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Cellular compatibility of a gamma-irradiated modified siloxane-poly(lactic acid)-calcium carbonate hybrid membrane for guided bone regeneration

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A bi-layered silicon-releasable membrane consisting of a siloxane-poly(lactic acid) (PLA)-vaterite hybrid material (Si-PVH) microfiber mesh and a PLA microfiber mesh has been developed by an electrospinning method for guided bone regeneration (GBR) application. The bi-layered membrane was modified to a three-laminar structure by sandwiching an additional PLA microfiber mesh between the Si-PVH and PLA microfiber meshes (Si-PVH/PLA membrane). In this study, the influence of gamma irradiation, used for sterilization, on biological properties of the Si-PVH/PLA membrane was evaluated with osteoblasts and fibroblasts. After gamma irradiation, while the average molecular weight of the Si-PVH/PLA membrane decreased, the Si-PVH/PLA membrane promoted cell proliferation and differentiation (alkaline phosphatase activity and calcification) of osteoblasts, compared with the poly(lactide-co-glycolide) membrane. These results suggest that the gamma-irradiated Si-PVH/PLA membrane is biocompatible with both fibroblasts and osteoblasts, and may have an application for GBR.

Keywords: Silicon, Poly(lactic acid), Guided bone regeneration, Membrane, Cellular compatibility

INTRODUCTION

Dental implants require sufficient alveolar bone levels for aesthetically and functionally successful placement; thus, these bone levels influence prognosis. Various procedures for alveolar ridge augmentation have been performed before placement of dental implants in severely resorbed bone areas following tooth loss. To provide a suitable environment for the implant placement, guided bone regeneration (GBR), a bone regeneration technique, is used frequently in clinical dentistry[1,2)].

GBR can support alveolar bone regeneration via the application of a biocompatible membrane[3-5)], which mechanically excludes surrounding soft connective tissue and maintains the space into which the desirable bone cells can migrate, thereby allowing the proliferation and differentiation of osteoblasts for bone formation. Critical criteria for ideal barrier membranes include biocompatibility, cell-occlusiveness, space-making function, tissue integration and clinical manageability[6)].

There are two types of membrane materials: non-resorbable materials, including expanded polytetrafluoroethylene; and resorbable materials, including poly(lactic acid) (PLA), poly(lactideco-glycolide) (PLGA) and poly(glycolide acid) (PGA). However, these membranes do not possess any bioactive properties to induce osteogenesis. Clinically, they are frequently used in combination with bone grafts for treatment of relatively large bone defects. Thus, it is thought that membranes with bioactive properties to induce bone formation are more suitable for bone regeneration.

Trace amounts of silicon species promotes bone formation[7-11)]. Carlisle have reported that silicon is associated with calcium in an early stage of calcification[7)]. Keeting *et al*. have demonstrated that the silicon-containing compound zeolite A (ZA) promotes proliferation, alkaline phosphatase (ALP) activity, and osteocalcin production of human osteoblast-like cells[8)]. Recently, Obata *et al*.[12)] developed a bi-layered silicon-releasable microfiber mesh with possible applicability for GBR, consisting of a siloxane-PLA-vaterite (Si-PVH) microfiber mesh and a PLA microfiber mesh, using an electrospinning method. As PLA is reported to be highly biocompatible, the PLA side of the bi-layered mesh comes into contact with connective tissue, while the Si-PVH side faces bone tissue to promote proliferation and differentiation of osteoblasts in bone defects.

For clinical applications, the meshes should be sterilized. Generally, gamma irradiation is used to sterilize GBR membranes, but in the case of synthetic polymers membranes, gamma irradiation may decrease their average molecular weight by molecular chain scission, resulting in the reduction of tensile strength and the acceleration of resolution of the membranes, which may decrease the functions of space-making and cell-occlusiveness[13,14)].

Fiber diameters and microfiber mesh pore sizes reportedly affect cell migration and transport or cell-occlusiveness function[15-17)]. Therefore, the bi-layered meshes were modified to produce three-layered microfiber meshes by inserting a PLA mesh with 2-µm-diameter

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fiber (PLA-2 microfiber mesh) between the PLA mesh with 10-µm-diameter fiber (PLA-10 microfiber mesh) and the Si-PVH microfiber mesh (Si-PVH/PLA membrane), to maintain functions of cell-occlusiveness and space-making after gamma irradiation.

Wakita *et al*. reported properties of Si-PVH microfiber mesh such as PLA degradability and time-dependent levels of calcium and silicon ions released from the mesh[12,18,19]. In the present study, we evaluated the cellular compatibility of gamma-irradiated Si-PVH/PLA membrane *in vitro* using osteoblastic cells and fibroblasts for the purpose of GBR application.

MATERIALS AND METHODS

Preparation and sterilization of Si-PVH/PLA membrane

The preparation methods for Si-PVH and PLA microfiber meshes were previously reported by Wakita *et al*.[12,18,19]. Briefly, Si-PVH, which is a composite consisting of PLA and siloxane-doped vaterite (SiV) powders, were prepared by blowing CO_2 gas in methanol $Ca(OH)_2$ slurry that included aminopropyltriethoxysilane. The Si-PVH microfiber meshes were prepared by an electrospinning method. Chloroform was used as the solvent for these mixtures to prepare a solution for electrospinning. The PLA-10 and PLA-2 microfiber meshes were also electrospun with the solution that PLA was dissolved in chloroform and methanol at different ratios. These three microfiber meshes were hot-pressed at 150°C for 10 seconds under a pressure of 200 kPa to produce Si-PVH/PLA membrane, as shown in Fig. 1; the resulting Si-PVH/PLA membrane was coated with hydroxyapatite (HA) by soaking in simulated body fluid (SBF) for 1 day.

