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이학석사학위논문

Prevention of capsular contracture on silicone implants in rats using biomembrane-mimicking coatings

2015년 2월

서울대학교 대학원

화학부 생화학 전공

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이 논문을 이학석사학위논문으로 제출함

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2015년 2월

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Abstract

Prevention of capsular contracture on silicone implants in rats using biomembrane-mimicking coatings

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Despite their popular use in breast augmentation and reconstruction surgeries, the limited biocompatibility of silicone implants can induce severe side effects, including capsular contracture – an excessive foreign body reaction that forms a tight and hard fibrous capsule around the implant. This study examines the effects of using biomembrane-mimicking surface coatings to prevent capsular formations on silicone implants. The covalently attached biomembrane-mimicking polymer, poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC), prevented nonspecific protein adsorption and fibroblast adhesion on the silicone surface. More importantly, in vivo capsule formations around PMPC-grafted silicone implants in rats were significantly thinner and exhibited lower collagen densities and more regular collagen alignments than bare silicone implants. The observed decrease in α-smooth muscle actin also sup-

ported the alleviation of capsular formations by the biomembrane-mimicking coating. Decreases in inflammation related cells, myeloperoxidase and transforming growth factor-β resulted in reduced inflammation in the capsular tissue. The biomembrane-mimicking coatings used on these silicone implants demonstrate great potential for preventing capsular contracture and developing biocompatible materials for various biomedical applications.

Keyword: Silicone, Capsular contracture, Phosphorylcholine, Surface modification, Foreign body reaction

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Contents

◈ Abstract

1. Introduction 4

2. Materials and Methods 8

3. Results 14

4. Discussion 22

5. Conclusion 27

6. References 28

7. Figures 33

◈ 국문 초록 (Abstract in Korean) 48

1. Introduction

Breast augmentation is the most common plastic surgery worldwide in 2013 and occupies about 15% of all surgical cosmetic procedures [1]. Furthermore the number of cases continues to increase with society's growing interest in beauty. In addition, demands for breast reconstruction surgery are increasing as a result of patients who have had mastectomies to remove cancerous tissues. Implants based on silicone elastomer bags that are filled with silicone gel, saline or other fillers are the most widely used implants for both breast augmentation and reconstructive surgical procedures [2]. Recipients are generally well satisfied with the breast-like mechanical properties and low cost of the silicone-based breast implants, but limited biocompatibility still provokes serious problems. Gabriel et al. [3] previously reported that, among 749 women who had breast implantation, 208 (27.8%) had received revision surgery due to single or multiple complications. Among them, capsular contracture – serious fibrous capsule formation around implants – was the most frequent complication, causing 131 women (17.5%) to undergo further surgical intervention. It has been reported that capsular contracture occurs over a time scale ranging from several months to years after breast implantation [4–7].

It has been hypothesized that capsular contracture might result from excessive foreign body reactions on the silicone surface, gel bleed, dust, glove powder, etc., or by subclinical infection by normal skin flora (usually by *Staphylococcus epidermidis*) [8–12]. The foreign body reaction include, in particular, the inflammatory process and exaggerated scar response to a foreign prosthetic material [13,14]. Here, a fibrous capsule

develops around the implant by the natural healing response to the presence of a foreign body, but results in excessive fibrotic scarring. Although the mechanism has not yet been elucidated in detail, the foreign body reaction is likely initiated by non-specific adsorption of proteins on the silicone surface within several minutes of implantation [15]. Macrophages are then recruited to the implantation site and form giant cells within 2 days due to their inability to successfully phagocytose the too-large foreign body. Collagenous encapsulation and excessive formation of fibrous tissue around the implant occur within 3 weeks.

Surface modifications of silicone implants have been studied as a means of reducing excessive foreign body reactions. Silicone implants coated with polyurethane [16] or fabricated with textured surfaces [17] have demonstrated limited success in clinical studies. However, the prevalence of capsular contracture after implantation remains significantly high [18], so the search for more biocompatible surfaces continues.

Among the various methods used to prepare biocompatible surfaces, coating with biomembrane-mimicking materials is very attractive [19]. Poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) mimics the head group of phosphatidylcholine in the cell membrane and exhibits exceptional anti-protein-adsorption activity, anti-thrombotic activity and hemocompatibility when used in coating materials for coronary stents [20], artificial joints [21], drug delivery carriers [22] and biomicrofluidics [23]. Increased hydrophilicity due to zwitterionic groups and biomembrane-mimicking phosphorylcholine moieties of PMPC are important contributors to the outstanding biocompatibility exhibited by PMPC-coated materials [24].

The present study examines the effects of PMPC coating on capsular formation around silicone implants inserted into rats (**Fig. 1**). Although

implants coated with other polymers, including hyaluronic acid (HA), polyethyleneglycol (PEG) and polyacrylamide (PAAm) [25], failed to alleviate capsular formation, I suspected that, given its biomembrane-mimicking properties, PMPC-coated silicone implants have the potential to modulate the initiation process and to reduce excessive capsular formation. It has been previously reported that the surface of polydimethylsiloxane (PDMS), a silicone elastomer, was successfully coated by PMPC, resulting in significantly reduced protein adsorption and cell adhesion [26,27]. In this study, successful PMPC coating of the silicone implants was confirmed via dynamic water contact angles and X-ray photoelectron spectroscopy (XPS). Subsequently, nonspecific protein adsorption and the adhesion of fibroblast cells, which were the primary collagen-producing cells, were measured. More importantly, PMPC-coated silicone implants were inserted subcutaneously into the backs of rats, and the resulting capsular formations were carefully compared to those observed on bare silicone implants. Various quantitative studies comparing capsular thickness, inflammatory cells, vascularity and amounts of transforming growth factor-β (TGF-β), α-smooth muscle actin, myeloperoxidase and CD34 were performed to examine the effects of PMPC coating on capsular formation.

In vivo analysis of PMPC-coated silicone implants is very important for finding ways to reduce the side effects of implantation, including capsular contracture, through a greater understanding of the mechanisms of foreign body reactions, and is crucial for establishing strategic footholds regarding the use of biocompatible materials in various biomedical applications.

2. Materials and methods

2.1. Materials

PDMS elastomer base and curing agent (Sylgard 184) were purchased from Dow Corning (USA). Benzophenone, bovine serum albumin (BSA) and bovine plasma fibrinogen (BPF) were purchased from Sigma-Aldrich (USA). 2-Methacryloyloxyethyl phosphorylcholine (MPC) monomer was purchased from KCI (Korea). Dulbecco's modified Eagle's medium (DMEM), Dulbecco's phosphate buffered saline (DPBS) and fetal bovine serum (FBS) were purchased from WelGENE (USA).

2.2. Preparation of silicone implants

The silicone implants were prepared from the silicone elastomer (PDMS) base (Sylgard 184) according to the manufacturer's protocol. A mixture of the base and the curing agent (10:1, w/w) was poured on a glass plate, degassed in a vacuum chamber and cured in an oven at 100 °C for 1 h. The cured silicone plate was cut into a disk (15 mm diameter, 0.5 mm thickness for in vitro and 2 mm thickness for in vivo) and preserved in acetone.

