

Efficacy study of the new polycaprolactone thread compared with other commercialized threads in a murine model

Seung Woo Cho MD¹  | Byung Ho Shin PhD¹ | Chan Yeong Heo MD, PhD^{1,2} | Jung Hee Shim PhD^{1,3}

¹Department of Plastic and Reconstructive Surgery, Seoul National University Bundang Hospital, Seongnam, Korea

²Department of Plastic and Reconstructive Surgery, Seoul National University College of Medicine, Seoul, Korea

³Department of Research Administration Team, Seoul National University Bundang Hospital, Seongnam, Korea

Correspondence

Jung Hee Shim, Department of Research Administration Team, Seoul National University Bundang Hospital, 82, Gumi-ro 173 Beon-gil, Seongnam-si, Gyeonggi-do 13620, Korea.
Email: xmylife@empas.com

Chan Yeong Heo, Department of Plastic and Reconstructive Surgery, Seoul National University College of Medicine, 103 Daehak-ro (Yeongeon-dong), Jongnogu, Seoul 03080, Korea.
Email: lionheo@snu.ac.kr

Abstract

Background: Polydioxanone (PDO) threads, poly-L-lactic acid (PLLA) threads, and polycaprolactone (PCL) threads have been used for lifting and antiaging purposes. The new PCL threads that have less residual monomer compared to the previous PCL are developed.

Aims: The efficacy of threads regarding collagen synthesis and wrinkle improvement was evaluated in vivo model.

Methods: In this study, threads were inserted into 30 six-week-old male SKH-1 hairless mice. One of four threads was implanted at either side of the spine of each mouse. Biopsy specimens obtained at 1, 4, and 8 weeks were examined using hematoxylin and eosin (H&E) and Herovici's stain. Additionally, immunoblot analysis was performed using primary antibody for collagen type III and transforming growth factor- β (TGF- β) and visualized by chemiluminescence and densitometric quantification. Finally, skin replicas were used to calculate total wrinkle area (mm²).

Results: Neocollagenesis was significantly increased by 50% in the new PCL and pre-existing PCL groups at 8 weeks (p value < 0.001). Additionally, new-PCL-implanted mice showed a significant increase in collagen type III and TGF- β expressions at 8 weeks (p value < 0.001). The number of inflammatory cells was also increased in the skin of PCL-implanted mice at 8 weeks. Finally, wrinkles were reduced about 20% in the new PCL group at 8 weeks.

Conclusions: The new PCL thread exhibited a superior skin rejuvenation effect. This suggests that the material processing technology can be applied not only to the thread but also to various products such as dermal filler and cosmetics.

KEYWORDS

collagen, PCL, TGF- β , thread lifting, wrinkle

Jung Hee Shim and Chan Yeong Heo contributed equally to this article as corresponding authors.

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1 | INTRODUCTION

Skin has an intact epidermis with layers that acts as a solid barrier to outside influences. It is composed of abundant components such as collagen, elastin, and glycosaminoglycans. As one grows older, the synthesis of collagen in skin reduces, especially type I and III collagen which results in uneven focal ptosis and laxity of the soft tissues around whole areas including infraorbital, buccal, mental, and submental parts.^{1,2}

A lot of facial rejuvenation techniques were developed to reverse or delay aging process such as lasers, fillers, botulinum toxin, fat grafts, and other soft tissue augmentation procedures.³⁻⁷ Various face lift surgeries have been the traditional methods of facial rejuvenation since the early 20th century.⁸ However, surgical methods with incisions have some disadvantages compared to nonsurgical methods such as difficulty of the techniques, high cost, long operation time and recovery periods, and surgical scars.⁹ With the advent of various facial rejuvenation techniques, minimally invasive methods have gained popularity.⁹ Especially, the thread lift is a safe and effective technique that lifts sagging skin on the face and neck using threads that are absorbed biologically. The evolution of thread lifting procedure is now in its third decade since Sulamanidze first proposed lifting using Aptos threads.¹⁰ Sulamanidze developed the first barbed (short) suture technique using the "Aptos" thread in the late 1990s.¹⁰ In 2002, the Woffles (long) thread, also known as Waptos was developed.¹¹ This thread was used as a suture suspension sling to lift facial soft tissues to the deep temporal fascia.¹¹ Isse reported an endo-progressive face lift suture in 2005.¹² This suture was fixed to the temporal fascia with a thread created by modifying the "Aptos" thread. Most of the techniques mentioned above involve a polypropylene thread which is nonabsorbable. Some have warned the dangers of nondegradable threads. They have a risk of migration, extruding from the skin, and may be visible under facial skin.¹³ Nowadays, the use of lifting threads made from biocompatible and biodegradable materials has received global attention.