The Si-PVH/PLA membranes were trimmed to circles of 10 mm in diameter to fit wells of 48-well tissue culture plates (Iwaki, Tokyo, Japan), and sterilized by gamma irradiation at the standard dose of 25 kGy (RIC1, Radia Industry, Gunma, Japan)[20]. The membranes were immersed for 1 day in the culture medium (as described in the Cell cultures section) to improve the hydrophilicity of the membranes before use.

Characterization of Si-PVH/PLA membranes

The molecular weights of Si-PVH, PLA-10, and PLA-2 microfiber meshes were determined by gel permeation chromatography (LC-20, Shimadzu, Kyoto, Japan). The weight-average molecular weight (*Mw*), the number-average molecular weight (*Mn*), and polydispersity index (*Mw*/*Mn*) were calculated. The microfiber meshes were characterized by X-ray diffractometer (XRD; RAD-B, Rigaku, Tokyo, Japan) and Fourier transform infrared spectrometer (FTIR; FT-IR-460 Plus, JASCO, Tokyo, Japan) before and after the gamma irradiation. A 48-well-sized Si-PVH/PLA membrane and PLGA membrane (GC membrane, GC Corporation, Tokyo, Japan) as controls were immersed for 9 days in 2.4 mL of α-modified minimum essential medium (α-MEM) (Sigma, St. Louis, MO, USA) containing 10% fetal bovine serum (FBS) (Hyclone Laboratories, Logan, UT, USA), 2 mM L-glutamine (ICN Biomedicals, Aurora, OH, USA), 100 units/mL penicillin G (Wako Pure Chemical Industries, Osaka, Japan), and 100 µg/mL streptomycin (Wako Pure

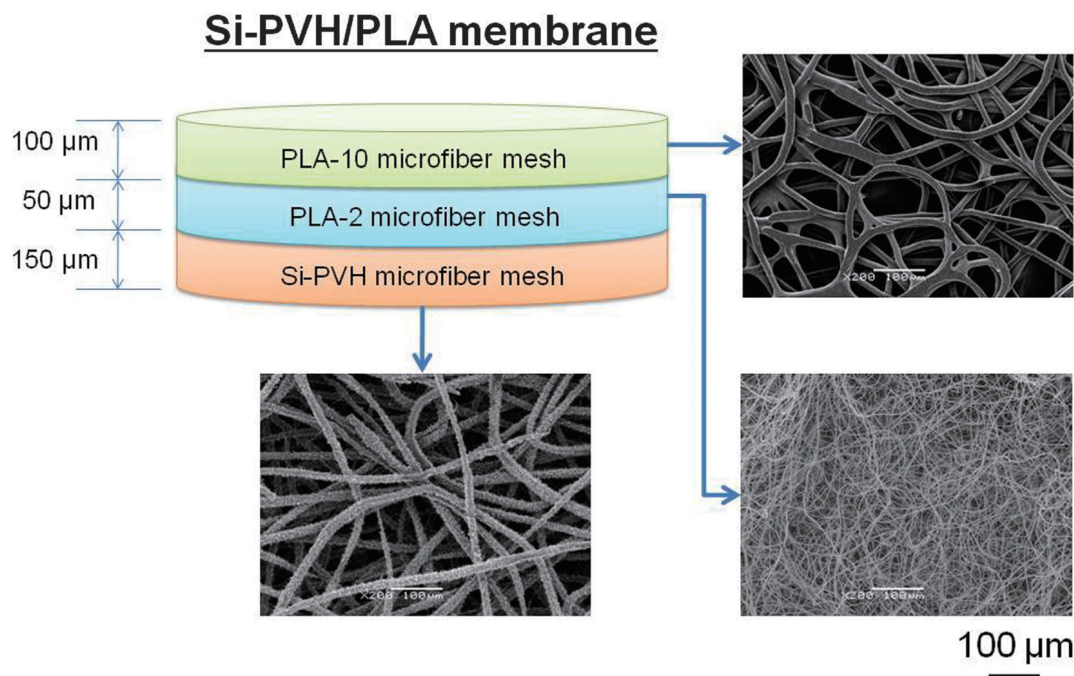

Fig. 1 Schematic drawings of the Si-PVH/PLA membrane and SEM images of PLA-10, PLA-2, and Si-PVH microfiber meshes.

Chemical Industries). Dissolution amounts of Si^{4+}, Ca^{2+}, and P^{5+} ions in the eluates were analyzed by inductively coupled plasma atomic emission spectroscopy (ICPS-7000, Shimadzu).

Cell cultures

Murine osteoblastic cell line MC3T3-E1 cells were obtained from Riken Cell Bank (Ibaraki, Japan) and cultured in α-MEM containing 10% FBS, 2 mM L-glutamine, 100 units/mL penicillin G and 100 μg/mL streptomycin at 37°C in a humidified atmosphere of 5% CO_2. Human gingival fibroblasts (HGFs) were isolated from healthy gingival tissues obtained during periodontal surgery after informed consent. The experimental protocol of this study was approved by the Ethical Committee of Kagoshima University Graduate School of Medical and Dental Sciences (22-81). The HGFs were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma) containing 10% FBS, 100 U/mL of penicillin, and 100 μg/mL of streptomycin under the same condition as MC3T3-E1 cells. The MC3T3-E1 cells and HGFs were detached by treatment with 0.25% trypsin (Gibco Laboratories, Paisley, UK) and 0.02% ethylenediaminetetraacetic acid (Nacalai Tesque, Kyoto, Japan) in phosphate-buffered saline (PBS) and subsequently were subcultured. The HGFs were used for experiments between their fifth and tenth passages.