2.3. PMPC coating on the silicone implants

The silicone implant was covalently coated with PMPC according to the method in the previous report [26]. The silicone implant was dipped into acetone-dissolved benzophenone (10 mg ml^{-1}) for 1 min. After drying in a vacuum chamber for 1 h, the benzophenone-adsorbed silicone implant was immersed in aqueous solutions containing various MPC monomer concentrations. The silicone implant was irradiated by UV from a

500W high-pressure mercury lamp (MS UV, Korea) for 15 min. Unreacted monomers, benzopinacol and excess benzophenone were removed by thorough washing with acetone and water. Finally, the coated silicone implant was soaked with water overnight to remove any remaining acetone and non-covalently attached polymers.

2.4. Measurement of the water contact angle

Dynamic water contact angles were measured to examine the hydrophilicity of the implant surfaces. Advancing contact angles were measured as the water volume was increased from 0 to 6 μl, whereas receding contact angles were measured as the water volume was decreased from 6 to 3 μl.

2.5. X-ray photoelectron spectroscopy (XPS)

Surface elemental analysis of the bare silicone implant and the PMPC-coated silicone implant was performed using XPS. The XPS instrument (AXIS-HIS, Kratos-Shimadzu) used an X-ray source of Mg Kα (15 kV) in a Mg/Al dual anode. The X-ray detector was located at a position 45° away from the normal. Each plate was cut into a 7 mm × 7 mm square and examined for C1s, O1s, Si2p, N1s and P2p.

2.6. Protein adsorption assay

BSA (4.5 mg ml^{-1}) and BPF (0.3 mg ml^{-1}) were dissolved in DPBS. Each silicone implant was incubated in the protein solution on an orbital shaker (200 rpm) at 37 °C for 1 h. After washing twice with fresh DPBS, the amount of adsorbed protein was quantified using a Micro™ BCA protein assay kit (Thermo Scientific). The absorbance at 570 nm was

measured using a spectrophotometer (V-650, Jasco).

2.7. Cell adhesion test

NIH 3T3 (mouse fibroblasts) cells were seeded on the silicone blocks in 24-well tissue culture dishes at 30,000 cells per well in 1 ml of DMEM containing 10% FBS. After incubation at 37 °C for 40 h, cells were gently washed with fresh DMEM containing 10% FBS. The adhered cells on the silicone implant were quantified using a cell counting kit (CCK, Dojindo).

2.8. Preparation of animals

Twenty female Sprague-Dawley rats, aged 8 weeks with an average body weight of approximately 250 g at the time of implantation, were used to evaluate capsular formation on the silicone blocks in vivo. All animals were free of specific pathogens and were maintained under the same food and environmental conditions. After an adaptation period of 1 week, healthy animals were selected for the experiment. The rats were housed in an animal facility and treated in accordance with the Guide for the Care and Use of Laboratory Animals of Seoul National University Hospital. This study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Seoul National University Hospital (IACUC No. 11-0383).

2.9. Insertion of the silicone implants

All surgical procedures were performed by the same individual (J.U.P.). The surgical field was prepared using 10% povidone-iodine, and a single dose of cefazolin (60 mg kg^{-1}) was administered intramuscularly for prophylaxis against infection. The animals were anesthetized using an intra-

peritoneal injection of Zoletil® (30 mg kg^{-1}) and Rumpun® (5 mg kg^{-1}). The two pockets for implant insertion were made at the back of each rat through two separate 2 cm vertical incisions, which were started at the lateral point 1.5 cm outside the midline and 1 cm below the shoulder bone (**Fig. 6A**). PDMS and PMPC–PDMS (coated using an MPC concentration of 0.50 M) (**Fig. 6B**) were implanted beneath the panniculus carnosus muscle. PDMS was positioned in the left back pocket and PMPC–PDMS was positioned in the right side pocket. Twenty replicates (10 for a 4-week analysis and 10 for a 12-week analysis) of each sample type were implanted. Muscle and skin incisions were closed using 4-0 Vicryl® and 5-0 Ethilon® sutures (Ethicon, Inc., USA).

2.10. Harvest of capsule from embedded silicone implants

After 4 or 12 weeks, the rats were sacrificed using CO_2 asphyxiation in accordance with AVMA (American Veterinary Medical Association) Guidelines for the Euthanasia of Animals. The capsular tissue formed near the implanted silicone implant was retrieved through a skin incision (**Fig. 6C**). The fibrous capsule around the silicone implant underwent gross examination before being harvested from the central portions of the upper and lower surfaces of the implant.

2.11. Histological analysis

Harvested specimens were fixed in 10% formalin. After 24 h, each specimen was embedded in paraffin and sections were cut transversely to visualize the architecture of the capsule. Histological analysis was performed using hematoxylin and eosin (H&E) staining. Each stained slide was examined at ×100 magnification using a Leica DM2500 microscope

(Leica Microsystems-Switzerland Ltd, Switzerland), and images were captured from three microscopic fields: right, center and left. The capsular thickness was measured at the maximal point using National Institutes of Health Image J 1.36b imaging software (National Institutes of Health, Bethesda, MD, USA). Next, the cellularity and vascularity were examined in each image. The number of cells per unit area (0.01 mm^2) was calculated automatically by the LAS Core Image Program (Leica Application Suite software, version 2.4.0, Leica Imaging Systems Ltd, Cambridge UK). The number of blood vessels per unit area (1 mm^2) was counted manually for each image and expressed as a vessel number.

Immunohistochemical staining was performed using rabbit anti-TGF-β (1:100; Abcam, UK), mouse anti-α-smooth muscle actin (1:200; DAKO, USA), rabbit anti-myeloperoxidase (1:300; DAKO, USA) and mouse anti-CD34 (1:500; Santa Cruz Biotechnology, USA) antibodies. After endogenous peroxidase quenching, the antigens were retrieved at high temperature (citrate buffer, pH 6.0). The slides were processed using Vectastain Elite ABC reagent (Vector Laboratories, USA) according to the manufacturer's instructions. After treatment with the appropriate biotinylated secondary antibody, sections were developed with 3,3-diaminobenzidine (DakoCytomation, Denmark) in chromogen solution and counter-stained with Harris's hematoxylin. Immunohistochemical staining was evaluated in three areas, as with H&E staining. The total pixel intensity was measured using Leica Q win image program V 3.2.0 (Leica Imaging Systems Ltd), and data were expressed as optical densities.

2.12. Statistical analysis

All data are expressed as means ± SEM (standard error of the mean). Data analysis was performed using GraphPad Prism (version 6.00 for

Windows, GraphPad Software, La Jolla, CA, USA). For all data, significant differences were determined using an unpaired t-test, assuming Gaussian distribution and that both populations have the same standard deviations. The accepted level of significant difference for the test was $p < 0.05$, and the degree of difference is indicated on the graph as ****, ***, ** and * for $p < 0.0001$, $0.0001 \leq p < 0.001$, $0.001 \leq p < 0.01$ and $0.01 \leq p < 0.05$, respectively. "No SD" indicates no significant difference.

3. Results

3.1. Surface coating of silicone implants with PMPC

UV-induced radical polymerization was used to covalently coat PMPC on the silicone surface, following the methodology of the previous report (**Fig. 2**) [26]. PDMS blocks were used as model silicone implants. Benzophenone was adsorbed on the surface of the PDMS as a photosensitizer, and the implant was irradiated with UV while in the MPC monomer solution. Benzophenone radicals were first formed by UV irradiation at a wavelength near 365 nm, and methylene radicals were successively formed on the PDMS surface. MPC monomers were polymerized on the surface, and PMPC-grafted silicone implants were obtained using varying initial concentrations of MPC (0.10, 0.25 and 0.50 M).