The absorbable threads make skin lifted immediately through mechanical effects.¹⁴ And these threads stimulate neocollagenesis process of tissues, which results in the production of new collagen.^{11,12} About 6 months after beginning of the implantation, the threads will degrade through hydrolysis.¹⁵ After this, synthetic absorbable thread-induced collagen synthesis will last about 2 ~ 3 months.⁸

There have been biodegradable thread materials such as polycaprolactone (PCL) and poly-L-lactic acids (PLLA) starting with polydioxanone (PDO).^{10,12,16} The PCL thread has received the most attention recently among them.¹⁵ PCL is very flexible and highly elastic substance; thus, it causes less pain and discomfort compared to PDO and PLLA. PCL is biodegradable and decomposed into CO₂ and H₂O. Its safety is proven from various biodegradable medical devices approved by the US Food and Drug Administration (FDA).¹⁷ Threads made from PCL are slowly absorbed into the body within 1 ~ 1.5 years compared to PDO (6 ~ 8 months) and PLLA (12 months).¹⁸ For functional improvement of the collagen synthesis

and wrinkle improvement, the new PCL thread with less residual monomers and a higher molecular weight has been developed expecting prolonged durability and enhanced efficacy.

There have been no other studies comparing antiaging effects on tissues of commercial threads focusing on collagen synthesis and wrinkle improvement. This study was performed to determine the efficacy of the new PCL thread compared to other commercial threads in vivo model. This preliminary study will provide guidance for future investigations.

2 | MATERIALS AND METHODS

2.1 | Insertion of threads and experimental plan

All animal experimental procedures were approved by the Animal Care and Use Committee of the hospital (permit number: 54-2017-003). 30 six-week-old male SKH-1 hairless mice (Orient Bio Inc) about 21-30 g were used in this study. Before the thread insertion, the mice were quarantined for a week and allowed free access to food and water to adapt to the laboratory environment. The mice were housed at a controlled temperature of 24°C, a relative humidity of 55%, and a 12-hour light cycle. One of four threads was implanted at either side of the spine (column) of each mouse. Mice were randomly allocated into the following five groups with 12 columns each; the new PCL (Glo-One) group, the PCL (Ultra-V) group, the PDO (Meta Biomed, Osong) group, PLLA (APROMEDION, Seoul, Korea) group, and a negative control group. All threads were 50 mm in length and 0.3 mm in diameter. After anesthesia with isoflurane, the dorsal skin of mice was disinfected with alcohol. Each thread was subcutaneously inserted under the dorsal skin on either side of the spine. Each thread was inserted 2 cm laterally from the spinal cord. Tissue samples from surrounding subcutaneous tissue, along with the thread, were harvested at 1, 4, and 8 weeks after implantation (3 × 9 cm size). Each sample was divided into three equal segments, each of which was used for staining, wrinkle measurement, and Western blotting, respectively.

2.2 | Histology

The specimens obtained from the dorsal skin were fixed in 10% buffered formalin (pH 7.1), embedded in paraffin, and sectioned at 6-10 μm for light microscopy. Sections were stained with hematoxylin-eosin (H&E) and Herovici's stain for collagen and connective tissue. The polychromatic Herovici's stain distinguishes young, newly formed type III collagen fibers (blue), from the mature, dense type I collagen fibers (red), making this stain very useful in the investigation of collagen synthesis.

The amount of type III collagen fibers in Herovici-stained sections was measured using an image analysis program (ImageJ software, National Institutes of Health, USA) and expressed as the percentage area occupied by each fiber around inserted thread. In

H&E-stained sections (100 × magnification), the number of inflammatory cells, which appear as blue dots around the thread, was measured using an image analysis program (ImageJ software, National Institutes of Health).

