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ORIGINAL ARTICLE

Experimental Investigation on the Tissue Response Induced by Face-Lifting Mesh Suspension Thread in Rats

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Background: Face-lifting procedures are often performed to hide the effects of aging. Thread-lifting, a minimally invasive technique for the correction of facial aging, has become increasingly popular, and various materials for the procedure have been developed. **Objective:** This study compared tissue responses to two types of threading sutures placed under rat skin: polypropylene (PP) monofilament mesh suspension thread (a novel face-lifting material) and polydioxanone (PDO) barbed thread. **Methods:** Eight rats each were assigned to the PP monofilament mesh suspension, PDO barbed thread, and control groups. Tissue reactions were evaluated 28 days after subcutaneous loading of the materials. **Results:** Significant increases in tensile strength and the mean area occupied by collagen fibers were evident in skin loaded with PDO barbed thread and PP monofilament mesh suspension thread compared to control skin ($p<0.05$). Compared to sites loaded with PDO barbed thread, those loaded with PP monofilament mesh suspension thread showed a significant increase in the number of collagen fibers and a lower grade of inflammation ($p<0.05$). **Conclusion:** PP monofilament mesh suspension thread has skin-rejuvenating effects comparable to those of PDO barbed thread, but induces a less severe inflammatory response. This indicates that it is a safe and effective material for use in thread-lifting procedures on aging skin. **(Ann Dermatol 31(6) 645~653, 2019)**

-Keywords-
Face-lifting, Mesh suspension thread, Polydioxanone thread, Thread-lifting

INTRODUCTION

Facial aging is a natural biological process that leads to the thinning, loss of elasticity, and increased laxity of facial skin together with the formation of wrinkles and the atrophy of soft tissues. With aging, connections between muscle and other tissues become weaker, resulting in the gradual development of brow ptosis, orbital rim prominence, deepening of the nasolabial folds, sagging skin and jowl formation[1-5]. Surgical correction of these involutional signs of facial aging[6,7] often yield dramatic improvements. However, there are risks associated with general anesthesia and potential perioperative complications, including hematoma, skin flap necrosis, scar formation, parotid fistulation, and facial nerve injury[8,9]. Thus, more durable and less invasive means of face-lifting have been sought[10,11].

One such procedure is thread-lifting, which is now widely used for brow-, midface-, jowl-, and neck-lifting[12,13]. A specific type of suture material is required to provide sufficient traction/holding power and thus long-lasting effects. Thread-lifting is continuously evolving, including new thread materials and shapes (e.g., by changing the directions of the barbs) and modified fixation techniques. The ideal thread-lifting material induces a minimal inflammatory response and achieves excellent cosmetic results.

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Recently, a polypropylene (PP) monofilament mesh suspension thread composed of an implantable distal mesh and a barbed thread became available commercially[14]. We compared the tissue responses to two types of threading suture after subcutaneous loading under rat skin: PP monofilament mesh suspension thread and polydioxanone (PDO) barbed thread.

MATERIALS AND METHODS

Animals and experimental groups

Twenty-four healthy male SPF/VAF outbred Crl:CD1 (Sprague Dawley, SD) rats (6 weeks old; OrientBio, Seongnam, Korea, ANNEX I and II) were housed in our animal facility. After 9 days of acclimatization, they were allocated to groups of four, with each group housed together in a polycarbonate cage placed in a temperature- (20°C～25°C) and humidity- (30%～35%) controlled room. The light: dark cycle was 12 hours:12 hours and food and water were supplied *ad libitum*. Body weights were measured (average, 251.25±11.09 g; range, 235.0～271.0 g) 1 day before placement of the test material (Table 1, Fig. 1). All experimental procedures were conducted in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals (NIH publication no. 85–23, reviewed in 1996). This study was approved by the Institutional Animal Care and Use Committee of Daegu Haany University, Gyeongsan, Korea (no. 88/2016). Then the 24 rats were divided into three groups of eight. Group A consisted of unloaded (control) rats, group B of rats subcutaneously loaded with PDO barbed thread (MIRACU™; Dongbang Medical Co. Ltd., Seongnam, Korea), and group C of rats subcutaneously loaded with PP monofilament mesh suspension thread (RaiseMeUp™; Prestige Medicare Co. Ltd., Seongnam, Korea) (Fig. 2).

