg721, (c) GHg712, (d) GHg532, and (e) GHg523.

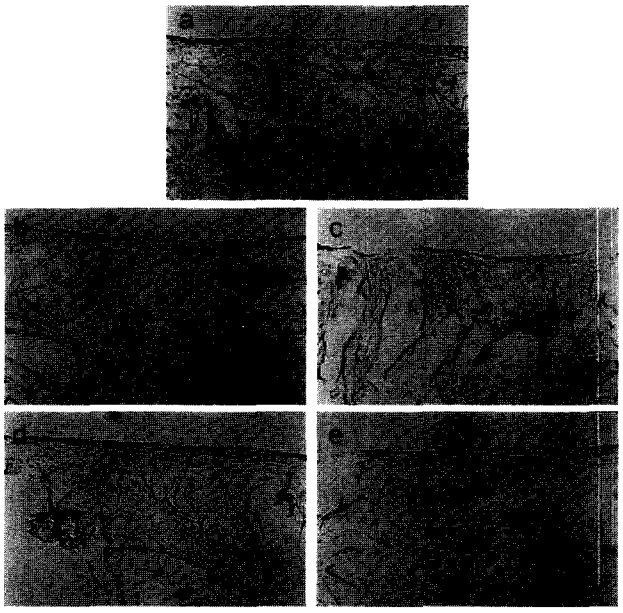

Figure 7. Histological staining with H&E of GHg scaffolds cultured with fibroblasts for eight weeks. (a) Gel100, (b) GHg721, (c) GHg712, (d) GHg532, and (e) GHg523.

of the culture media such as the serum may be accumulated by the scaffold, so influencing the adhesion and proliferation of the cells. However, the matrix with fibroblasts is not completely degradated and exchanged with the neo-dermal collage secreted from the fibroblasts. Although the matrix incompletely forms the skin structure, it is applied for the wound bed, because a dermal substitute only functions as a guide for cells moving into the repair area, serves as a scaffold for cells such as fibroblasts, and helps to synthesize extra-cellular matrix (ECM) components.[26-28]

Animal Tests. Figure 8 shows the histological results of acellular Gel100, cellular Gel100 and GHg721 scaffolds applied to the dorsal skin wound of athymic mice after one week of dressing. The skin defects for acellular Gel100 containing fibroblasts had almost completely regenerated and showed only small defect at the center. The wound dressed by GHg721 containing fibroblasts had re-epithelialized, although re-epithelialized length in the wound was shorter than that of cellular Gel100. On the other hand, in the case of acellular Gel100, the surface was covered with a thick zone of acute inflammatory exudates with underlying exuberant granulation tissue that had not yet epithelialized.

From the *in vivo* study, it was found that the artificial dermis rather than the acellular scaffold improved the re-epithelialization on the full-thickness skin defect. This was caused by the effects of the fibroblasts on the artificial dermis. Several studies have indicated that the presence of fibroblasts in a dermal equivalent stimulates epidermal differentiation.[25,29,30] It is assumed that the presence of fibroblasts in a dermal equivalent accelerate the healing process by reducing the time needed for the fibroblasts to invade the wound tissue and by early synthesis of new skin tissue, because the fibroblasts on the artificial dermis can release biologically-active substances, such as cytokines. These cytokines promote healing by stimulating the production of components in the basement membrane, so preventing dehydration and increasing inflammation, and promoting the formation of granulation tissue.[31,32] In addition, the cytokines are stimulated by the increased proteolytic activity leading to the fragmentation of more proteins, and the activation of larger numbers of latent growth factors, resulting in a higher chemotactic activity of the wound tissue.[29]

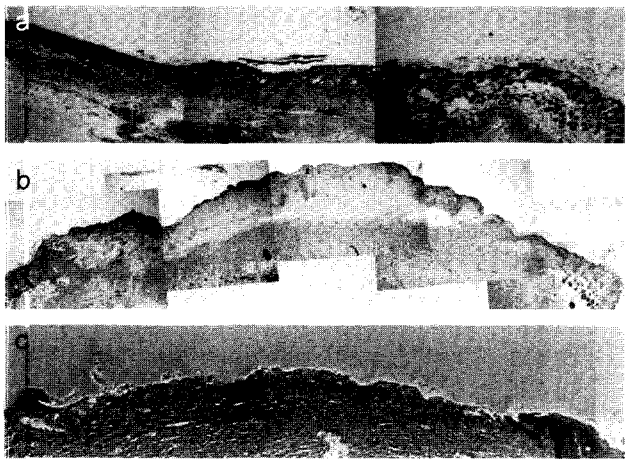

Figure 8. Histological cross-sections of a mouse skin wound after one week (H&E staining). Artificial dermis (a) Gel100, (b) GHg721 and (c) Acellular Gel100.

Conclusions

Porous scaffolds composed of gelatin and polysaccharides such as HA and β-glucan had the inter-connected pore structure and average pore size of 90~150 μm. The initial modulus of scaffolds increased with polysaccharides content of scaffold. The cell attachment and proliferation improved in the gelatin alone, whereas water uptake increased with HA and β-glucan contents. *In vitro* culture study revealed that the fibroblasts showed a good affinity to, and distribution on Gel100 rather than on other compositions during the two-week culture periods, whereas an eight-week long-term culture study revealed that seeded fibroblasts proliferated and were well distributed in all samples. *In vivo*, the artificial dermis rather than the acellular scaffold improved the re-epithelialization on the full-thickness skin defect. Therefore the artificial dermal skin composed of gelatin and polysaccharides such as HA and β-glucan will be useful to promote wound healing.