2.3 | Immunoblot analysis

The dorsal skin of thread inserted area was washed with ice-cold PBS and lysed in a RIPA buffer (Cell Signaling Technology) containing protease inhibitor cocktails (Roche Applied Science) for 60 minutes on ice. The skin was homogenized, followed by centrifugation at 16 000 g for 30 minutes at 4°C. Protein concentration was determined by the Bradford method. Protein samples were separated on an 8%-16% Tris-glycine gel (Invitrogen, USA), blotted onto polyvinylidenedifluoride (PVDF) membrane (Bio-Rad, Hercules). Primary antibodies used were as follows: transforming growth factor-β (TGF-β), type III collagen (Abcam). Blots were probed with anti-goat or anti-rabbit IgG-HRP and visualized by ECL chemiluminescence (GE Healthcare). Membranes were exposed to BioMax Light Kodak films. Densitometric quantification of the bands was performed using an image analyzer system (Multi Gauge version 2.3 software). These experiments were performed in triple.

2.4 | Wrinkle analysis

Lee and colleagues measured wrinkles using a SILFLO impression material (FLEXICO, England) and the Visioline® VL650 (Courage & Khazaka, Koln, Germany) in a hairless mouse.¹⁹ Wrinkles were measured by similar methods in this study. Skin replicas were obtained from the dorsal skin using a SILFLO impression material. The Visioline® VL650 was used to assess the skin surface. Total wrinkle

area (mm²) was calculated. The analyses using Visioline® VL650 were performed in Daegu Technopark, Korea.

2.5 | Statistical analysis

All quantitative data were presented as mean ± SEM based on data derived from four experiments at each group. Statistical comparisons were carried out using the analysis of variance (ANOVA). In all analyses, $P < .05$ was considered to be statistically significant.

3 | RESULTS

3.1 | Gross observation

After implantation of the threads into each mouse, changes in the weight and morphology of the skin were evaluated at 1, 4, and 8 weeks. Mice exhibited no gross changes of the skin in the insertion area of each thread during the experimental period. All experimental groups had no noticeable change in body weight (Figure 1).

3.2 | Neocollagenesis

All groups induced predominantly young collagen in the dermal layer of mice at all of the time points examined. The amount of type III collagen was significantly increased in the new PCL group compared to the other groups at 4 weeks. The amount of new collagen was significantly increased about 50% in both PCL thread groups compared with the PDO or PLLA thread group at 8 weeks (Figure 2). Values represent means ± SEM from three independent experiments. (* $P < .001$) (Young collagen: blue, mature collagen: red).

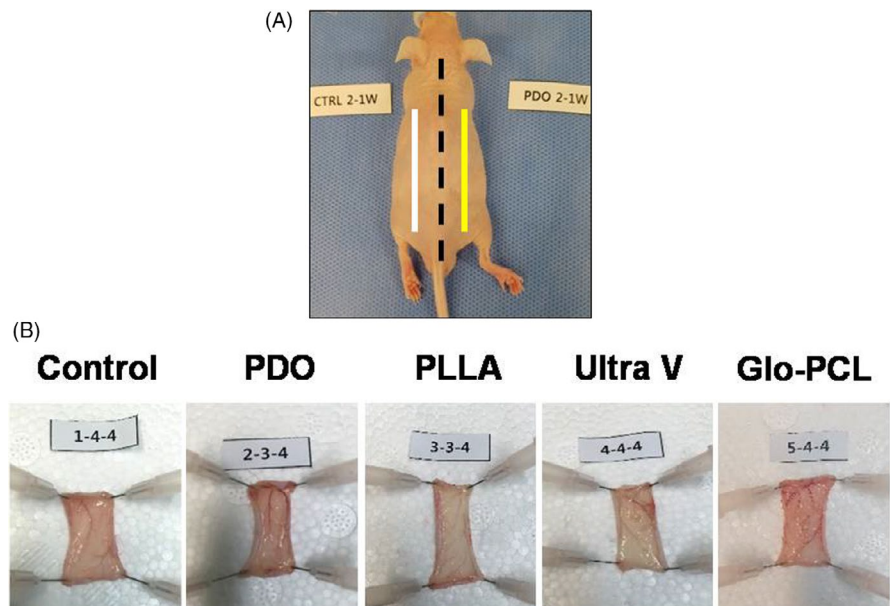


FIGURE 1 Thread implantation in mice and harvested tissues. A, Each thread was inserted at subcutaneous layer 2 cm laterally from the midline. B, Tissue samples were harvested along with the threads at 1, 4, and 8 weeks after implantation (3 × 9 cm size), followed by division into three equal segments. Each figure shows a segmented sample