Preparation of test materials

PP monofilament mesh suspension thread and PDO barbed thread were supplied as complete kits, which were stored at room temperature with protection from light and moisture.

Subcutaneous loading of test materials

The two test materials were subcutaneously loaded using needle-type probes after the dorsal hair of the rats was clipped and the area sterilized with povidone iodine (BetadineTM; Korea Pharma Co., Hwaseong, Korea). During this procedure, the rats were placed under inhalation anesthesia with 3% (v/v) isoflurane (Hana Pharm. Co., Hwaseong, Korea) in a mixture of 70% N_2O and 28.5%

Table 1. Experimental design used in this study

Group		Animal treatment	Animal No.
Group A	Intact (control)	No loading	R01～R08
Group B	PDO barbed thread	Reference material, subcutaneously loaded	R09～R16
Group C	PP monofilament mesh suspension thread	Test material, subcutaneously loaded	R17～R24

PDO: polydioxanone, PP: polypropylene, group A: unloaded (control), group B: subcutaneously loaded with PDO barbed thread (MIRACU™), group C: subcutaneously loaded with PP monofilament mesh suspension thread (RaiseMeUp™).

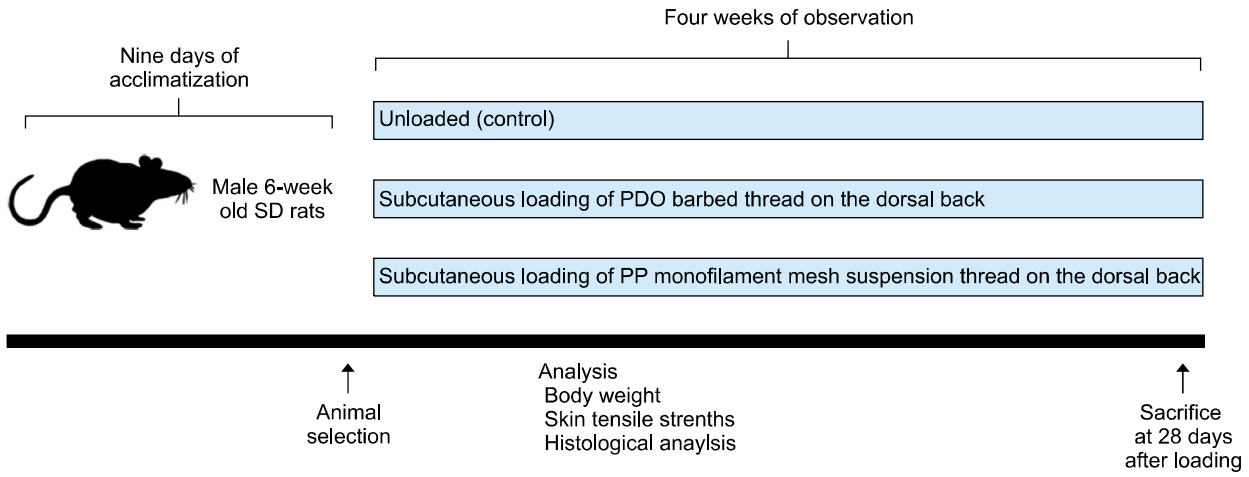

Fig. 1. Experimental design of the study. SD rats: Sprague Dawley rats, PDO: polydioxanone, PP: polypropylene.