Cell proliferation assay

The MC3T3-E1 cells and HGFs (5×10^3 cells/well; n=4) were plated on Si-PVH and PLA sides of Si-PVH/PLA membranes in 48-well tissue culture plates, respectively. For comparison, both cell types were plated on PLGA membranes and culture plates. Cell proliferation was assessed at 3, 6, and 9 days after incubation using Cell-Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions. The absorbance of the formazan dye produced in the cultures was measured at a wavelength of 450 nm. Cell number was calculated, based on a calibration curve which was prepared using the data obtained from the wells that contained the known numbers of viable cells.

In some experiments, the effects of eluates from Si-PVH/PLA membranes or PLGA membranes on proliferation of MC3T3-E1 cells were investigated. After MC3T3-E1 cells (5×10^3 cells/well; n=4) were cultured in 48-well tissue culture plates for 1 day, medium change was performed every 3 days using medium containing eluates after immersion of the 48-well sized membrane for 9 days, and cell proliferation was assessed at 3, 6, and 9 days.

Morphology of the cells on the membrane

The cells that attached onto the membranes at days 3, 6, and 9 after incubation were pre-fixed with 2.5% glutaraldehyde containing 0.1 M sodium cacodylate buffer (pH 7.4) for 20 minutes, fixed for 1 hour with 0.1 M sodium cacodylate buffer and post-fixed for 30 minutes with 1% osmium tetroxide containing 0.1 M cacodylate buffer. After dehydration using graded ethanol series, the membranes were transferred into *t*-butyl alcohol, freeze-dried, and sputter-coated with gold. The morphology of the cells was observed by scanning electron microscopy (SEM; JSM-5510LV, JEOL, Tokyo, Japan).

ALP activity assay

MC3T3-E1 cells were seeded at a density of 1×10^4 cells in 48-well tissue culture plates and cultured for 1 day. The medium was changed to the medium containing eluates after immersion of the 48-well sized membrane for 9 days with 0.2 mM ascorbic acid and 10 mM β-glycerophosphate at 3-day intervals. The ALP activity was measured with a 4-nitrophenyl phosphate colorimetric assay at days 3, 9, and 15 after changing to the stimulating medium. The cells were washed with PBS twice before addition of lysis buffer (1.5 M Tris-HCl (pH 9.2), 0.1 M $ZnCl_2$, 0.5 M $MgCl_2$-$6H_2O$, and Triton X-100), and the cells in each well were sonicated for 10 seconds. The sonicated samples were added to substrate solution consisting of 4-nitrophenyl phosphate (Sigma), 1.5 M alkaline buffer solution (Sigma) and H_2O and incubated for 20 minutes at 37°C. Subsequently, 2N NaOH was added to the samples to stop the reaction. Absorbance of the samples was measured at 405 nm to obtain ALP activity per unit protein content. The total protein was quantified with DC protein assay (BIO-RAD, Hercules, CA, USA). The ALP activity was calculated with protein revision.

Calcification assay

The MC3T3-E1 cells were cultured for 21, 27, and 33 days to observe time dependence of calcification as described in the ALP activity assay, except for the addition of 10^{-7} M dexamethasone (Sigma) to the medium. After culture, the cells were washed with PBS twice, fixed with formalin for 20 minutes, washed with distilled water, and treated with alizarin red S stain solution (pH 6.37) for 5 minutes. Subsequently, stained calcium deposits were washed with distilled water twice and dye was extracted with 10% (w/v) cetylpyridinium chloride in 10 mM sodium phosphate (pH 7.0) for 20 minutes at room temperature. The concentration of the alizarin red S was determined by measuring the absorbance at 540 nm using an alizarin red S standard curve[21].

Statistical analyses

These data were statistically analyzed by means of the Bonferroni method. Differences were considered significant at $p<0.05$.

RESULTS

Characterization of Si-PVH/PLA membrane

Table 1 shows the molecular weights (*Mw* and *Mn*) and the polydispersity index (*Mw*/*Mn*) before and after gamma irradiation of PLA-10, PLA-2, and Si-PVH microfiber meshes. While the molecular weights of all samples after the irradiation decreased to approximately one third of those before the irradiation, the polydispersity

index increased after the irradiation.

Figure 2a shows that there were no remarkable changes in XRD patterns before and after gamma irradiation on Si-PVH and PLA-10 microfiber meshes, whereas the sharp diffraction peak assigned to PLA on the PLA-2 microfiber mesh was changed to a broad diffraction peak by the gamma irradiation. Figure 2b shows no changes in FTIR spectra before and after the gamma irradiation. Absorptions in -C-O- stretching bands at approximately 1,080–1,200 cm^{-1} and -C=O stretching band around 1,750 cm^{-1} were not shifted by the gamma irradiation.

Figure 3 shows levels of Si^{4+}, Ca^{2+}, and P^{5+} ions in eluates from Si-PVH/PLA membranes and PLGA membranes for 9 days. While Si^{4+} levels were significantly higher in eluates from Si-PVH/PLA membranes than in eluates from PLGA membranes, concentrations of Ca^{2+} and P^{5+} ions were lower in eluates from Si-PVH/PLA membranes.

