Biodegradation and Biocompatibility of Poly L-lactic Acid Implantable Mesh

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Purpose: The purpose of this study was to evaluate the biodegradation and biocompatibility of poly L-lactic acid (PLLA) implantable mesh under *in vitro* and *in vivo* conditions.
Methods: PLLA mesh was examined for changes in weight and tensile strength. The histology of the tissue around the PLLA implant was also evaluated.
Results: The weight and tensile strength of the PLLA prosthesis was stable for 180 days. In addition, the surface of the PLLA mesh was not digested under *in vitro* or *in vivo* conditions as determined by scanning electron microscope. Histologically, there were no significant changes in the diameters of implanted PLLA mesh and subtype fibers over the course of 180 days. Likewise, there were no significant changes in the number of inflammatory and mast cells after 180 days, nor was there an increase in the percentage of collagen surrounding the PLLA mesh.
Conclusions: The results indicate that PLLA prostheses have good rigidity and biocompatibility *in vivo* and *in vitro*.

Keywords: Biodegradation; Biocompatibility; Polylevolactic acid; Mesh

- ***HIGHLIGHTS***
  - The tensile strengths of PLLA mesh were not decreased after *in vitro* incubation for 180 days.
  - The diameter of PLLA fibers was unchanged *in vitro* and *in vivo* as assessed by SEM and histological examination.
  - PLLA mesh did not induce inflammation in subcutaneous tissue.

INTRODUCTION

Various types of scaffolds have been developed for both research and clinical applications of skin tissue engineering [1-3].

For example, synthetic biodegradable polymers such as poly L-lactic acid (PLLA) and poly(glycolic acid) (PGA) have been used to prepare porous scaffolds for skin reconstruction [4,5]. A requirement of such scaffolds is mechanical strength, which

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is necessary to maintain the structure and shape for implantation. The characteristics of an ideal implant material have been defined, and include biocompatibility, sufficient mechanical strength, physical resistance to the surrounding tissue, and availability in a convenient format that is also affordable for clinical use [6,7].

Lactic acid-based polymers (PLA) are degraded at a slower rate than lactic acid-glycolic acid copolymers. PLLA mesh is more resistant to degradation, and its tensile strength can be long-lasting, up to 8 months [8,9]. PLLA also exhibits better biocompatibility and produces a lower inflammatory response compared to polypropylene (PPL), a reference nonabsorbable material [10]. L-lactic acid-rich PLA-based materials have been used for more than 10 years in orthopedic surgery, where they have gained a reputation for good biocompatibility in applications such as bone fixation, bone substitutes, and repair of osteochondral defects, ligaments, and tendon reconstructions [11,12]. Recently, PLLA stents have also been studied in cardiovascular surgery applications [13].

Biodegradable mesh for skin plastic surgery should not only be able to support the tension applied to the tissue, but also inhibit any inflammatory responses that occur at the site of implantation. PLLA is a good candidate for such artificial subcutaneous prostheses, owing to its high bio-compatibility and favorable biophysical properties.

The goal of this study was to characterize biodegradable PLLA small-diameter artificial prostheses for skin plastic surgery and to assess their biocompatibility in subcutaneous tissue. *In vitro* studies were carried out either in the presence of lysozyme or phosphate buffer saline (PBS). In addition, the biocompatibility of a PLLA prosthesis was evaluated over the course of 180 days using a subcutaneous rat model.

MATERIALS AND METHODS

Preparation of PLLA Mesh
A PLLA cylindrical mesh implant was provided by Prestige Medicare Co., Ltd (Raise Me Up, Seongnam, Korea). The product was $\Phi\,0.8 \times 300$ mm in size, and its purpose is to support or protect soft tissue by acting as a biodegradable polymeric surgical mesh.

In Vitro Degradation of PLLA Prosthesis
For the degradation test, the PLLA mesh ($\Phi\,0.8 \times 50$ mm) was first weighed and then placed in sealed polyethylene bottles filled with PBS in the presence of 50 units/mL of lysozyme at 37°C, pH 7.2 for 30, 60, 90, 120, 150, and 180 days (n = 5). At specific time points, the specimens were washed thoroughly with deionized water and dried at 50°C in an oven for 12 hours. Finally, the samples were weighed and prepared for scanning electron microscopy.