Measurements of water contact angles supported the formation of PMPC grafts on the silicone implants (**Fig. 3**). As the concentration of MPC monomer was increased, the water contact angle decreased, indicating increasing surface hydrophilicity. The advancing contact angle changed from 108° (Noncoated) to 81° (0.50 M MPC), and the receding contact angle changed from 88° (Noncoated) to 38° (0.50 M MPC). As zwitterionic phosphorylcholine residues of PMPC are more hydrophilic than methyl residues of PDMS, the measured increase in hydrophilicity supported successful coating of PMPC on the PDMS surface.

The existence of a PMPC graft on the silicone implant was also confirmed using XPS (**Fig. 4**). The range of binding energies was selected for the detection of carbon, oxygen, silicone, nitrogen and phosphorus. The presence of nitrogen and phosphorus signals and the reduction of

silicone signals in PMPC−PDMS (coated PDMS in an MPC concentration of 0.50 M) provided evidence that the PDMS surface was covered by phosphorylcholine moieties. In addition, the PDMS surface showed a carbon peak at the C1s binding energy for only methylene ($-CH_2-$) or methyl ($-CH_3$) groups, whereas the PMPC−PDMS sample showed two other peaks at the C1s binding energies for a carbon−oxygen single bond (-C-O-) and a double bond (-C=O). Moreover, a shoulder O1s peak could be observed in PMPC−PDMS, providing evidence of carbon−oxygen bonds in PMPC. Both water-contact-angle data and XPS spectra strongly support the successful introduction of PMPC to silicone implants using UV-induced polymerization.

3.2. In vitro protein adsorption and cell adhesion test

It was previously reported that PMPC-coated PDMS could prevent non-specific protein adsorption on the silicone surface [26]. Similarly, I analyzed the adsorption of albumin and fibrinogen, two of the most abundant proteins in serum. As shown in **Fig. 5A**, PMPC−PDMS exhibited adsorptions of BSA and BPF reduced by 52 and 63%, respectively, compared to PDMS. **Fig. 5B** shows the adhesion of mouse fibroblast cells (NIH-3T3) observed on the silicone implants. It is clear that the PMPC coating can prevent the adhesion of fibroblasts.

3.3. In vivo capsular formation

After it was confirmed that protein adsorption and cell adhesion on silicone implants were inhibited by PMPC coating, Implantations for PDMS and PMPC−PDMS beneath the panniculus carnosus muscle on the back of rats were conducted so that I could observe capsular formation

around the implants (**Fig. 6**). After 4 or 12 weeks, tissues around the silicone implants were carefully obtained in order to compare capsular formations.

First, I compared the capsular thickness around PDMS and PMPC–PDMS. Histological estimation of the peri-implant capsular thickness showed significant differences between PDMS and PMPC–PDMS at both time points (**Fig. 7**). The capsules around PDMS were significantly thicker than those around PMPC–PDMS. After 4 weeks, the average capsular thicknesses were 369 μm in the PMPC–PDMS group and 509 μm in the PDMS group. After 12 weeks, the capsular thicknesses were 207 and 247 μm, respectively. Upon gross examination, the tissues around the PMPC–PDMS implant demonstrated a more parallel arrangement of collagen fibers and lower collagen density compared to the tissues around the PDMS implant, which showed a denser, more irregular collagen-fiber arrangement at each of the two time points.

In addition, investigating capsular formation based on the contact site of the implant, I compared capsular thicknesses on the superficial and deep surfaces of the silicone implants. In all groups, no remarkable differences in capsular thickness were observed between superficial and deep sections (**Fig. 8**).

3.4. Cellularity and vascularity

Inflammatory cells, such as neutrophils and macrophages, act as major mediators in inflammatory reactions by secreting various cytokines, recruiting fibroblasts and activating collagen synthesis, resulting in capsule formation. Estimation of the numbers of intracapsular inflammatory cells was conducted using the LAS Image Analysis Program (**Fig. 9A**). At the 4-week point, the PDMS group (52 counts per unit area) showed sig-

nificantly higher numbers of inflammatory cells than the PMPC–PDMS group (41 counts per unit area). After 12 weeks, the PDMS group (29 counts per unit area) also exhibited a significantly higher count than the PMPC–PDMS group (24 counts per unit area). The inflammatory cell counts were in accordance with the capsular thickness results. The higher numbers of inflammatory cells observed in the non-coated PDMS group were directly related to thick capsular formations at both time points.

I also compared the vascularity of capsular tissues around the silicone implants. There were no significant differences between the PDMS group and the PMPC–PDMS group at either time point, although the PDMS group showed slightly higher vascular numbers than the PMPC–PDMS group (**Fig. 9B**).

3.5. Immunohistochemistry analysis in capsular formation

I performed immunohistochemistry (IHC) to obtain a more detailed analysis of the capsular formation around our silicone implants (**Figs. 10-13**). Transforming growth factor-β (TGF-β) is a main growth factor secreted from inflammatory cells and functions in fibroblast chemotaxis, activation of extracellular matrix deposition, increased collagen synthesis and down-regulation of matrix metalloproteinases. At the 4-week point, the optical density of TGF-β in the PDMS group (mean optical density; 2.05) was significantly higher than that in the PMPC–PDMS group (mean optical density; 1.05) (**Fig. 10**), providing evidence (in addition to the results obtained for capsular thickness and cellularity) of a more severe inflammatory reaction against PDMS than against PMPC–PDMS. At the 12-week point, the PDMS group demonstrated a mean optical density of 1.30, compared to 0.857 for the PMPC–PDMS group. Although the differ-

ence is not significant, the optical density of the PDMS group was still higher than that of the PMPC–PDMS group. I expected that the low titer of TGF-β on the surface of PMPC-coated silicone implants would contribute to the down-regulation of inflammation and the suppression of capsular formation.

Regarding α-smooth muscle actin as a sign of the formation of myofibroblasts, I did not observe any difference between the PDMS group (mean = 1.54) and the PMPC–PDMS group (mean = 1.41) at the 4-week point. In contrast, at 12 weeks, the PMPC–PDMS group (mean = 1.44) showed a significantly lower level of α-smooth muscle actin than the PDMS group (mean = 2.21) (**Fig. 11**).

Myeloperoxidase levels could also be used to approximately quantify local inflammatory reactions. At both time points, the PMPC–PDMS group showed a significantly lower level of myeloperoxidase than the PDMS group (**Fig. 12**). The low myeloperoxidase level in the PMPC–PDMS group indicates a reduced inflammatory reaction with less capsular tissue formation.

The vascularity of capsular tissues around each silicone implant was further confirmed by IHC using an anti-CD34 antibody as the marker of endothelial cells. At both time points, I observed no significant difference in CD34 levels between the PDMS group and the PMPC–PDMS group (**Fig. 13**). These results are further supported by the lack of vascularity differences observed via H&E staining (**Fig. 9B**).

4. Discussion

Although the cause and exact mechanism of capsular contracture are still controversial, I hypothesized that the reduction of excess foreign body reactions is one of the key factors to alleviate the capsular contracture. It was expected that the surface modification of silicone implants with a biomembrane-mimicking polymer, PMPC, can suppress the induction of the excess foreign body reaction due to its resemblance to cell surfaces.