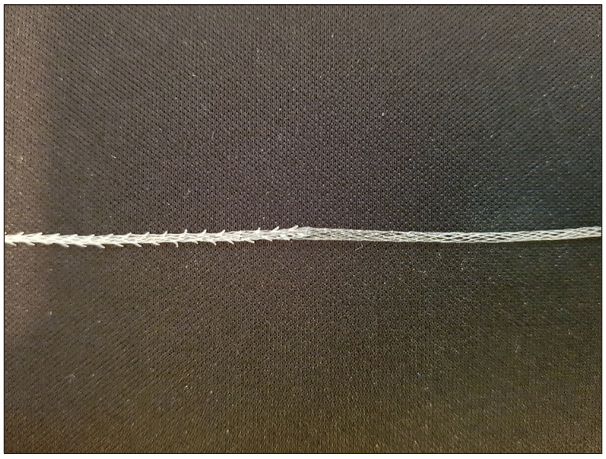

Fig. 2. Photograph of polypropylene monofilament mesh suspension thread (RaiseMeUp™; Prestige Medicare Co. Ltd., Seongnam, Korea).

O_2 (both v/v), administered using a rodent inhalation anesthesia apparatus (Surgivet, Waukesha, WI, USA) and a rodent ventilator (model 687; Harvard Apparatus, Cambridge, UK). The test materials were loaded longitudinally, on the right and left sides of each rat, and then fixed to the loaded sites by skin sutures made with 3–0 black silk, with two ties per test material, at the cephalic and caudal sites. Control rats (group A) underwent hair clipping and skin sterilization with no material loading.

Changes in body weight

Body weights were measured at day −1, day 0 (the day of subcutaneous loading) and days 7, 14, 21, and 28 after loading, using an automatic electronic balance (Precisa Instrument, Zurich, Switzerland). All animals were fasted overnight (for 18 hours; water was not restricted) prior to test material loading to reduce individual differences caused by feeding behavior.

Gross inspection and skin tensile strength measurement

The rats were sacrificed under inhalation anesthesia and squares of dorsal skin (from both the right and left loading sites) were inverted to observe the subcutaneous regions. Skin tensile strength was measured (in Newtons; N) using a computerized testing device (SV-H1000; Japan Instrumentation System Co., Tokyo, Japan). Equally sized squares from the right side of the dorsal skin, with (groups B, C) or without (group A) loaded test material, were sampled 28 days after subcutaneous loading. The tissue samples were fixed into the machines at two points (cephalic and caudal sites). Peak tensile loads were recorded as skin tensile strength apparent during a 10 mm expansion.

Table 2. Classification of inflammation grade

Grade	Response
0	No inflammatory response
1	Mild inflammatory response, with low cell density present in up to 25% of the analyzed area
2	Moderate inflammatory response, with medium cell density present in 26%～75% of the analyzed area
3	Severe inflammatory response, with high cell density present in more than 75% of the analyzed area

Histopathology

The dorsal skin on the left side (containing the test materials) was sampled 28 days after subcutaneous loading and fixed in 10% (v/v) neutral buffered formalin (NBF) for 24 hours. Individual samples were trimmed and re-fixed in 10% (v/v) NBF. After paraffin embedding, 3 to 4 μm-thick sections were obtained and stained with hematoxylin and eosin (H&E) and Masson's trichrome (MT). The latter identifies collagen fibers. Mast cells were identified with toluidine blue (TB) stain[15-18]. An experienced pathologist observed the mounted slides under a light microscope (Model Eclipse 80*i*; Nikon, Tokyo, Japan) at 40×, 100×, and 400× magnification. More detailed information was obtained by determining the mean diameters of the remnant-loaded test materials (in μm), the amount of mast cell infiltration, the inflammatory response around the loaded test materials, and the percentage of tissue occupied by collagen fibers. The inflammatory response was graded according to the greater or lesser presence of inflammatory cells, principally lymphocytes and macrophages under H&E stain (Table 2). Microscopy-based counts from five high-power fields in skin regions with a predominance of inflammatory cells were averaged. The number of mast cells, averaged over eight high-power fields, was counted on TB-stained sections. The percentage of skin region occupied by collagen fiber (per square millimeter of dermis) was calculated from MT-stained sections.

Histomorphometric analyses were done using a computer-assisted image-analysis program (*i*Solution FL ver. 9.1; IMT *i*-solution Inc., Vancouver, QU, Canada) as described previously[15-18] but with a few modifications. One histological field running from the epidermis to the hypodermis in each section, from around the centrally located loading sites, and eight dorsal skin samples from each group, were histopathologically evaluated. At least five repeat measurements were conducted, whenever possible, on the same specimens to calculate mean histomorphometric values. The pathologist was blinded to the group.