Cell proliferation

Figure 4 shows the proliferation of HGFs on the PLA sides of Si-PVH/PLA membranes, PLGA membranes and culture dishes. The cell proliferation on Si-PVH/PLA membranes was higher than that on PLGA membranes at 6 and 9 days, but was lower than that on the culture dishes. Figure 5 shows the proliferation of MC3T3-E1 cells on the Si-PVH sides of Si-PVH/PLA membranes, PLGA membranes, and culture plates. Cell proliferation on both the membranes was inferior to that on the

Table 1 The weight-average molecular weight (*Mw*), the number-average molecular weight (*Mn*), and polydispersity index (*Mw*/*Mn*) before and after the gamma irradiation of PLA-10, PLA-2 and Si-PVH microfiber meshes, respectively

Specimen	Gamma irradiation	*Mw* (kDa)	*Mn* (kDa)	Polydispersity index (*Mw*/*Mn*)
PLA-10	before	318	214	1.49
	after	115	66	1.76
PLA-2	before	357	230	1.55
	after	128	72	1.80
Si-PVH	before	317	176	1.80
	after	119	60	1.99

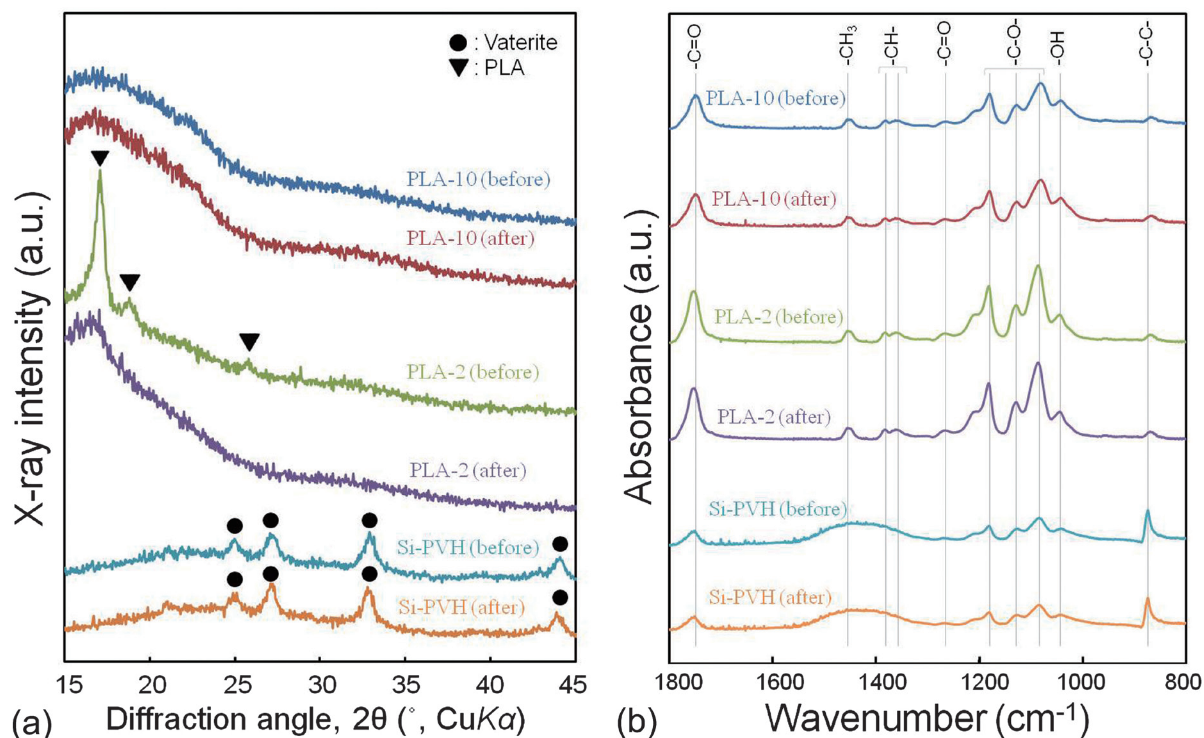

Fig. 2 XRD patterns (a) and FT-IR spectra (b) of PLA-10, PLA-2, and Si-PVH microfiber meshes before and after gamma irradiation.

culture plates. However, the number of cells on the Si-PVH/PLA membranes was higher than that on the PLGA membranes at 6 and 9 days. Figure 6 represents the proliferation of MC3T3-E1 cells cultured in eluates from the membranes and control medium alone. At 6 and 9 days after the stimulation, MC3T3-E1 cells proliferated well in eluates from the Si-PVH/PLA membranes, compared with eluates from PLGA membranes or medium alone.