Various methods, including oxidation, non-covalent adsorption and covalent grafting, have been used for the surface modification of silicone. Most of the methods introduced hydrophilic surfaces on silicone. Oxidation through oxygen plasma or water vapor plasma treatment was shown to produce hydroxyl groups (-OH) on the PDMS surface temporarily [28] or semi-permanently [29]. A solvent vaporization method [30] and simple dipping or swelling of PDMS platforms in polymeric solutions [31,32] have also been used for non-covalent modifications. In this study, I selected the covalent grafting method because it produces a modified surface with the highest durability for semi-permanent use of silicone implants in the body.

As shown in **Fig. 3**, hydrophilicity was clearly increased in PMPC-coated surfaces. Adsorption of albumin and fibrinogen was successfully prevented and adhesion of fibroblasts was significantly inhibited by the PMPC coating. Given that protein adsorption is considered to be the first step in the foreign body reaction and that fibroblasts play an important role in capsular formation [15], PMPC-coated silicone implants were expected to be able to alleviate excessive capsular formation.

Although PMPC coating produced hydrophilic surfaces similar to other methods, the resulting surfaces have a different tendency to adhesion of cells. Hydroxyl-group-modified silicone surfaces exhibited enhanced adhesion of fibroblasts as the hydrophilicity increased [33]. The attachment of fibroblasts was facilitated even more on the amine-group- or carboxylic-acid-group-modified surfaces compared to the hydroxyl-group-modified surfaces [34]. However, the PMPC-coated surfaces with zwitterionic phosphorylcholine groups showed dramatically reduced adhesion of fibroblasts regardless of the surface charges [35], which represents the different characteristics of the biomembrane-mimicking PMPC-coated surfaces.

When the implant was inserted in vivo, a foreign body reaction was triggered, leading to a cascade of inflammatory cell recruitment, fibroblast proliferation, collagen synthesis and capsular formation. A stronger foreign body reaction leads to more excessive capsular formation, such that the capsular thickness and density and the collagen regularity can provide a road map regarding foreign body reactions against implanted materials. Moreover, capsular thickness is positively related to the occurrence of capsular contracture [36]. The capsular thickness decreased during the period between 4 and 12 weeks in the PDMS and PMPC–PDMS groups (**Fig. 7**). In addition, PMPC-coated silicone implants resulted in less excessive extracellular matrix formation than uncoated silicone implants. Considering that the capsular contracture normally proceeds through 1 year [6], the analyses at the 4- and 12-week points are relatively short, but the initial process of capsular formation can be observed in this model system. I supposed that a proliferation phase was activated after the inflammatory phase, resulting in vigorous collagen production and accumulation at 4 weeks, and that collagen maturation and rearrangement mainly occurred at 12 weeks. This short-term trend

had also been reported in a previous article [36]. Given the similarity in decreased capsular thicknesses observed for the PDMS and PMPC–PDMS groups (51% decrease for PDMS and 44% decrease for PMPC–PDMS during 8 weeks), I expected that the overall durations of the respective foreign body reaction procedures were also likely to be similar.

Many pieces of evidence for the course of capsular formation (inflammation, fibroblast proliferation, and then capsule formation and maturation) were also found in the cellularity and IHC analyses (**Figs. 9-13**). The PMPC–PDMS group clearly showed lower numbers of inflammatory cells (**Fig. 9A**) and smaller amounts of inflammatory markers such as TGF-β and myeloperoxidase (**Figs. 10** and **12**) than the PDMS group, which strongly supported the reduction of inflammation around the PMPC-coated implants at both time points.

The number of inflammatory cells and the amount of TGF-β and myeloperoxidase were decreased during the period between 4 and 12 weeks. Thus, I supposed that the most relevant event occurring at the 4-week point was the inflammatory cell response, including the migration of inflammatory cells and the release of cytokines. There were then significant decreases in inflammatory cell number and myeloperoxidase amount from 4 to 12 weeks. These decreases may reflect the transition from the inflammatory and proliferation phase to the maturation phase. At the 12-week point, the formation of an extracellular matrix by myofibroblasts and collagen maturation may be the most relevant events in the peri-implant tissue, which showed an increased level of α-smooth muscle actin (**Fig. 11**).

The correlation between vascularity and capsular formation is the subject of controversy. In this study both the vascularity and CD34 data showed no differences between PDMS and PMPC–PDMS, and between 4

and 12 weeks (**Figs. 9B** and **13**). In a clinical study, Rubino et al. and Wynn et al. reported that capsules without contracture were thinner and less vascularized than those with contracture and suggested that vascularization could facilitate the development and growth of contracture capsules [37,38]. However, Vieira et al. reported that more vascularized tissue resulted in a softer capsule and a lower probability of capsular contracture in breast augmentation [36]. More research is required to determine the relationship between neoangiogenesis and capsular formation in implantations.

A previous study using silicone implants coated with other hydrophilic polymers, such as PEG, HA and PAAm, failed to alleviate capsular formation [25]. Hydroxylated silicone implants showed a similar decrease in capsular thickness with PMPC-coated silicone implants in this study, but the inflammation score was not different from that of the untreated silicone [39]. Plasma- and collagen-coated silicones enhanced adhesion of cells and increased angiogenesis in peri-implant tissues [40]. In another study, silicone implants thickly coated with a spider silk protein (eADF4) showed a similar reduction in both capsular formation and inflammation with PMPC-coated silicone implants [41]; however, eADF4 has the drawbacks of being somewhat unstable and expensive [42].

The comparison of in vivo results of capsular formation using diverse treatments is actually not very simple because each study has many variables, like types of implant, kinds of animal, and types of implantation site. In order to attribute more definite effects to capsular contracture, long-term in vivo tests, including the measurement of actual pressure upon miniaturized fluidic hemisphere-shaped silicone implants inserted beneath the breast of larger animals rather than solid plate-shaped ones inserted in the backs of rats, will be necessary. However, the silicone

implants semi-permanently coated with the biomembrane-mimicking polymer PMPC, which showed significant alleviation of capsular formation and excessive inflammation in this study, have good potential as a platform for future development of biomedical implants with completely biocompatible surfaces.

5. Conclusion

In the present study, I covalently coated silicone implants with a biomembrane-mimicking polymer, PMPC, and confirmed a reduction in the adhesion of proteins and fibroblasts and in vivo peri-implant capsular formation through 12 weeks experiments. PMPC-coated silicone implants showed a significant decrease in capsular thickness compared to non-coated implants. The accompanying decrease in inflammation-related cells, TGF-β and myeloperoxidase strongly supported the reduction of inflammation in the tissues surrounding the implants. Moreover, significant decreases in α-smooth muscle actin and collagen density around the PMPC-coated implants also supported the alleviation of capsular formation by the biomembrane-mimicking coating. Although longer-term analysis will be required, the biomembrane-mimicking coating could well be a foothold for suppressing breast capsular contracture as well as understanding the mechanism(s) of foreign body reactions in other biomedical applications.