Statistical analyses

All numerical data are expressed as means±standard deviations. Multiple comparison tests were conducted. Homogeneity of variance was examined using Levene's test[19]. If the result indicated no significant deviation, the data were analyzed using a one-way ANOVA followed by the least-significant difference multi-comparison test to identify pairs of groups that differed significantly. If the Levene test revealed a significant deviation from homogeneity, a non-parametric Kruskal–Wallis H test was conducted. If the latter revealed a significant difference, a Mann–Whitney U test was performed[20]. The level of significance was set at $p<0.05$. All statistical analyses were conducted using IBM SPSS Statistics ver. 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Changes in body weight and weight gain

Body weight gain during the 28 days was observed in the rats of all three groups. The differences ($p>0.05$) in body weight or weight gain between rats in groups B or C and those in group A were not significant throughout the 28 days of the loading period (Table 3, Fig. 3).

Gross findings and changes in skin tensile strength

There were no serious gross findings in the loaded regions of group B and C rats compared to group A control rats, at least not macroscopically (Fig. 4). Significant ($p<0.05$) increases in skin tensile strength were evident in groups B and C compared to group A, regardless of whether the test materials were intact or remnant (Fig. 5). However, there were no significant differences in the skin tensile strength between groups B and C ($p>0.05$). After removal of the remnant test materials, significant ($p<0.05$) decreases in skin tensile strength were evident in both experimental groups compared to the similarly treated control group.

Histolopathological findings

1) Remnant-loaded test materials

The mean single-filament diameter of remnant material in group B rats was 586.25±26.25 μm, and the mean diameter of remnant bundles in group C rats 574.77±31.6 μm. The bundles in group C rats were composed of fibers of 12 subtypes, with a mean diameter of 120.77±6.25 μm (Table 4, Fig. 6A). The two types of remnant material were observed in the respective rats 28 days after loading, in close proximity to the loading sites.

2) Inflammatory response

Inflammatory response grades were significantly ($p<0.05$)

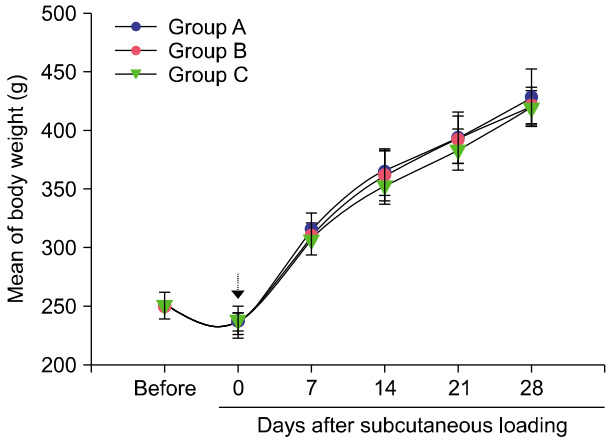

Fig. 3. Body weight changes. There were no significant changes in the body weights of rats in groups B or C compared to rats in group A throughout the 28-day loading period. Before: 1 day before subcutaneous loading. The rats were sacrificed on day 28 after loading. All animals were fasted overnight before subcutaneous loading (dotted arrow). Group A: unloaded (control), group B: subcutaneously loaded with polydioxanone barbed thread (MIRACU™), group C: subcutaneously loaded with polypropylene monofilament mesh suspension thread (RaiseMeUp™).