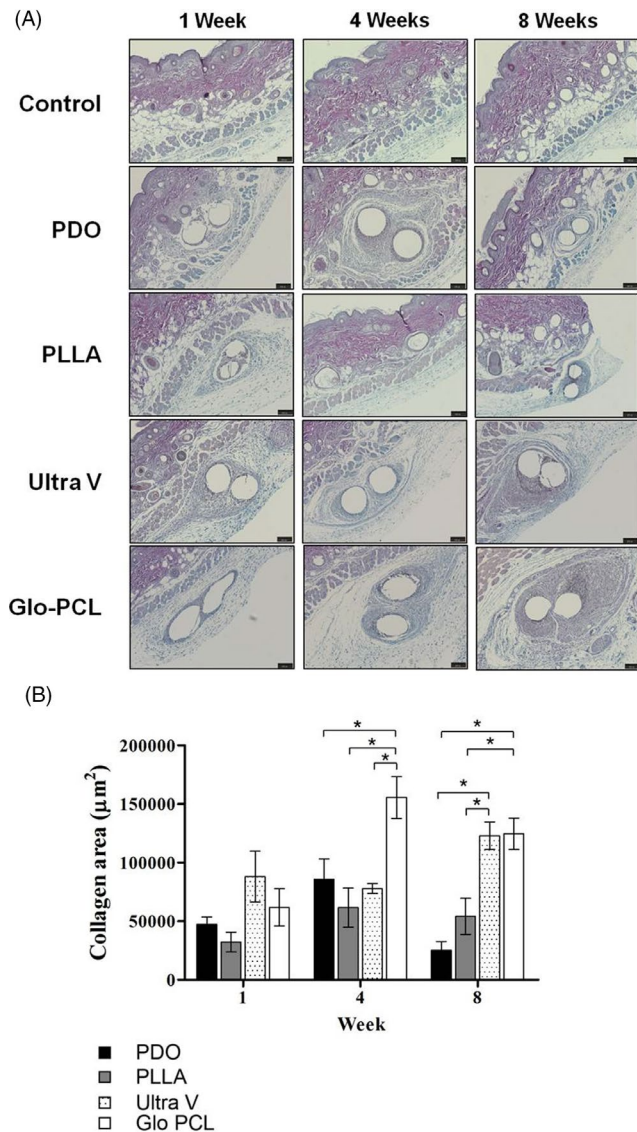


FIGURE 2 Histological evaluations of collagen fibers. A, Young, newly formed collagen fibers appearing blue through Herovici's staining were selectively stained and quantified. Black bar = 500 μm . B, Statistical analyses were performed to compare cross-sectional area of young collagen in each group at 1, 4, and 8 weeks. (* $P < .001$)

3.3 | Collagen synthesis mediated by TGF- β

The expressions of TGF- β and type III collagen were measured. There were no significant differences among the groups at 1 week. At 4 weeks, although there were no significant differences of type III collagen among the groups, significant increases of TGF- β were observed in the new PCL group. Though it is not statistically significant, type III collagen showed about 1.5-fold increases in the new PCL group relative to that of nonimplanted mouse at 4 weeks. Especially, mice with the new PCL showed significant increases (above 250% and 300%) in TGF- β and type III collagen expressions at 8 weeks, even though there was no statistically significant difference between the PCL groups. (* $P < .001$) However, the PDO and PLLA

groups showed similar levels of TGF- β and type III collagen relative to nonimplanted mouse (Figure 3).

3.4 | Inflammation

The number of inflammatory cell was significantly increased only in the new PCL group at 4 weeks. At 8 weeks, there was statistically significant increase in the number of inflammatory cells in both PCL groups compared with the PDO and PLLA groups (above 200%) (Figure 4).

3.5 | Wrinkle analysis

There was no significant difference of the total wrinkle areas among the groups. However, wrinkles were reduced about 20% in the new PCL group at 8 weeks (Figure 5).