6. References

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7. Figures

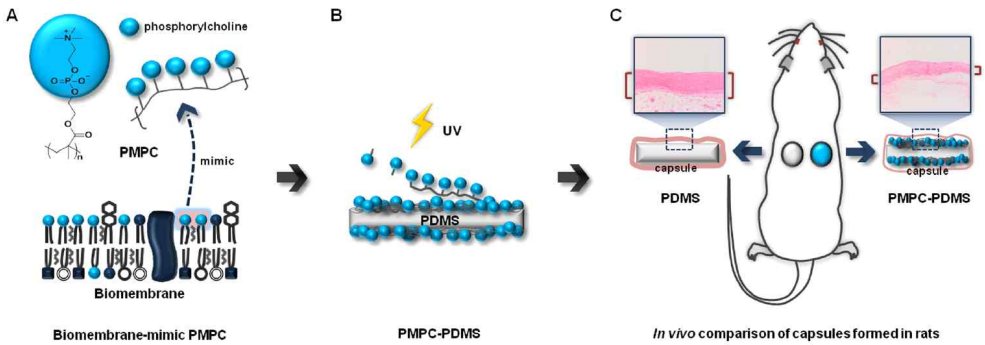

Fig. 1. Schematic illustration of silicone-implant coating and implantation. **(A)** Biomembrane-mimicking PMPC, a hydrophilic and biocompatible polymer containing the head group of the most abundant phospholipid in cell membranes. **(B)** Preparation of PMPC–PDMS via UV-induced surface polymerization of MPC on PDMS. **(C)** In vivo comparison, for the purpose of examining biocompatibility, of capsules formed on PDMS and PMPC–PDMS in rats.

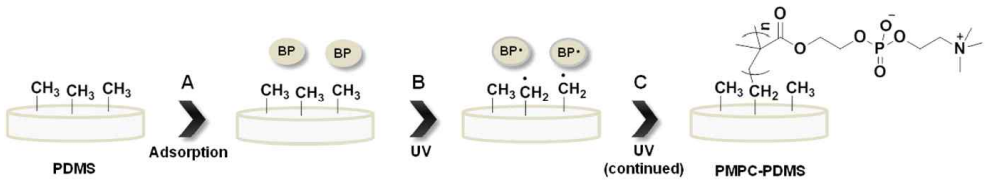

Fig. 2. Preparation of PMPC–PDMS. **(A)** Physical adsorption of benzophenone (BP). **(B)** Initiation of polymerization. **(C)** Formation of PMPC-grafted PDMS.

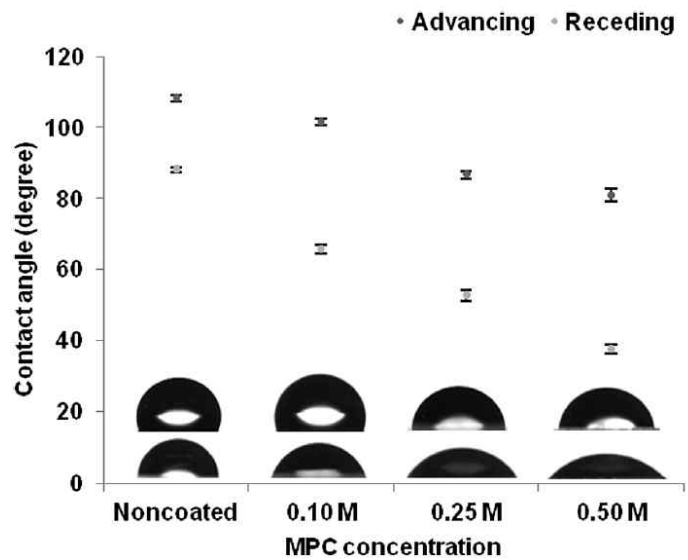

Fig. 3. Water contact angle based on MPC concentration. Data are represented as mean ± SEM (n = 18).

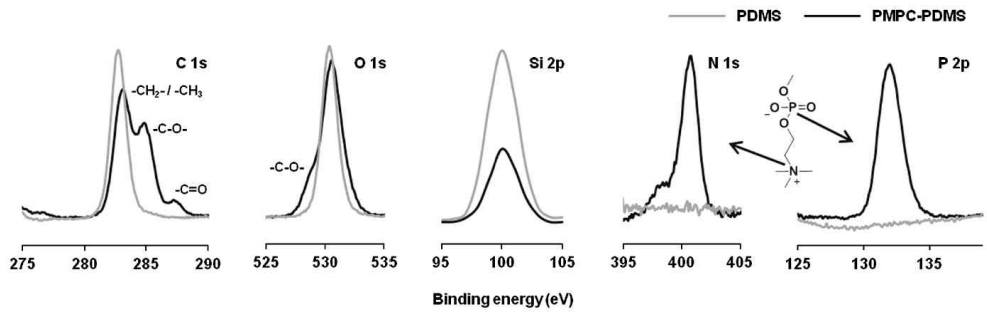

Fig. 4. XPS data obtained for PDMS and PMPC–PDMS (coated using an MPC concentration of 0.50 M).

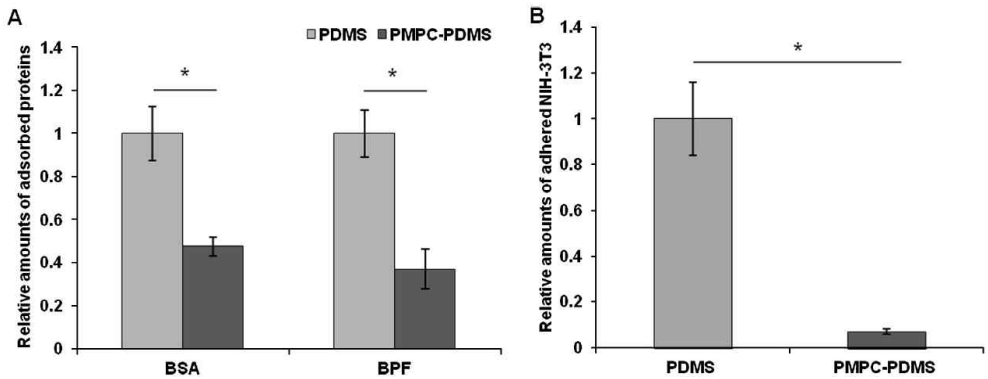

Fig. 5. In vitro protein adsorption and cell adhesion onto PDMS and PMPC–PDMS (MPC concentration = 0.50 M). **(A)** Relative amounts of adsorbed BSA and BPF. **(B)** Relative amounts of adhered mouse fibroblasts (NIH-3T3). Data are represented as means ± SEM (n = 3). The marker (*) indicates $0.01 \leq p < 0.05$.

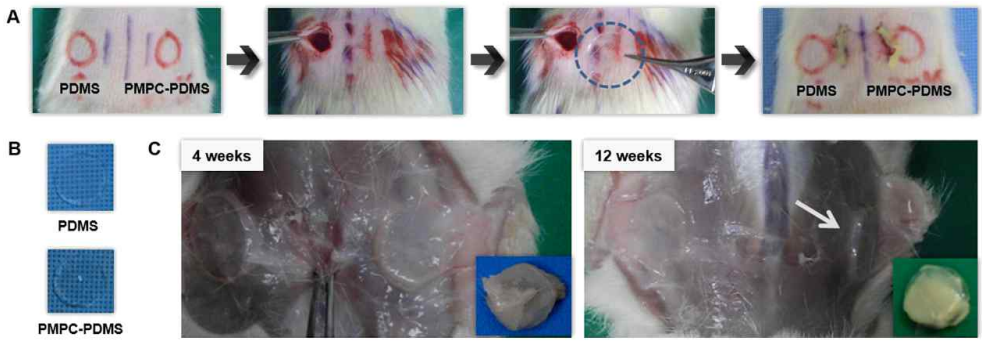

Fig. 6. In vivo experiment to investigate capsular formation on PDMS and PMPC–PDMS (MPC concentration = 0.50 M) using a rat model. **(A)** Insertion of silicone implants in the back of each rat. On the third image from the left, the dashed circle indicates the PDMS plate. **(B)** PDMS and PMPC–PDMS silicone implants. **(C)** Harvest of silicone implants from rats sacrificed after 4 or 12 weeks. An arrow indicates the plate-lying side in the 12-week image. The small images depict the representative shapes of capsules around the silicone implants.