In Vivo Study
Thirty male Sprague-Dawley rats (250–300 g) were anesthetized with xylazine and ketamine prior to abdominal skin implantation of the biodegradable PLLA prosthesis. Two mesh implants with dimensions of $\Phi\,0.8 \times 50$ mm were placed in the abdominal subcutaneous tissue of each rat via 2 small holes parallel to the midline. Rats were sacrificed with anesthesia and cervical dislocation at 30, 60, 90, 120, 150, and 180 days after implantation. Tissue samples impregnated with PLLA mesh were taken from the left side of the abdomen and fixed with 10% neutral formalin for histological examination. On the right side, PLLA mesh was separated from the subcutaneous tissue, wiped with gauze, and prepared for scanning electron microscopy and analysis of tensile strength. The procedures in this study were approved by the Institutional Animal Care and Use Committee of Kyungpook National University (2016-0086).

Evaluation for Tensile Strength of PLLA Mesh
The tensile strength of the PLLA mesh explanted from the abdominal skin tissue was measured with a tensiometer (JSV-H1000, JISC Ltd., Tokyo, Japan). The mesh was secured tightly in a clamp in order to prevent slipping during the test, and measurements were performed at a rate of 30 mm per minute. The tensiometer was used to measure the breakage strength of specimens, and all analyses were performed at room temperature.

Scanning Electron Microscopy
The PLLA mesh specimens were washed in PBS (pH, 7.2) and fixed in 2.5% glutaraldehyde overnight prior to dehydration in increasing concentrations of ethanol (30%, 50%, 70%, 90%, 95%, and 100%, 1 hour each). The samples were then dried with critical point drying. The dried specimens were coated with gold before being observed under a scanning electron microscope (SEM) to investigate the surface morphology at the lumen of the prosthesis.

Histological Staining
The skin samples were fixed in 10% neutral buffered formalin

for 24 hours and then prepared as paraffin blocks. Sections 3–4 μm thick were subsequently prepared, and representative sections were stained with hematoxylin and eosin for general histopathology, Masson's trichrome (MT) stain for collagen fiber, and Toluidine blue (TB) stain for mast cells according to previously established methods [14-17]. The stained histological sections were observed by light microscopically (Model Eclipse 80i, Nikon, Tokyo, Japan). For analysis of more detailed changes, the diameter of PLLA bundles and subtype fibers were measured. In addition, the number of inflammatory cells and mast cells around PLLA bundles was counted. The percentage of collagen fiber around PLLA bundles was calculated by histomorphometrical analysis using a computer-assisted image analysis program (iSolution FL ver 9.1, IMT i-solution Inc., Vancouver, BC, Canada) as described previously [14-17] with some modifications.

Statistical Analysis

All data are expressed as the mean ± standard deviation. Data were statistically evaluated by paired Student t-test. P-values less than 0.05 were considered statistically significant.

RESULTS

Weight of PLLA Implants *In Vitro*

After digestion with lysozyme *in vitro*, the weight of the PLLA implants were 0.0100 ± 0.00017 g (day 0, before), 0.0103 ± 0.00021 g (day 30), 0.0102 ± 0.00025 g (day 60), 0.0109 ± 0.00074 g (day 90), 0.0100 ± 0.00051 g (day 120), 0.0104 ± 0.00047 g (day 150), and 0.0102 ± 0.00033 g (day 180). There were no significant differences in the weights of PLLA mesh

compared to weight at the initial (day 0) time point (Fig. 1).

The Tensile Strength of PLLA Mesh

The tensile strengths of the PLLA mesh were 29.87 ± 2.03 N (day 0), 29.63 ± 4.61 N (day 30), 25.17 ± 9.59 N (day 60), 26.70 ± 3.68 N (day 90), 26.23 ± 6.77 N (day 120), 28.59 ± 7.10 N (day 150), and 27.51 ± 4.33 N (day 180). There were no significant changes in the tensile strength for each time point compared with the initial (day 0) time point (Fig. 2). These results suggest that the PLLA mesh maintains its strength in soft tissue for 180 days.

Scanning Electron Microscopy

SEM observation of the PLLA prostheses after 180 days showed that the surface was not digested *in vitro*, and had a similar shape as the implant prior to digestion. The surface of *in vivo* PLLA implants was not able to be examined due to ingrowth of the tissue around the mesh fiber. However, the PLLA mesh fibers did appear to maintain their diameter compared to preimplantation specimens (Fig. 3).