Morphology of the cells on the membrane

Figure 7a shows the morphology of HGFs on the PLA sides of Si-PVH/PLA and PLGA membranes at 3, 6, and 9 days. Similarly, Figure 7b exhibits the morphology of MC3T3-E1 cells on the Si-PVH sides of Si-PVH/PLA and PLGA membranes at 3, 6, and 9 days. The HGFs and MC3T3-E1 cells on Si-PVH/PLA membranes proliferated in a time-dependent manner, and needle-like microspikes extending from the cells ran along or across the fibers of Si-PVH/PLA membrane. The cells spread forming

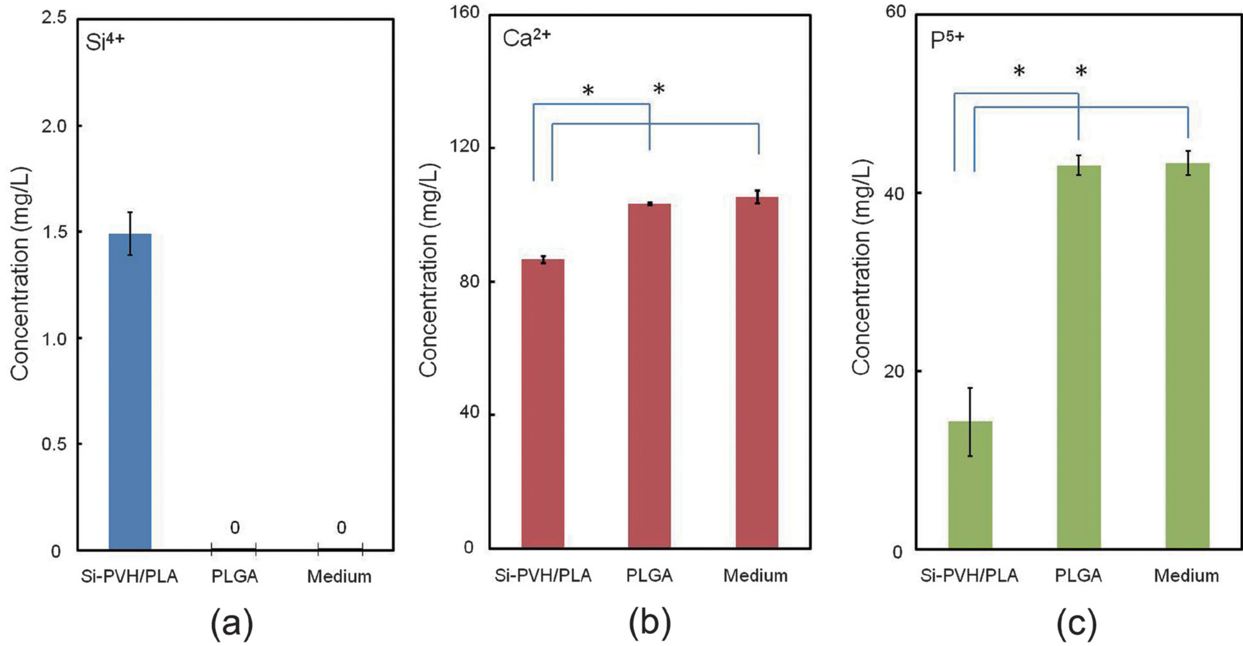

Fig. 3 Concentrations of Si^{4+} (a), Ca^{2+} (b), and P^{5+} (c) in eluates from Si-PVH/PLA and PLGA membranes after 9 days of soaking and medium alone (*$p<0.05$).

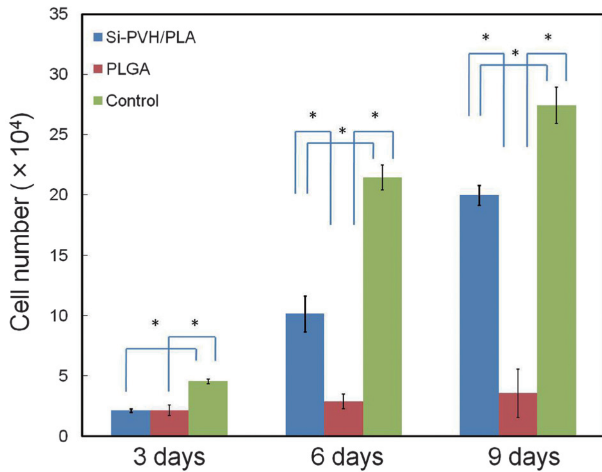

Fig. 4 Proliferation of HGFs cultured on PLA side of Si-PVH/PLA membrane, PLGA membrane and culture plate at 3, 6, and 9 days after incubation (*$p<0.05$).

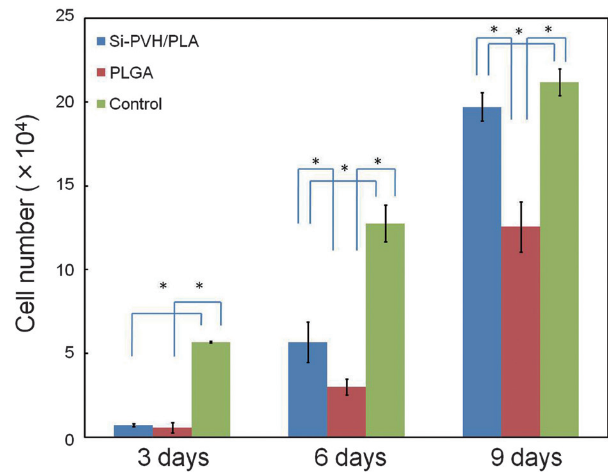

Fig. 5 Proliferation of MC3T3-E1 cells cultured on Si-PVH side of Si-PVH/PLA membrane, PLGA membrane and culture plate at 3, 6, and 9 days after incubation (*$p<0.05$).