Table 3. Body weight gains in intact rats and those loaded with test materials

Group	Body weight on:				Body weight gain [B–A]
	1 day before subcutaneous loading	Day of subcutaneous loading [A]*	21 days after subcutaneous loading	28 days after loading, at sacrifice [B]	
Group A	251.0±11.6	238.8±11.9	315.4±14.9	394.3±21.9	189.4±19.6
Subcutaneously loaded rats					
Group B	250.8±11.6	237.5±7.7	392.6±20.1	421.8±15.3	184.3±18.6
Group C	252.0±11.5	237.6±13.7	384.1±17.1	419.3±14.3	181.6±11.6

Values (g) are expressed as the mean±standard deviation from eight rats. Group A: unloaded (control), group B: subcutaneously loaded with polydioxanone barbed thread (MIRACU™), group C: subcutaneously loaded with polypropylene monofilament mesh suspension thread (RaiseMeUp™), *All animals were fasted overnight (~18 hours; water was not restricted).

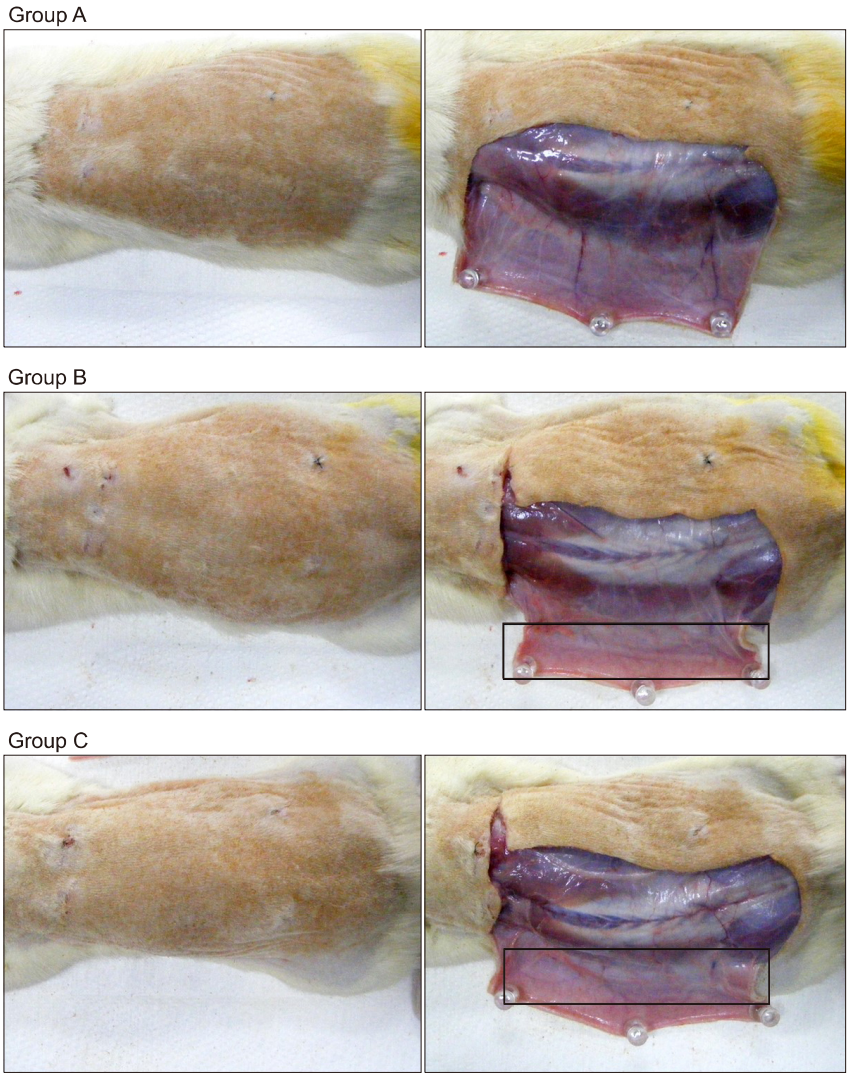

Fig. 4. Representative gross findings. There were no serious gross findings around sites loaded with polydioxanone (PDO) barbed thread or polypropylene (PP) monofilament mesh suspension thread compared to control sites. Squares indicate remnant loaded materials. Group A: unloaded (control), group B: subcutaneously loaded with PDO barbed thread (MIRACU™), group C: subcutaneously loaded with PP monofilament mesh suspension thread (RaiseMeUp™).

higher around the loaded sites in group B than in those of either group C or group A (Table 4). Most of the infiltrated cells were lymphocytes. (Fig. 6A) Whereas the three groups did not significantly differ ($p>0.05$) with respect to the mean number of mast cells (Table 4, Fig. 6B).