4 | DISCUSSION

Aging of facial skin results from elastic tissue and collagen degradation, which develops fine-to-deep wrinkles.^{9,20} In addition to decreased elasticity, gradual thinning of subcutaneous fat layer causes volume depletion and sagging of mid-face.²⁰ Many facial rejuvenation techniques were developed to reverse or delay this aging process. As minimally invasive techniques replace surgical methods of facial rejuvenation, thread lifting has gained in popularity.⁹ In recent years, biodegradable materials have been used to make threads.²¹

Polydioxanone (PDO) is a synthetic polymer of multiple repeating ether-ester units that is slowly hydrolyzed to a 2-hydroxyethoxy-acetic monomer over 6 months and work by triggering fibroblasts to produce more collagen in a targeted area.¹⁶ After PDO threads, poly-L-lactic acids (PLLA) threads were developed. They are made from a polymer derived from lactic acid that has been widely used as orthopedic pins and sutures.²² PLLA threads produce collagen over a longer period than PDO threads. They have cones to hang to the tissue and increase the volume of saggy areas helping not only to provide a lift but to restore shape to the facial area.²² Polycaprolactone (PCL) threads are the latest monofilament suspension threads of synthetic origin (caprolactone).¹³ They regenerate collagen for a longer period than PDO and PLLA threads. The breakdown process of threads produces small molecular weight molecules which gradually induce the production of collagen and hyaluronic acid by the skin. As a result, skin becomes more moisturized, revitalized, and firm.²³ The new PCL thread used in this experiment was developed changing the ratio of the solvent during the polymerization. It resulted in reduction of the amount of residual monomer compared to the previous PCL. This means the new PCL thread has a higher polymer concentration and a higher molecular weight which helps resist against tensile stress.²⁴

The threads have lifting effects immediately through mechanical effects by increasing the tonus of tissues, followed by

FIGURE 3 Quantitative evaluation of type III collagen and TGF- β expressions. A, Immunoblot analysis was performed to evaluate type III collagen and TGF- β expressions at 1, 4, and 8 weeks. B, Densitometric quantification of the bands was performed by an image analyzer system using Multi Gauge version 2.3 software

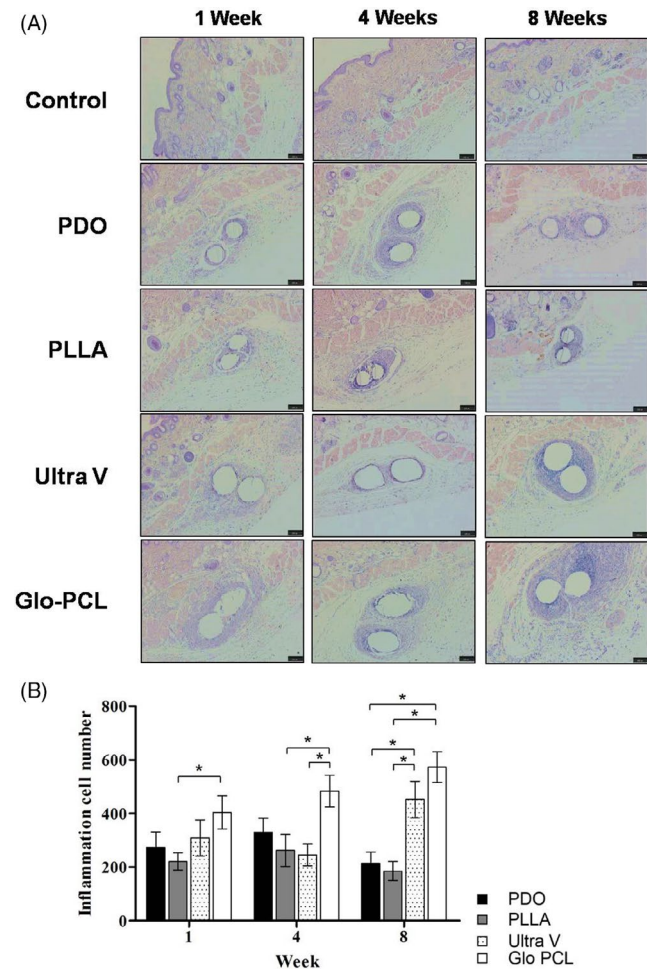
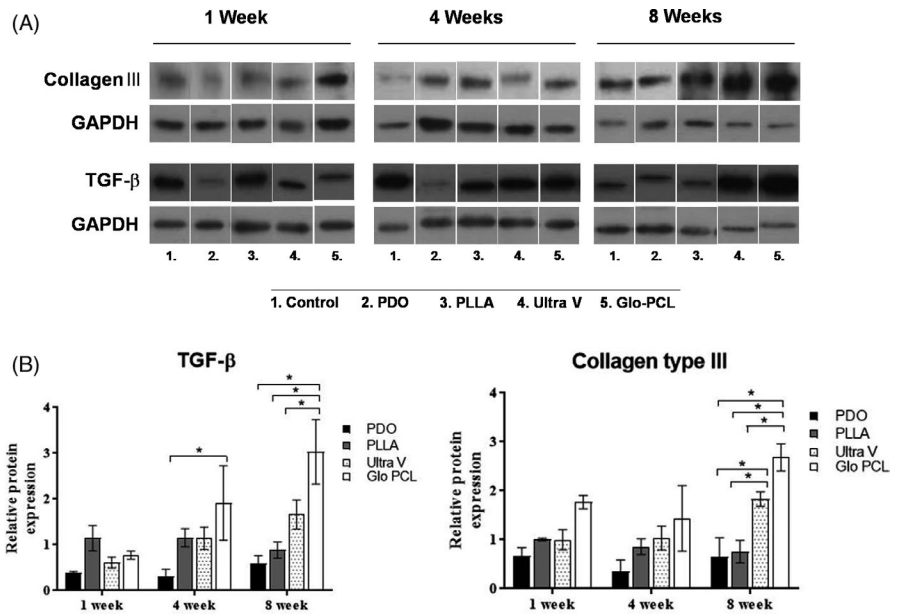