Histological Findings

The diameters of the PLLA mesh implants were 566.62 ± 22.19 μm (day 30), 563.42 ± 21.83 μm (day 60), 570.95 ± 26.69 μm (day 90), 564.30 ± 18.50 μm (day 120), 562.71 ± 21.32 μm (day 150), and 558.53 ± 13.34 μm (day 180). The diameters of the PLLA subtype fibers were 151.01 ± 9.12 μm (day 30), 150.44 ± 9.40 μm (day 60), 150.92 ± 3.81 μm (day 90), 153.32 ± 9.35 μm (day 120), 153.76 ± 4.16 μm (day 150), and 153.32 ± 8.94 μm (day 180). The number of inflammatory cells around the PLLA mesh was 85.40 ± 35.66 (day 0), 85.20 ± 54.27 (day 30),
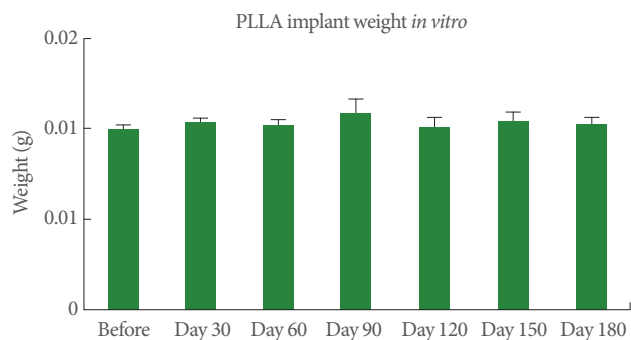

Fig. 1. Poly L-lactic acid (PLLA) implant weight according to incubation time. The weight of the PLLA implant was measured after digestion for 30, 60, 90, 120, 150, and 180 days in phosphate buffer saline with lysozyme.
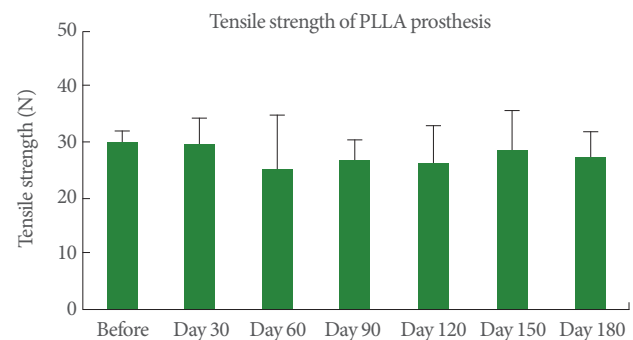

Fig. 2. The tensile strength of poly L-lactic acid (PLLA) implants was measured after tissue implantation for 30, 60, 90, 120, 150, and 180 days.