3) Area of collagen fiber

Significant increases in the mean area occupied by collagen fibers were evident in the skin of group B and C rats compared to the skin of group A rats (Table 4). Moreover, compared to the loaded sites in group B, those in group C were characterized by a significant increase in collagen fibers ($p<0.05$) (Fig. 6C).

DISCUSSION

In face-lift procedures, the use of less invasive techniques with minimal risk and short recovery times, such as the S-lift, delta-lift, lower superficial musculoaponeurotic system (SMAS) lift, and more recently, percutaneous suture suspension, are in increasing demand. Percutaneous suspension sutures afford gentle rejuvenation of the face, improving folding, soft-tissue ptosis, and skin redundancy[21]. Barbed sutures were originally developed in 1992 by Ruff and independently by Sulamanidze et al. in 1996[1]. Wu[22] used another type of barbed suture, one that effectively suspends sagging tissue from the stable tissue of the temporal scalp. Isse developed the Isse endo-progressive face-lift suture, made of PP filaments with unidirectional barbs that become anchored to the temporalis fascia. Contour threads consist of 25 cm lengths of 2–0 PP suture material including a central 10 cm segment that contains 50 unidirectional, helicoidally configured barbs. The silhouette lift suture is a nonabsorbable PP 3–0 suture featuring small knots and flexible cones[23].

The many types of threads that are currently available

have been developed using different materials and different synthetic cog structures. The latter can be generally classified into three types: non-barbed sutures, bidirectionally barbed non-anchored sutures, and unidirectionally barbed anchored sutures[24]. Recently, a PP mesh suspension thread featuring an implantable distal mesh and a barbed thread was introduced. PP meshes are used in many fields of surgery besides face-lifts. For example, the use of a mesh supporting system in a double-skin technique for mammoplasty and in the closure of the abdominal wall in numerous other operations has been reported[25,26]. The advantages of PP monofilament mesh suspension thread include increased tensile strength and placement in the sub-SMAS plane. The tensile strength (apart from that afforded by the cogs) is increased by the distal mesh while the pore size (<1 mm) of the mesh results in less-extensive scar formation. Furthermore, a separate mesh segment is used for fixation to the deep temporal fascia, which increases both thread stability and the longevity of the lifting effect[27]. Kwon et al.[28] demonstrated that a PP mesh had sufficient mechanical strength and stretching force for use in brow suspension. Pak et al.[25] also used PP mesh in nasolabial folds and reported that there were no serious adverse effects in treated patients. However, as with every newly developed procedure and the associated materials, there are benefits and drawbacks that must be carefully evaluated.

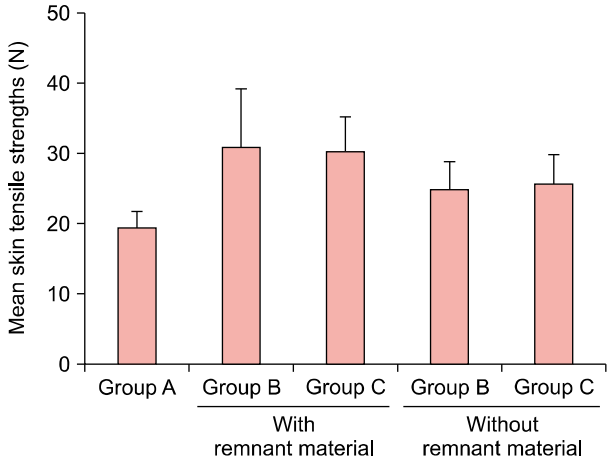

Fig. 5. Tensile strength on the dorsal skin. Significant increases in skin tensile strength were determined in groups B and C compared to group A, with and without remnant loaded thread. Values are expressed as the mean±standard deviation of the data from eight rats. Group A: unloaded (control), group B: subcutaneously loaded with polydioxanone barbed thread (MIRACU™), group C: subcutaneously loaded with polypropylene monofilament mesh suspension thread (RaiseMeUp™).