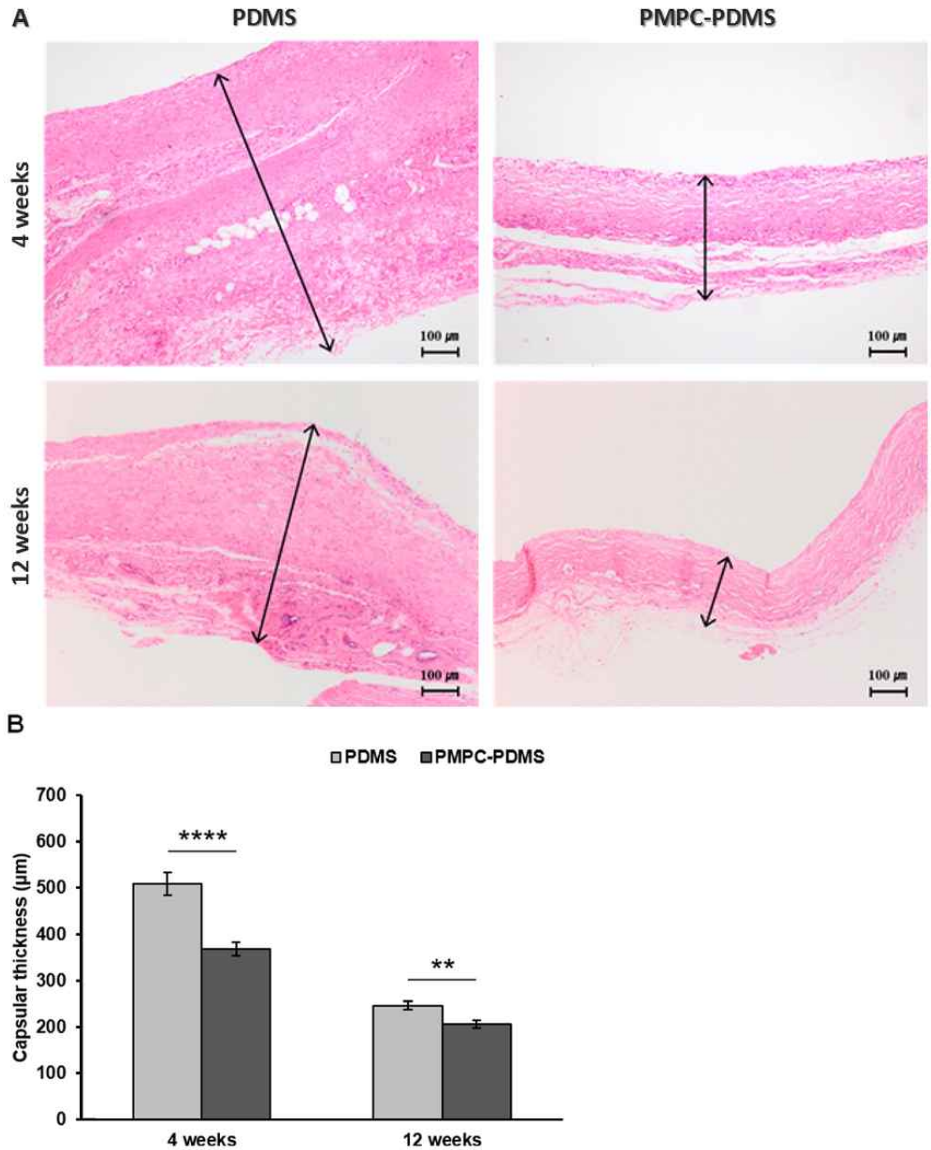

Fig. 7. Capsular thicknesses around the PDMS and PMPC–PDMS implants after 4 and 12 weeks in rats. **(A)** H&E staining images. The region of each capsule was indicated with an arrow. **(B)** Thickness of the capsule formed after 4 weeks and 12 weeks (n = 60). Data are represented as means ± SEM. The markers (****) and (**) indicate $p < 0.0001$ and $0.001 \leq p < 0.01$, respectively.

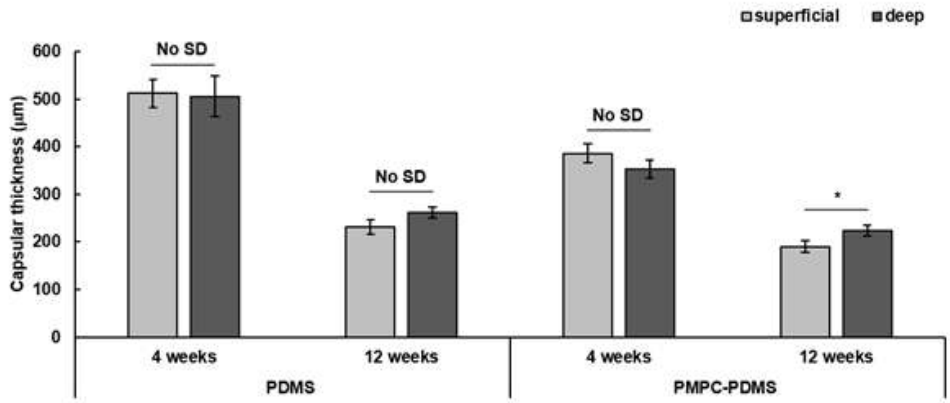

Fig. 8. Comparison of capsular thicknesses measured at superficial and deep surface levels. Data are indicated as means ± S.E.M (n=30). The marker (*) indicates $0.01 \leq p < 0.05$.

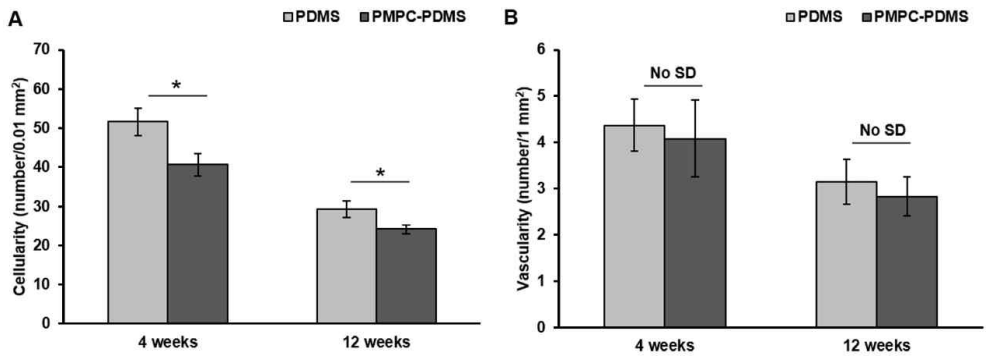

Fig. 9. In vivo analysis of intracapsular inflammatory cells and vascular formations. **(A)** The number of inflammatory cells (n = 60) and **(B)** the number of developed vessels (n = 60). Data are represented as means ± SEM. The marker (*) indicates $0.01 \leq p < 0.05$. "No SD" means there is no significant difference.