SEM figures *in vitro*   SEM figures *in vivo*

Before

Day 30

Day 60

Day 90

Day 120

Day 150

Day 180
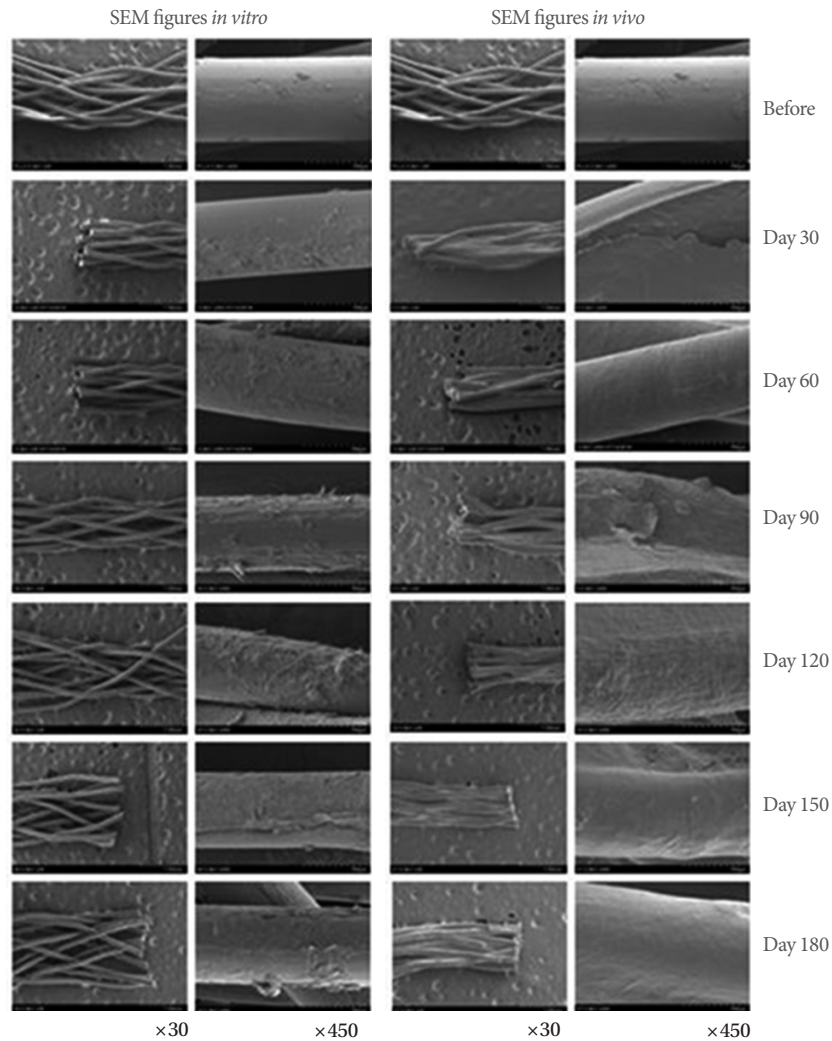
×30   ×450   ×30   ×450

Fig. 3. Scanning electron microscopy. Samples were collected at the indicated time points and observed by scanning electron microscope (SEM) to collect information on physical aspects of the mesh and identify any changes in fiber surfaces and sections.

Table 1. Histomorphometrical analysis on the tissue around PLLA subcutaneous implantation

Time	Diameter of implanted PLLA		Tissue adjacent to PLLA implantation		
	Bundles (μm)	Subtype fibers (μm)	Inflammatory cells (cells/mm²)	Mast cells (cells/mm²)	Collagen fibers (%/mm²)
Day 0	NC	NC	85.40 ± 35.66	45.60 ± 12.20	75.20 ± 3.93
Day 30	566.62 ± 22.19	151.01 ± 9.12	85.20 ± 54.27	42.80 ± 7.82	74.88 ± 9.00
Day 60	563.42 ± 21.83	150.44 ± 9.40	96.60 ± 50.45	41.80 ± 7.50	74.15 ± 6.52
Day 90	570.95 ± 26.69	150.92 ± 3.81	90.60 ± 39.15	38.40 ± 10.04	74.59 ± 3.39
Day 120	564.30 ± 18.50	153.32 ± 9.35	82.60 ± 17.40	43.60 ± 14.03	73.25 ± 4.46
Day 150	562.71 ± 21.32	153.76 ± 4.16	89.80 ± 27.11	43.20 ± 8.44	73.10 ± 5.93
Day 180	558.53 ± 13.34	153.32 ± 8.94	88.20 ± 14.86	40.00 ± 2.00	74.29 ± 6.06