In the present work, significant increases ($p<0.05$) in skin tensile strength and the mean area occupied by collagen fibers were evident 28 days after subcutaneous loading with PP monofilament mesh suspension thread and PDO barbed thread, compared to control rats. In addition, significant increases in collagen fibers were seen in the sites loaded with PP monofilament mesh suspension thread vs. PDO barbed thread ($p<0.05$). However, the difference in tensile strength between skin loaded with PDO barbed thread and PP monofilament mesh suspension thread was not significant ($p>0.05$). Increases in tensile strength and collagen fibers have been associated with skin rejuvenation[17,24,27,29-33]. Our results suggest that the two test materials induce similarly potent collagen deposition and increases in skin tensile strength, both of which are closely related to skin rejuvenation.

Materials appropriate for the treatment of skin aging should remain at the loading site and should not cause serious local irritation[15,34,35]. In this study, compared to control skin, sites loaded with PDO barbed thread exhibited a significantly ($p<0.05$) more intense inflammatory response, whereas this was not the case in sites loaded with PP monofilament mesh suspension thread. In their study of meshes used to repair abdominal wall incisional defects,

Table 4. Histomorphometric analysis of regions around subcutaneously loaded sites

Item	Group A	Subcutaneously loaded rats	
		Group B	Group C
Remnant material diameter (μm)			
Bundles	-	586.3±26.3	574.8±31.6
Fiber subtypes	-	-	120.8±6.3
Grade of inflammatory response	1.0±0.0	2.1±1.0	1.3±0.5
Mast cells (count/high-power field)	11.8±2.7	12.4±2.7	11.2±2.6
Collagen fibers (%/mm^2 dermis)	59.4±3.0	81.3±7.5	100.4±8.6

Values are expressed as the mean±standard deviation from eight rats. Group A: unloaded (control), group B: subcutaneously loaded with polydioxanone barbed thread (MIRACU™), group C: subcutaneously loaded with polypropylene monofilament mesh suspension thread (RaiseMeUp™).