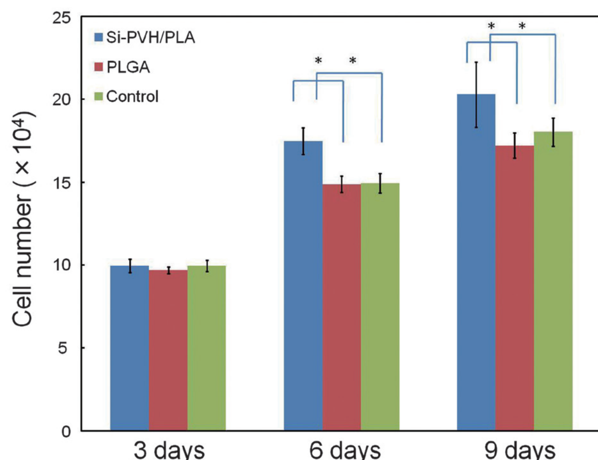

Fig. 6 Proliferation of MC3T3-E1 cells cultured in the eluates from Si-PVH/PLA and PLGA membranes, and medium alone at 3, 6, and 9 days after stimulation (*p<0.05).

cell-cell contacts over multiple fibers and filled in gaps between the fibers with cell-cell binding after 9 days of incubation. In contrast, the number of cells on PLGA membranes appeared to be fewer than those on Si-PVH/PLA membranes. Cells were observed only on small pores and along edges of large pores; scarcely any cell-cell interactions were found, even at 9 days.

ALP activity assay

Figure 8 shows ALP activity of MC3T3-E1 cells cultured in eluates from Si-PVH/PLA and PLGA membranes, and medium alone. Cellular ALP activity in each medium increased in a time-dependent manner. At 15 days after stimulation, Cellular ALP activity in eluates from Si-PVH/PLA membranes was similar to that of cells in eluates from PLGA membranes, which was significantly higher than that of the cells cultured in medium alone.

Calcification assay

Figure 9 shows the calcification of MC3T3-E1 cells in eluates from Si-PVH/PLA and PLGA membranes and medium alone. The calcium deposits of cells cultured in eluates from Si-PVH/PLA membranes were significantly higher than those of cells cultured in eluates from PLGA membranes or with control medium alone at all the time points.

DISCUSSION

Gamma irradiation is very frequently used for sterilization of polymer-based medical materials including GBR membranes. It reportedly reduces molecular weight by breaking molecular chains of PLA and PLGA, thus decreasing tensile strength and accelerating resolution of PLA[13,14]. This finding suggests that gamma irradiation may reduce the space-making and the cell-occlusiveness functions in polymer-based GBR membranes. Therefore, bi-layered membrane consisting of Si-PVH and PLA-10 microfiber meshes need to be modified. A PLA-2 microfiber mesh was inserted for the purpose of cell-occlusiveness between Si-PVH and PLA-10 microfiber meshes; the outer meshes encourage cell proliferation and differentiation.

Although the Mw and Mn of PLA-2, PLA-10, and Si-PVH microfiber meshes after the gamma irradiation decreased to approximately one third of those before the gamma irradiation and the number of short molecules increased, it is believed that membrane with Mw>100 kDa can maintain mechanical properties, including space-making, for the period required for bone regeneration[22].

XRD analysis (Fig. 2a) showed that gamma irradiation vitrified PLA-2 with crystalline PLA phase, whose formation seems to be induced by large tensile stress generated in thin fibers during the electrospinning. Meanwhile, no significant changes in the XRD patterns of PLA-10 and Si-PVH microfiber meshes occurred. No chemical reaction was apparently caused by the gamma irradiation in PLA-2, PLA-10, and Si-PVH microfiber meshes because there were no new peaks in FTIR spectra before and after the gamma irradiation (Fig. 2b).

There are several polymer-based materials including PLGA, which show favorable biocompatibility. Proliferation of HGFs on the PLA side of Si-PVH/PLA membranes was superior at 6 and 9 days, compared with that on PLGA membranes. The minimum recommended pore size for cell migration and transport is 25–100 μm[15]. As PLA-10 microfiber meshes have an approximate pore size of 74 μm on average, it is thought that HGFs could bridge among fibers and proliferate three-dimensionally. However, we could not detect any HGFs beneath the Si-PVH sides of the membranes, suggesting that the membranes are cell-occlusive. Fujikura *et al.* have demonstrated that fibroblasts can infiltrate into PLA-10 microfiber mesh but not into PLA-2-μm microfiber mesh[16].

Furthermore, the 10-μm-diameter fibers of Si-PVH/PLA membrane are wide enough for cells to extend along the fibers[17], and needle-like microspikes extending from HGFs ran along or across fibers of Si-PVH/PLA membranes, whereas HGFs were observed only on small pores and along edges of large pores; scarcely any cell-cell interactions were found in PLGA membranes (Fig. 7a). Therefore, these findings imply that PLA-10 microfiber mesh provides a scaffold where HGFs adhere and proliferate well three-dimensionally, compared with PLGA membrane with a two-dimensional structure, while the PLA-2-μm microfiber mesh prevents HGFs from invading into bone defects.