Values are presented as mean ± standard deviation.
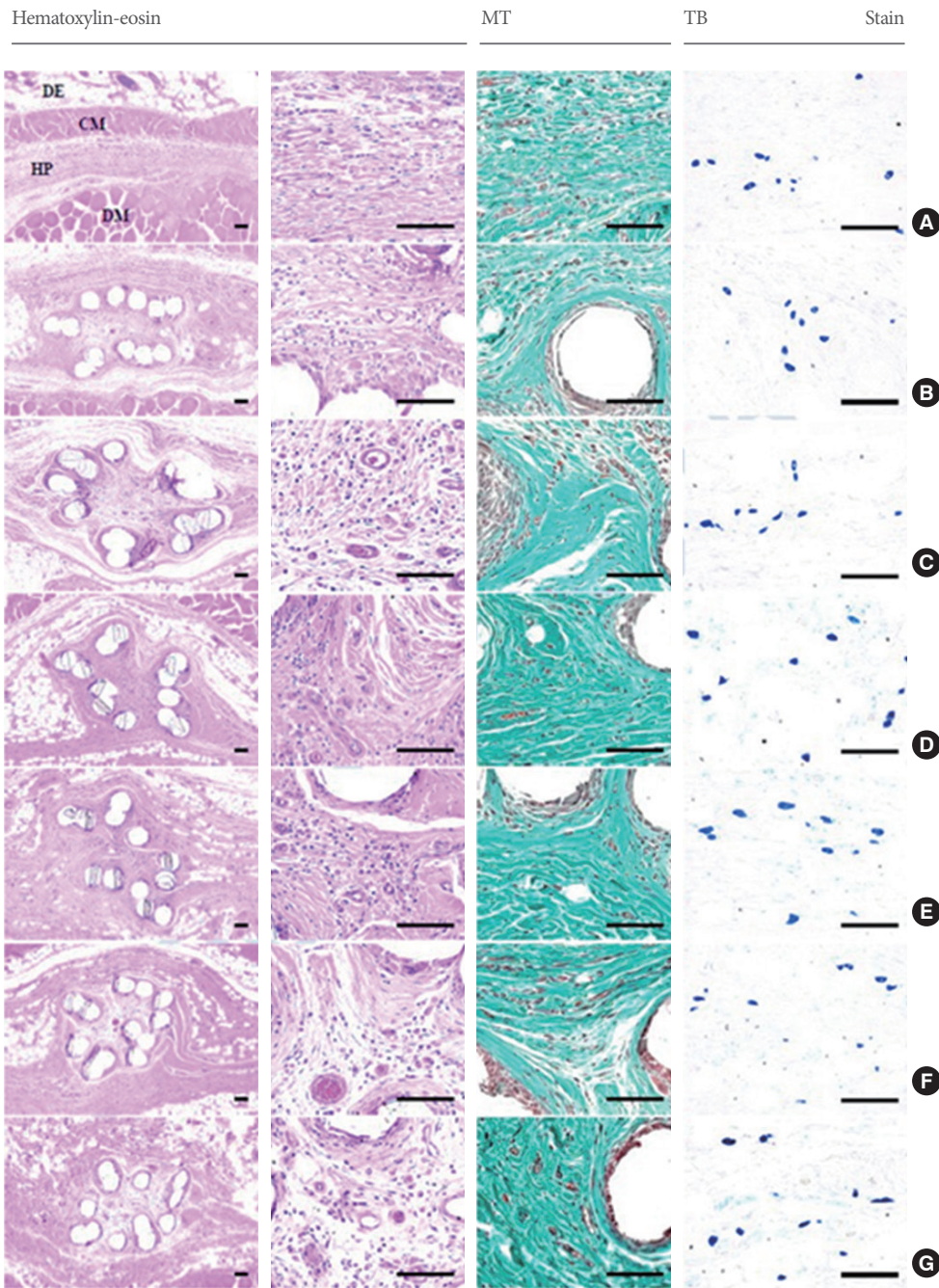PLLA, poly L-lactic acid; NC, not calculated.

Fig. 4. Representative histopathological images of subcutaneous tissues surrounding poly L-lactic acid (PLLA) implants. The PLLA mesh consisted of 12 subtype fibers, all of which were present at each time point of 30, 60, 90, 120, 150, and 180 days. There were no significant changes in the diameters of the implanted PLLA mesh or subtype fibers at 60, 90, 120, 150, and 180 days after implantation. In addition, there were no significant changes in the number of inflammatory and mast cells, and the percentage of collagen around PLLA mesh remained stable over 180 days. (A) Normal control (day 0). (B) Tissue collected 30 days after PLLA implantation (day 30). (C) Tissue collected 60 days after PLLA implantation (day 60). (D) Tissue collected 90 days after PLLA implantation (day 90). (E) Tissue collected 120 days after PLLA implantation (day 120). (F) Tissue collected 150 days after PLLA implantation (day 150). (G) Tissue collected 180 days after PLLA loading (day 180). CM, cutaneous muscle; DM, dorsal back muscle; DE, dermis; HP, hypodermis; MT, Masson's trichrome; TB, Toluidine blue. Scale bars = 80 μm.