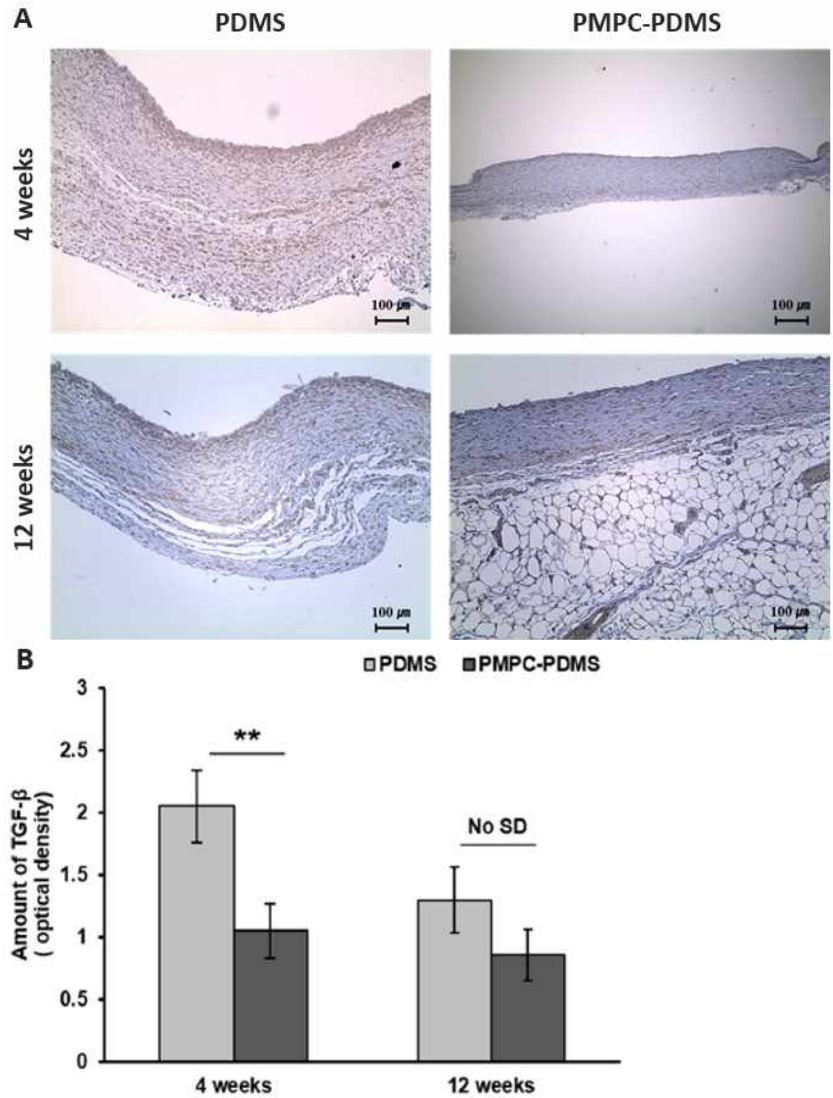

Fig. 10. Analysis for TGF-β using in vivo IHC analysis of tissues surrounding the PDMS and PMPC–PDMS implants. **(A)** Representative IHC images indicating amount of TGF-β (brown) as the key landmark of an inflammatory reaction. **(B)** Amount of TGF-β measured by optical densities. Data are indicated as means ± SEM (n = 20). The marker (**) indicates $0.001 \leq p < 0.01$. "No SD" means there is no significant difference.

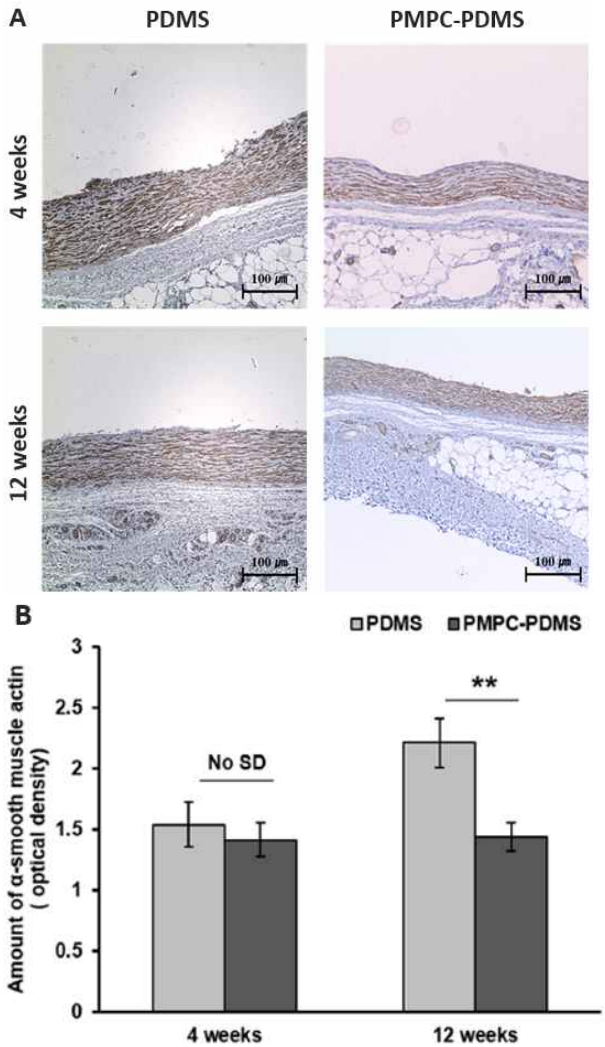

Fig. 11. Analysis for α-smooth muscle actin using in vivo IHC analysis of tissues surrounding the PDMS and PMPC–PDMS implants. **(A)** Representative IHC images indicating amount of α-smooth muscle actin (brown). The fibrous capsular layer of spindle-like and α-smooth muscle actin-containing cells is shown. **(B)** Amount of α-smooth muscle actin measured by optical densities. Data are indicated as means ± SEM (n = 20, but n = 18 for 4-week data). The marker (**) indicates $0.001 \leq p < 0.01$. "No SD" means there is no significant difference.