Proliferation of MC3T3-E1 cells on Si-PVH/PLA membrane was superior to that on PLGA membrane after 6-day culture and approximately equal to that on the culture plate after 9-day culture. As observed on SEM micrographs, cells bridged in the areas where fibers were dense; in contrast, they adhered along the fibers in the areas where fibers were sparse (Fig. 7b). Cells could

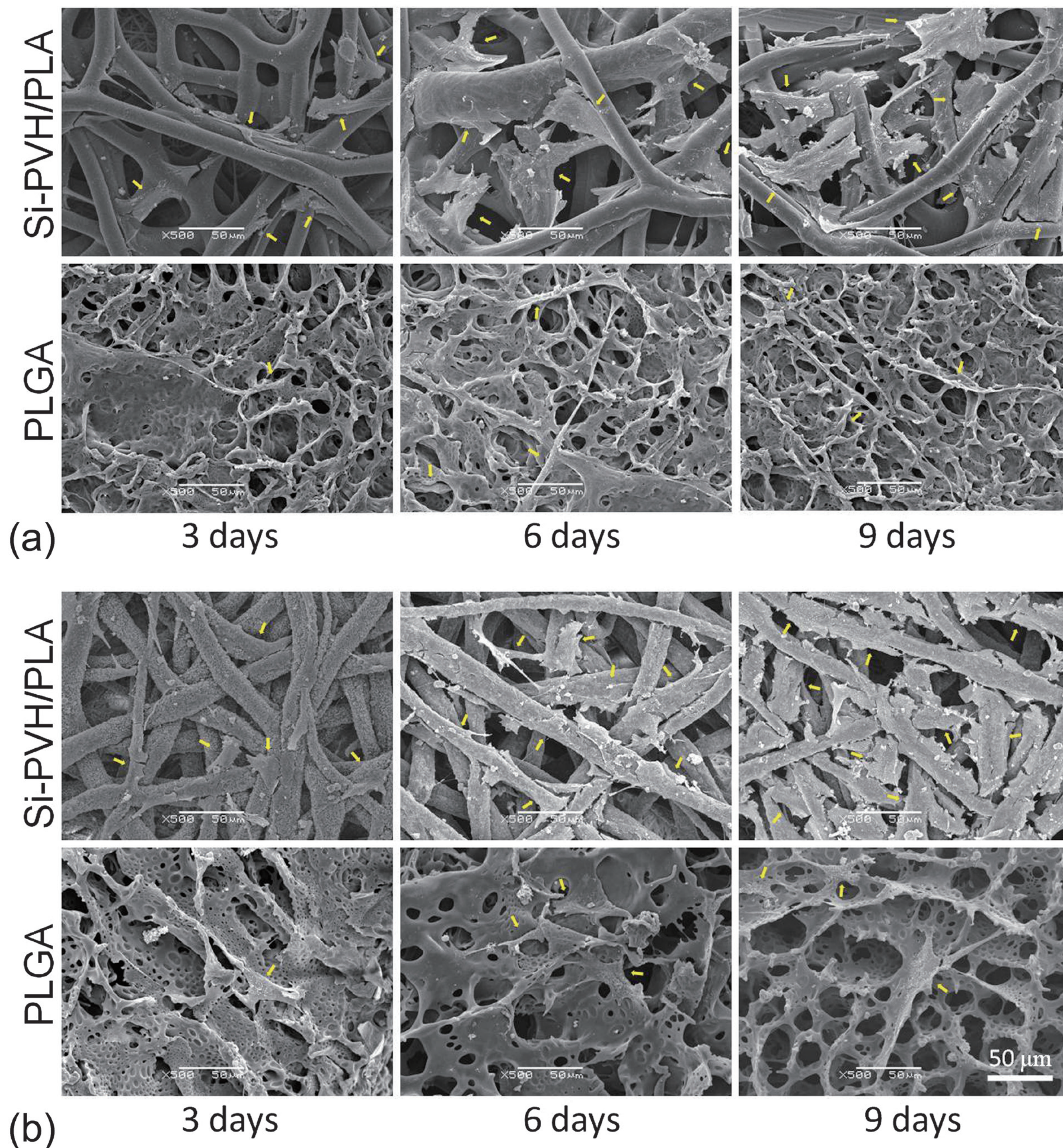

Fig. 7 SEM photographs of HGFs cultured on PLA side of Si-PVH/PLA membrane and PLGA membrane (a) and MC3T3-E1 cells cultured on the Si-PVH side of the membrane and PLGA membrane (b) at 3, 6, and 9 days after incubation. Arrows show definite HGFs and MC3T3-E1 cells in (a) and (b), respectively. (Bar: 50 μm)

apparently multiply three-dimensionally in the fiber structure of Si-PVH meshes, similar to proliferation of HGFs in PLA meshes. Although the surface structures of Si-PVH fibers were visually changed after gamma irradiation (data not shown), adherence and proliferation of MC3T3-E1 cells were favorable, similar to the report of Obata *et al.*[12].

The features of Si-PVH/PLA membrane can be summarized as follows: the membrane has a tri-layered structure consisting of PLA-10, PLA-2, and Si-PVH microfiber meshes whose functions are connective tissue integration, cell-occlusiveness, and osteoblastic cells proliferation, respectively.

The Si-PVH/PLA membrane was developed with the

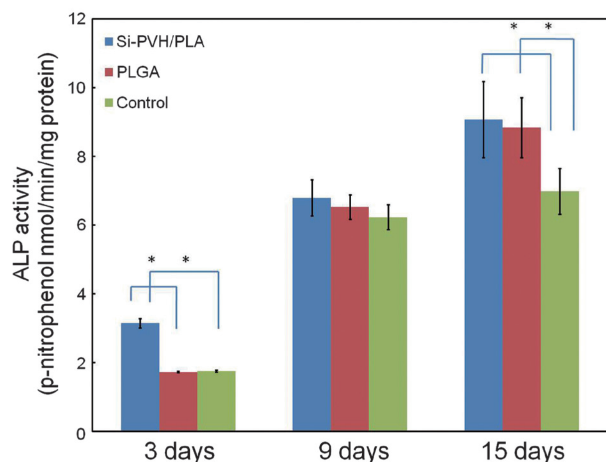

Fig. 8 ALP activity of MC3T3-E1 cells cultured in eluates from Si-PVH/PLA and PLGA membranes, and medium alone at 3, 9, and 15 days after stimulation (*p<0.05).