FIGURE 4 Histological evaluations of inflammatory cells. A, Hematoxylin-and-eosin-stained sections of samples were obtained at 1, 4, and 8 weeks. Black bar = 500 μ m. B, The number of inflammatory cells in each group of mouse was measured

neocollagenesis by fibroblasts.¹⁴ Collagen is essential for contiguous formation of the interstitium in the skin and a major structural component of the skin that prevents wrinkle formation.²⁵ Though there are several studies comparing the shapes of threads such as Cog, monofilament, and multifilament threads focusing on the mechanical effects, few studies have investigated the materials of threads focusing on the production of collagen.^{16,26} However, the new PCL has a high molecular weight and was expected to have more lifting effect by producing more collagen for a longer period. Therefore, this study was designed to determine whether there are any differences in neocollagenesis among the thread materials. In previous animal studies, the thickest capsule formation around the thread was observed and the tensile strength was maximum at 4 weeks.^{26,27} Thus, in this study, histological and molecular changes were investigated for 8 weeks after the implantation of PDO, PLLA, and PCL threads to evaluate the aspect of collagen production with residual tensile strength.

After thread insertion, the biostimulatory reaction starts with subclinical inflammation by macrophages and multinucleated giant cells.²⁸ It continues to microparticle encapsulation followed by collagen production by fibroblasts.²⁸ Consiglio and colleagues implanted PLLA threads in the abdominal region, which were removed at 1, 3, 6, and 12 months, followed by histological evaluation.²⁹ Intense inflammatory cell infiltration was normally observed at 1 and 3 months in a similar way with this study. Kapicioglu and colleagues inserted Cog threads and PLLA threads 2 cm lateral to the spinal cord of rats.²² Skin samples were analyzed at 1, 3, and 6 months using light microscope and transmission microscope. They revealed that dermis thickness, numbers of fibroblasts, and numbers of collagen fibrils were significantly increased with threads like this study. However, there was no significant difference between Cog groups and PLLA groups unlike this study. In the present study, histopathologic review