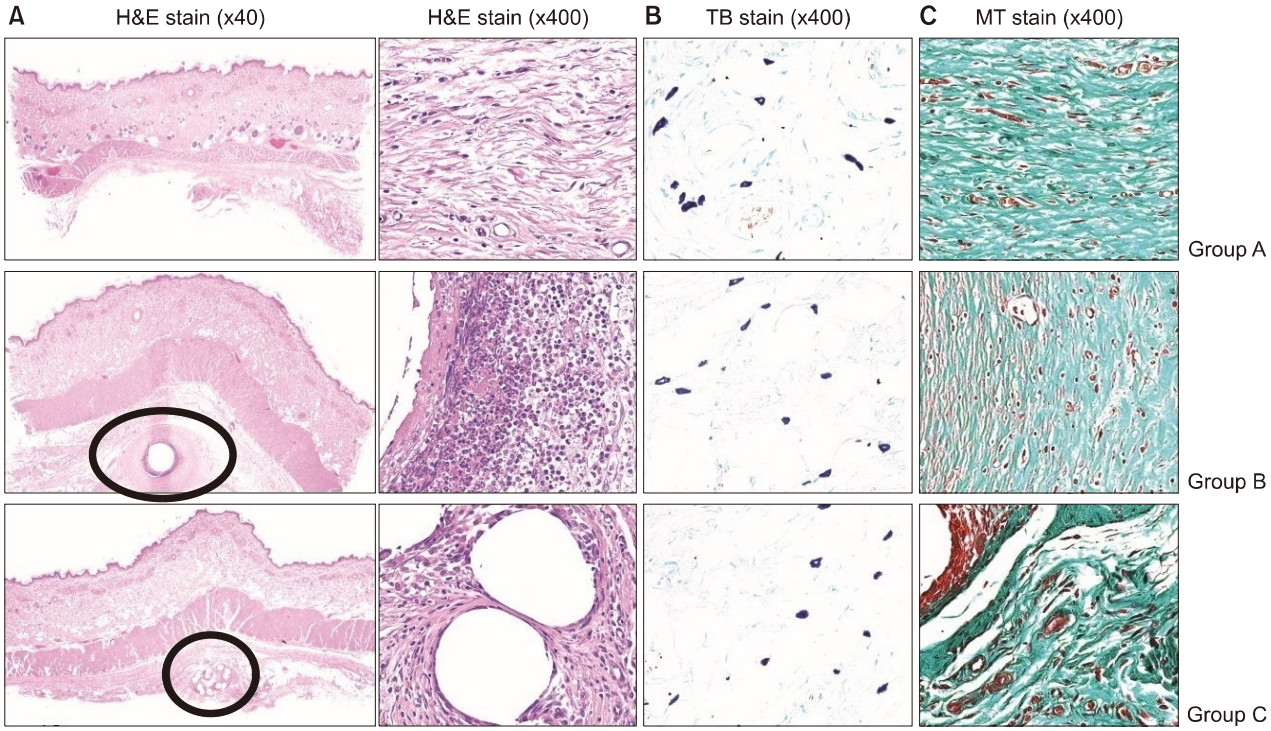

Fig. 6. Histological profiles of dorsal skin tissues around loading sites. Based on measurements from the hematoxylin and eosin (H&E) stained sections (A), the mean diameter of the remnant material in group B was 586.25 ± 26.25 μm, and the mean diameter of the remnant bundles in group C was 574.77 ± 31.60 μm. Bundles with a mean diameter of 120.77 ± 6.25 μm were detected 28 days after loading and were well localized to the loading sites. The mean inflammatory cell grade around loading sites was significantly higher in group B than in group A rats. On the toluidine blue (TB) stained sections (B), the mast cell infiltration around the loaded regions of the dorsal skin did not significantly differ between groups B and C and control group A rats. In the Masson's trichrome (MT) stained sections (C), significant increases in collagen fibers were evident in group C compared to either group A or group B ($p < 0.05$).

Pereira-Lucena et al.[36] suggested that the absorbable materials in composite meshes prolong the inflammatory tissue reaction and that an intense inflammatory reaction might reduce tissue maturation and collagen deposition. Pascual et al.[37] also demonstrated that the use of meshes containing absorbable biological materials could increase the production of inflammatory mediators. Maeda et al.[38] compared four different types of mesh: high-density PP, low-density PP, PP mesh encapsulated with oxidized cellulose–coated PDO, and expanded polytetrafluoroethylene. On postoperative day 28, the inflammatory scores of the low-density PP group were lower than those of all other groups[38]. Our work also suggests that the use of absorbable biological materials, such as PDO, can prolong the inflammatory tissue reaction compared to PP. Long-term safety is the another important concern in the use of non-absorbable threads for aesthetic purposes. However, recent studies using PP monofilament suspension thread reported no major side effects at 6 or 12 months[14,27]. Mutaf[39] also reported the PP mesh lifting in the brow did not induce a foreign-body reaction during the 6 months to 4 years of follow-up. A study of 350 patients who underwent an Aptos procedure using PP barbed monofilament also reported no major complication over a 43-month period[12].

Overall, compared to PDO barbed thread, PP monofilament mesh suspension thread resulted in better collagen deposition while affording comparable skin tensile strength. The PP monofilament mesh suspension thread was also well preserved and remained at the loading site, where at 28 days it was associated with a less inflammatory response than seen at sites loaded with PDO barbed thread. Thus, PP monofilament mesh suspension thread may be a safe and effective thread-lifting material for the treatment of aging skin.

This study had several limitations, including the small number of animals in each group and the relatively short follow-up period. In addition, because we compared PP monofilament mesh suspension with PDO barbed thread rather than traditional PP thread, whether the difference in efficacy and safety were due to the PP material itself or to the newly developed implantable mesh could not be

determined. Further work is needed to evaluate the longer-term efficacy and safety of PP monofilament mesh suspensions in human facial skin.

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CONFLICTS OF INTEREST

The authors have nothing to disclose.

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