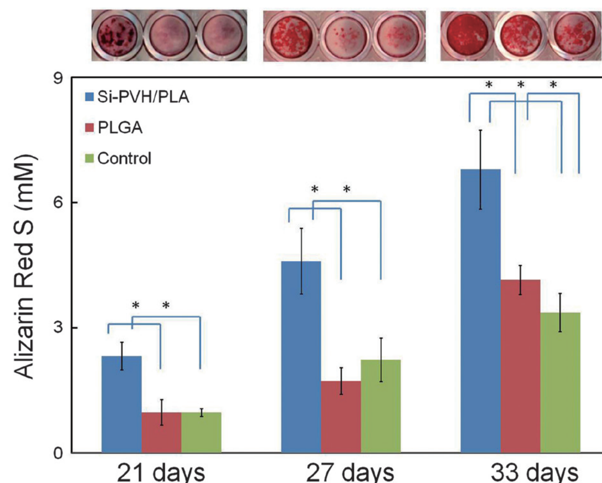

Fig. 9 Calcification of MC3T3-E1 cells cultured in eluates from Si-PVH/PLA and PLGA membranes, and medium alone at 21, 27, and 33 days after incubation (*p<0.05).

expectation that its components' inherent silicon species will induce bone formation. Ca^{2+} and P^{5+} ions are also involved in osteoblast[12] proliferation or differentiation. Since the Si-PVH/PLA membrane consists of SiV and PLA with HA coating, concentrations of Si^{4+}, Ca^{2+}, and P^{5+} ions released from the membrane were evaluated. The levels of ionic silicon species released from Si-PVH/PLA membrane are controlled by soaking the membrane in SBF and coating with HA[12]. Various studies have reported concentrations of silicon species that are effective for activation of osteoblastic cells[8-12,18,23]. In this study, the levels of Si^{4+} ions released from the membrane immersed in medium for 9 days was 1.49 mgL^{-1} (Fig. 3a). Effective concentrations of Si for cell proliferation and differentiation reported by Tsigkou *et al.*[9], Xynos *et al.*[10], and Reffitt *et al.*[11] are 14.5–19.4 µg/mL, 16.58 ppm, and 11.7–21.9 µM respectively. Variations in optimal concentrations of Si reported by these studies seem to be due to the differences of coexistence ions, solution pH, and form of Si^{4+} ion in the solution. The Ca^{2+} and P^{5+} ion concentrations were lower than those in medium alone (Fig. 3b and c), and thought to be within cellular tolerance levels for proliferation and differentiation[24,25]. Eluates from Si-PVH/PLA membranes enhanced proliferation and calcification of MC3T3-E1 cells, compared with those from PLGA membranes. Keeting *et al.*[8] showed that the silicon-containing compound ZA increases the mRNA levels of transforming growth factor β_1 and induces proliferation and differentiation of human osteoblast-like cells. Furthermore, orthosilicic acid stimulates collagen type I synthesis in human osteoblast-like cells and enhances osteoblastic differentiation[11]. Therefore, silicon released from Si-PVH/PLA membrane may be involved in collagen synthesis, growth factor production and calcification of bone matrix. The ALP activity of MC3T3-E1 cells after stimulation with eluates from Si-PVH/PLA membranes was almost the same as that after stimulation with eluates from PLGA membranes. It has been reported that differentiation of MC3T3-E1 cells can be enhanced by PLLA[26,27]. It is likely that enhancement of ALP activity of MC3T3-E1 cells is caused not only by silicon species but also by lactic acid. We suggest that structure and eluates of Si-PVH/PLA membrane are conductive to osteoblast proliferation and differentiation, and that the membrane promotes bone formation with bioactive properties.

Our preliminary study, aimed at evaluating GBR in intrabony defects in dogs, showed that new bone formation extended along the Si-PVH/PLA membrane and that no inflammatory cells were observed around the membrane (data not shown). Although the *Mw* of the Si-PVH/PLA membrane after the gamma irradiation was reduced to approximately one third of the pre-irradiation *Mw*, the Si-PVH/PLA membrane maintained adequate strength for clinical manipulation in GBR operation. In addition, according to histological preparations, the Si-PVH/PLA membrane seemed to have sufficient mechanical properties required for space-making and cell-occlusiveness. Further *in vivo* studies to reveal the effectiveness of Si-PVH/PLA membrane on GBR procedures are in progress in our laboratory.

CONCLUSIONS

Gamma irradiation for sterilization induced some structural changes in the modified three-layer structure membrane. However these changes seemed to cause negligible degradation of biological properties. Proliferation of MC3T3-E1 cells and HGFs on Si-PVH/

PLA membranes was superior to that on PLGA membranes. Moreover, proliferation and differentiation of MC3T3-E1 cells in eluates from Si-PVH/PLA membranes were significantly greater than those in eluates from PLGA membranes. Our data suggest that the gamma-irradiated Si-PVH/PLA membrane has favorable cellular compatibility with fibroblasts and osteoblasts, and may be suitable for GBR.

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