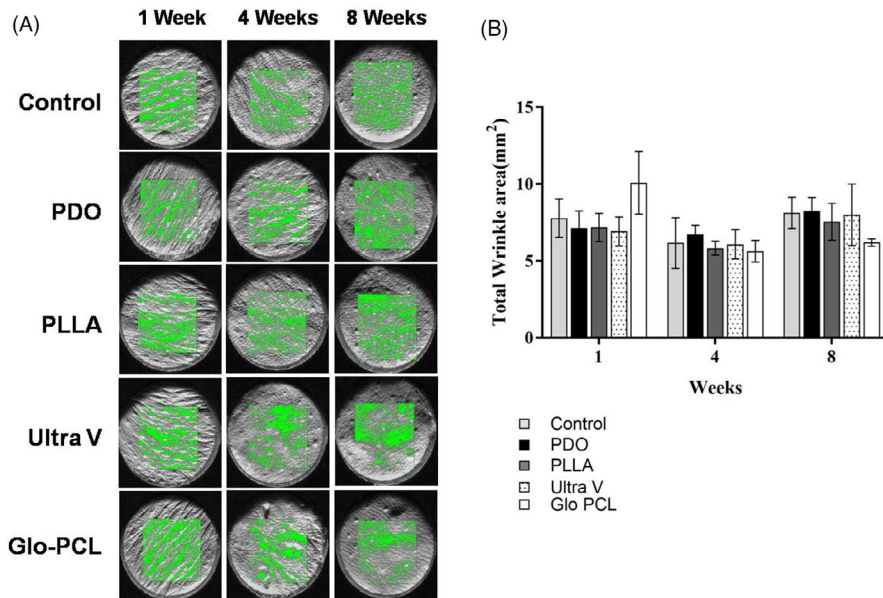


FIGURE 5 Evaluation of total wrinkle areas from skin replicas. A, Skin replicas were obtained from the dorsal skin of mice using a SILFLO impression material. B, Total wrinkle area (mm²) of each group was measured

revealed that inflammatory reaction was induced after the implantation of all kinds of threads as in the previous study.²⁷ However, the number of inflammatory cells was significantly increased in the new PCL group at 4 weeks (Figure 4). The new PCL has a higher molecular weight and this helps to resist against tensile stress.²⁴ It means pre-existing PCL threads may start to degradate earlier than the new PCL threads resulting in lower level of biostimulation at earlier stage such as 4 weeks. Furthermore, it also explains higher expressions of TGF- β and type III collagen in the new PCL group at 4 weeks, though they are not statistically significant among the thread inserted groups (Figure 3). Because inflammation induces fibroblast to activate TGF- β pathway, both TGF- β and type III collagen expressions were increased.²⁸ Eventually, the amount of young collagen stained blue in Herovici's stain was significantly increased in the new PCL group at 4 weeks (Figure 2).

At 8 weeks, the pre-existing PCL and the new PCL groups showed a significantly higher level of inflammatory cells after implantation compared with the other groups. However, there was no significant difference between the PCL groups (Figure 4). Therefore, it is assumed that biostimulation reached at almost same level in both PCL groups considering the similar level of inflammatory cells. It is also explaining increased expressions of TGF- β and type III collagen in both PCL groups (Figure 3). Consequently, newly formed collagen fibers were increased in both groups (Figure 2).

Previous studies have demonstrated an increase in collagen production with the expression of various signaling molecules.³⁰ Among them, TGF- β pathway is considered to play a key role in wound healing process, stimulating fibroblasts proliferation in the dermis, followed by the synthesis of several proteins including collagen.³¹ Therefore, the findings of present study are strengthened by correlating TGF- β expression with the level of newly synthesized collagen fibers. In addition to the filling effect of collagen fibers, it is postulated that the immediate lifting effect by mechanical anchorage is sequentially

reinforced with fibrous tissues around the thread caused by initial inflammation.

In spite of an increase in collagen production, total wrinkle area was not significantly decreased in all groups at every time point (Figure 5). It can be assumed that reinforcement of mechanical anchorage and filling effect of collagen fibers are not enough to show significant difference on the surface of the skin in 8 weeks. Nonetheless, a longer period of study is needed because there were differences of expressions of TGF- β and type III collagen at 8 weeks between among the groups and it may induce greater differences as time goes by.

This study has some limitations. First, present study included a small number of samples and a relatively short period of investigation. Second, all threads used in this study were monofilaments. Further research with other shapes of threads for a longer period is required to demonstrate its clinical effectiveness.

5 | CONCLUSIONS

Here, the efficacy of the new PCL threads in SKH-1 hairless mouse has been confirmed. First, collagen synthesis was significantly increased in the new PCL group. The new PCL thread showed a significant increase at 4 weeks and both PCL groups demonstrated significant increases compared with the other groups at 8 weeks. Second, wrinkle areas of the skin were decreased in the new PCL group at 8 weeks compared with threads of various materials. However, there should be a long-term assessment of the effects for its clinical effectiveness.

The new PCL thread exhibited a skin rejuvenation including neo-collagenesis and wrinkle improvement compared with other types of threads. Furthermore, the material processing technology can be applied not only to the thread but also to various products such as dermal filler and cosmetics.

CONFLICT OF INTEREST

This study was funded by Glo-one from 2015 to 2017. This included all materials, subjects, and students fee. Glo-one started its thread lifting business in 2017. Since 2017, the study was conducted independently. We previously secured our rights to publish free and independently. The funding source had no involvement in the collection, analysis, and interpretation of data; in writing of the report; and in the decision to submit the article for publication.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Seung Woo Cho  <https://orcid.org/0000-0001-8266-8415>

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