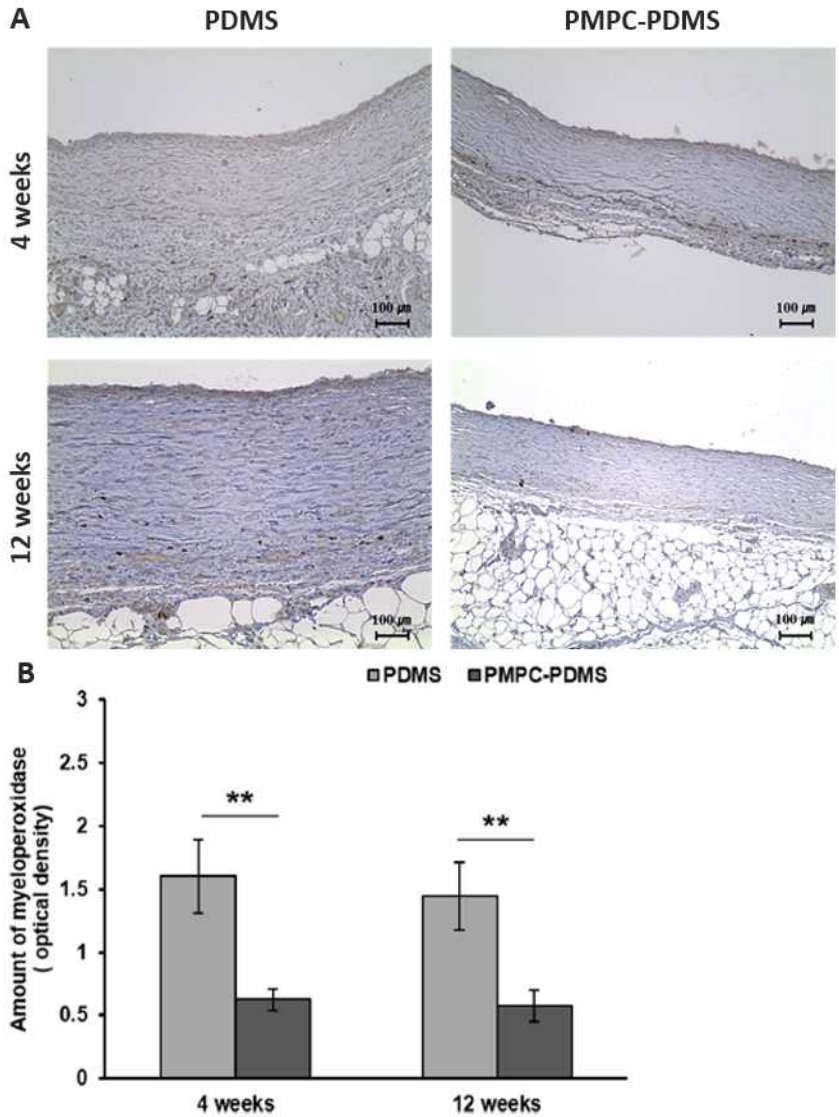

Fig. 12. Analysis for myeloperoxidase using in vivo IHC analysis of tissues surrounding the PDMS and PMPC–PDMS implants. **(A)** Representative IHC images indicating amount of myeloperoxidase (brown), which was mainly expressed in inflammatory cells such as neutrophils. **(B)** Amount of α -smooth muscle actin measured by optical densities. Data are indicated as means ± SEM (n = 20). The marker (**) indicates $0.001 \leq p < 0.01$.

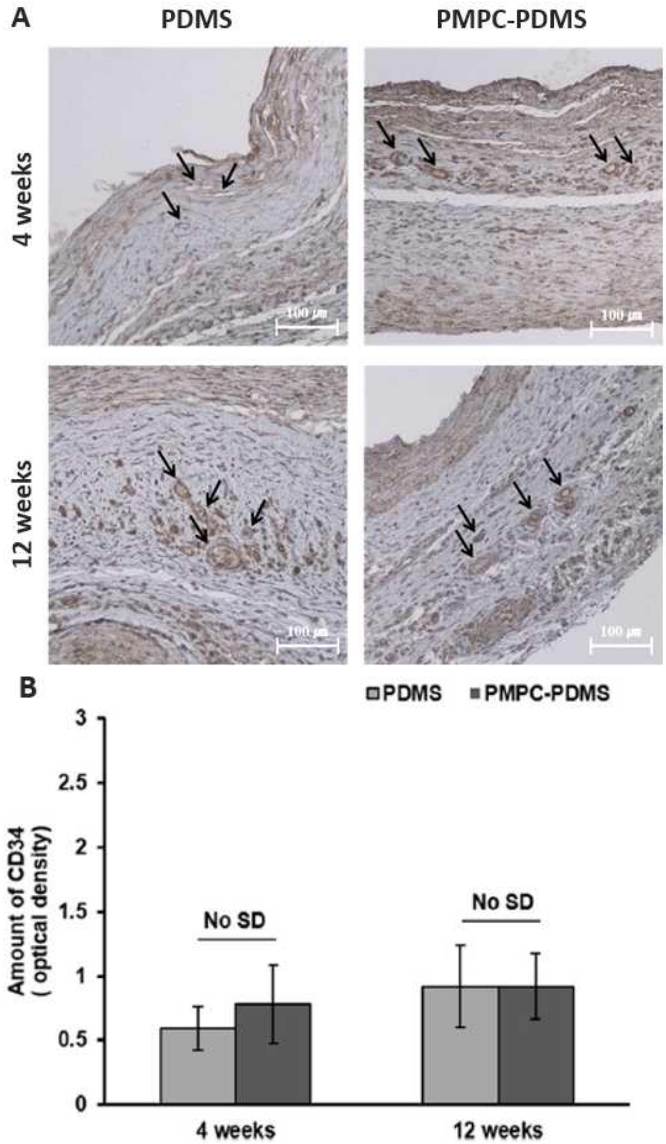

Fig. 13. Analysis for CD34 using in vivo IHC analysis of tissues surrounding the PDMS and PMPC–PDMS implants. **(A)** Representative IHC images indicating CD34 (brown)-stained endothelial cells. **(B)** Amount of α -smooth muscle actin measured by optical densities. Data are indicated as means ± SEM (n = 16). "No SD" means there is no significant difference.

국문 초록

유방 확대술과 재건술이 널리 시술되고 있으며 그 수요가 날로 증가하는 반면 이에 쓰이는 실리콘 보형물의 낮은 생체적합성은 환자들에게 고통스러운 부작용을 가져다준다. 가장 널리 알려진 부작용 중 하나는 구형 구축 (capsular contracture)이며 이는 과도한 이물 반응 (foreign body reaction)에 의해 보형물에 딱딱하고 두꺼운 섬유 조직이 캡슐로 둘러싸게 되면서 발생한다. 본 연구는 실리콘 보형물 위의 과도한 섬유 조직 형성을 막기 위해 생체막 모방 고분자를 이용해 보형물의 표면을 코팅하는 것이 주목적이다. 실리콘 보형물의 표면에 공유결합으로 강하게 결합된 고분자 PMPC (Poly(2-methacryloyloxyethyl phosphorylcholine))는 실리콘 표면의 단백질 흡착과 섬유아세포 점착을 막아준다. 또한 쥐 (rats)의 등 (back) 피하에 삽입된 PMPC로 코팅된 실리콘 보형물은 일반 실리콘 보형물에 비해 현저히 얇은 캡슐을 형성하는 것을 확인했으며, 생성된 캡슐 속의 콜라겐의 밀도가 더욱 낮고 잘 정렬되어 있는 것을 확인할 수 있었다. 또한 코팅된 실리콘에서 α-smooth muscle actin이 훨씬 적은 양이 존재한다는 점이 캡슐 형성이 완화되었다는 증거를 보충해준다. 뿐만 아니라, 코팅된 실리콘에서의 염증세포 수의 감소, myeloperoxidase 양의 감소, transforming growth factor-β의 감소는 염증반응 또한 적게 일어났음을 보여준다. 이러한 생체막 모방 고분자를 이용한 실리콘 보형물의 코팅은 구형 구축을 막을 수 있는 실현가능한 대안을 제시하고 있으며 다른 생체·의학적 적용을 위한 생체에 적합한 물질을 만드는 데에도 널리 쓰일 수 있을 것이라고 예상한다.

주요어: 실리콘 보형물, 구형 구축, PMPC, 표면 개질, 이물 반응

학번: 2012-23054