96.60±50.45 (day 60), 90.60±39.15 (day 90), 82.60±17.40 (day 120), 89.80±27.11 (day 150), 88.20±14.86 (day 180). Likewise, the number of mast cells around the PLLA mesh was 45.60±12.20 (day 0), 42.80±7.82 (day 30), 41.80±7.50 (day 60), 38.40±10.04 (day 90), 43.20±8.44 (day 120), 43.20±8.44 (day 150), and 40.00±2.00 (day 180). Lastly, the percentage of collagen fibers in the tissue was 75.20%±3.93% (day 0), 74.88%±9.00% (day 30), 74.15%±6.52% (day 60), 74.59%±3.39% (day 90), 73.25%± 4.46% (day 120), 73.10%±5.93% (day 150), and 74.29%±6.06% (day 180) (Table 1, Fig. 4).

The PLLA mesh contained 12 fiber subtypes at each of the time points analyzed. There were no significant changes in the diameters of the implanted PLLA mesh and subtype fibers for samples collected at 60, 90, 120, 150, or 180 days postimplantation. In addition, there were no significant changes in the number of inflammatory or mast cells or percentage of collagen abundance around the PLLA mesh at 180 days (Fig. 4).

DISCUSSION

Among various scaffold materials, collagen and gelatin are commonly used to prepare scaffolds due to their favorable intrinsic properties such as low antigenicity and ability to promote cell adhesion and proliferation [18]. However, collagen and gelatin-based scaffolds have a low mechanical strength, meaning that they are rapidly absorbed in tissue. For these reasons, various alternative scaffolds of biodegradable synthetic polymers have been developed, such as PLLA, PGA, poly(lactic-co-glycolic acid), and poly(ε-caprolactone), which have favorable mechanical and biocompatible properties [4,5].

PLLA has been widely studied for bone regeneration due to its degradability, biocompatibility, and process ability [19-21]. However, there are several aspects of PLLA that remain problematic with respect to its application in biomedical fields, including hydrophobicity, lack of bioactivity, and release of acidic degradation by-products that may cause inflammation when implanted *in vivo* [22,23]. In the present study, *in vitro* and *in vivo* studies were used to determine whether PLLA mesh, when used as a subcutaneous implantable scaffold for plastic surgery, undergoes mechanical and biocompatible changes.

It was found that the weight and microstructure of PLLA were not significantly altered during *in vitro* experiments. In addition, PLLA fibers were not digested by lysozyme after 180 days as determined by SEM. This result is consistent with the findings of de Tayrac et al. [10], who observed degradation of

PLLA mesh only after 8 months at a pH of 7.4 and temperature of 37°C. *In vivo* implantation experiments were also performed to evaluate the biocompatibility of PLLA mesh, as well as to determine whether PLLA mesh is biodegraded or becomes weakened with respect to mechanical strength after prolonged implantation.

Under *in vitro* conditions, the tensile strengths of the PLLA mesh did not decrease after 180 days. Consistent with this finding, a previous study showed that the tensile strength of a PLLA scaffold developed for vaginal surgery begins to decrease under 20 N of force only after implantation for 8 months [10]. In addition, this study found that the diameter of PLLA fibers did not decrease during the experiments as determined by SEM and histological examination. These findings suggested that the PLLA mesh used in the present study was not digested in living tissue.

Histological examination showed that there were no significant time-dependent changes in the number of inflammatory and mast cells induced by the implanted PLLA mesh. Likewise, there were no changes in the percentage of collagen fibers in subcutaneous tissue around the PLLA mesh during experiments. Conversely, it has been reported that PLLA may induce an inflammatory reaction due its hydrophobicity, low bioactivity, and release of acidic degradation by-products in musculoskeletal tissue and *in vitro* when incubated with gastric juice [22,23]. Taken together with the results of this study, PLLA mesh may not cause inflammation in subcutaneous tissue, but may cause inflammation in other tissues or under specific incubation conditions.

In summary, by using *in vitro* and *in vivo* models, the current study showed that the weight and microstructure of PLLA is not degraded over time. Specifically, the tensile strengths of PLLA mesh did not decrease after *in vitro* incubation for 180 days. In addition, the diameter of PLLA fibers was unchanged *in vitro* and *in vivo* during the study period as assessed by SEM and histological examination. The PLLA mesh did not induce inflammation in subcutaneous tissue; however, according to the literature, it may cause inflammation in different tissues or under specific incubation conditions. Therefore, PLLA mesh may be very useful as a subcutaneous implantable device for skin plastic surgery. Future studies on long term application of PLLA mesh in the subcutaneous space will be useful to more clearly determine the biodegradable and biocompatible properties of PLLA.

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