Acknowledgements. SBL appreciates the fellowship from BK21 program. Financial support from the Korea Food and Drug Administration (KFDA) under the program year 2002 is greatly appreciated.

References

(1) J. S. Mao, L. G. Zhao, Y. J. Yiu, and K. D. Yao, *Biomaterials*, **24**, 1067 (2003).
(2) G. Khang, J. M. Rhee, P. Shin, I. Y. Kim, B. Lee, S. J. Lee, Y. M. Lee, H. B. Lee, and I. Lee, *Macromol. Res.*, **10**(3), 158 (2002).
(3) S. J. Lee, Y. M. Lee, G. Khang, I. Y. Kim, B. Lee, and H. B. Lee, *Macromol. Res.*, **10**(3), 150 (2002).
(4) Y. S. Choi, S. R. Hong, Y. M. Lee, K. W. Song, M. H. Park, and Y. S. Nam, *Biomaterials*, **20**, 409 (1999).
(5) Y. S. Choi, S. R. Hong, Y. M. Lee, K. W. Song, M. H. Park, and Y. S. Nam, *J. Biomed. Mater. Res.*, **48**, 631 (1999).
(6) Y. S. Choi, S. B. Lee, S. R. Hong, Y. M. Lee, K. W. Song, M. H. Park, and Y. S. Nam, *J. Mater. Sci.-Mater. M.*, **11**, 1 (2001).
(7) S. R. Hong, S. J. Lee, J. W. Shim, Y. S. Choi, Y. M. Lee, K. W. Song, M. H. Park, Y. S. Nam, and S. I. Lee, *Biomaterials*, **22**, 2777 (2001).
(8) S. Meier and E. D. Hay, *Dev. Biol.*, **38**, 249 (1974).
(9) G. O. Gey, M. Svoelis, M. Foard, and F. B. Bang, *Exp. Cell. Res.*, **84**, 63 (1974).

(10) A. E. Postlethwaite, J. M. Seyer, and A. H. Kang, *Proc. Natl. Acad. Sci. USA*, **75**, 871 (1978).
(11) J. K. Law, J. R. Parsons, F. H. Silver, and A. B. Weiss, *J. Biomed. Mater. Res.*, **23**, 961 (1989).
(12) L. Benedetti, R. Cortivo, A. Berti, and F. Pea, *Biomaterials*, **14**, 1154 (1993).
(13) N. E. Larsen, C. T. Pollak, K. Reiner, E. Leshchiner, and E. A. Balazs, *J. Biomed. Mater. Res.*, **27**, 1129 (1993).
(14) G. Karlsson and R. Bergman, *J. Chromatogr. A*, **986**, 67 (2003).
(15) T. Sawai and M. Uzuki, *International Congress Series*, **1223**, 273 (2001).
(16) G. Marchini, M. Marraffa, C. Brunelli, R. Morbio, and L. Bonomi, *J. Cataract Refr. Surg.*, **27**, 507 (2001).
(17) J. A. Bohn and J. N Bemiller, *Carbohyd. Polym.*, **28**, 3 (1995).
(18) K. Yang, Y. K. Seo, H. H. Youn, D. H. Lee, S. N. Park, and J. K. Park, *Artif. Organs*, **24**, 7 (2000).
(19) R. L. Freshney, in *Culture of Animal Cells, A Manual of Basic Technique*, 4th edition, Wiley-Liss Inc., 2000, pp 345.
(20) M. L. Martine, Lafrance, and D. W. Armstrong, *Tissue. Eng.*, **5**, 153 (1999).
(21) K. S. Khang, S. J. Lee, J. H. Jeon, J. H. Lee, and H. B. Lee, *Polymer (Korea)*, **24**, 869 (2000).
(22) Y. Tamada and Y. Ikada, *J. Colloid Interf. Sci.*, **155**, 334 (1993).
(23) N. DAN, *Biochimica et Biophysica Acta (BBA) - Biomembranes*, **1564**, 343 (2002).
(24) R. A. Quirk, W. C. Chen, M. C. Davies, S. J. B. Tendler, and K. M. Shakesheff, *Biomaterials*, **22**, 865 (2001).
(25) Y. Kuroyanagi, *J. Artif. Organs.*, **2**, 97 (1999).
(26) B. Chevallay, N. A. Malak, and D. Herbage, *J. Biomed. Mater. Res.*, **49**, 448 (2000).
(27) M. D. Harriger, A. P. Supp, G. D. Warden, and S. T. Boyce, *J. Biomed. Mater. Res.*, **35**, 137 (1997).
(28) M. Tanaka, N. Nakakita, and Y. Kuroyanagi, *J. Biomat. Sci.-Polym. E.*, **10**, 433 (1999).
(29) D. Y. Lee, H. T. Ahn, and K. H. Cho, *J. Dermatol. Sci.*, **23**, 132 (2000).
(30) C. Bernard, L. Corinne, and D. Louis, *J. Invest. Dermatol.*, **92**, 122 (1989).
(31) A. J. Singer and R. A. F. Clark, *New. Engl. J. Med.*, **341**, 738 (1999).
(32) W. H. Eaglstein, M. Iriondo, and K. Laszlo, *Dermatol. Surg.*, **21**, 839 (1995).