Novel Polydioxanone Multifilament Scaffold Device for Tissue Regeneration

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BACKGROUND Facial aging is the result of intrinsic and extrinsic factors that lead to gradual reduction of dermal extracellular components and skin elasticity and wrinkle formation. A novel stent-shaped biodegradable and biocompatible scaffold device braided with absorbable polydioxanone (PDO) multifilaments was recently marketed for tissue suturing and augmentation.

OBJECTIVE To explore tissue regeneration profiles following implantation of the stent-shaped hollow scaffold in rats and mini-pigs.

MATERIALS AND METHODS The scaffold device was implanted under the panniculus carnosus of rat dorsal skin and in the subcutaneous layer of mini-pig dorsal skin. Tissue samples were harvested and histologically evaluated after 3 days and 1, 2, 4, and 12 weeks for rats and after 1, 2, 4, 8, and 12 weeks for mini-pigs.

RESULTS Type III collagen was slowly replaced by Type I collagen in the scaffold. Cells from the surrounding tissue infiltrated the hollow space of the scaffold, which induced de novo tissue regeneration in this space.

CONCLUSION The novel stent-shaped scaffold used here may be useful for stimulated tissue remodeling of aged skin, collagen synthesis, and partial restoration of dermal matrix components. The cosmetic purpose of this novel soft tissue augmentation device should be clinically investigated in long-term studies.

The authors have indicated no significant interest with commercial supporters.

As humans age, wrinkles on the face or body deepen and make people look older than they are chronologically. Fine and deep wrinkles have many intrinsic and extrinsic causes,[1] including lifestyle habits and behaviors, such as tanning, smoking, and sleeping positions, which can increase the risk of premature skin aging.

Several methods are available for non-surgical correction of wrinkles. Botulinum toxin Type A or B injections are one of the most common and effective approaches for wrinkle correction. However, it is limited by incomplete alleviation of wrinkles caused by underlying muscle contraction and its short-term (3–5 months) effects. Use of hyaluronic acid-based injectable fillers is another safe and effective method for correction of wrinkles caused by loss of collagen and skin elasticity, but like botulinum toxin injections, its relatively short-term efficacy (12–18 months) is a limitation. Additionally, the hyaluronic acid tends to migrate toward the direction of muscle contraction after injection.[2]

Polydioxanone (PDO) is a reabsorbable polymer that remains in position for a considerable time (approximately 180–230 days).[3] Biodegradable PDO has been used mainly in laparotomy sutures and esophageal stents, but more recently, PDO monofilaments have been implanted to cosmetically enhance the skin.[4] Cosmetic PDO monofilaments can be used for minimally invasive lifting of various types of wrinkles on the neck and face, including sagging

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brows, malar folds, and noticeable nasolabial and marionette folds. Absorption of the PDO threads accelerates the production of new dermal matrix.

Recently, we successfully developed a novel tissue regeneration system comprising a stent-shaped hollow scaffold device braided with multiple PDO filaments that remain secure after implantation into the subcutaneous layer. This stent-shaped structure, which is designed to lift wrinkles, creates a hollow space in which newly formed collagen can accumulate, in addition to collagen deposition on the exterior of the implant. In the present study, we explored the in vivo efficacy of this new device in rats and mini-pigs.

Materials and Methods

Morphology

The absorbable stent-shaped hollow multifilament device used in this study was originally designed and manufactured by Metabio Med (Osong, South Korea) and is marketed as Retense (Aestura; Seoul, South Korea). This scaffold comprises a thin, long mesh tube that penetrates the subcutaneous tissue and through-holes that guide tissue cells surrounding the scaffold into the hollow portion to form fibrous tissue. The through-holes provide lengthwise conduits from the outer surface to the hollow portion of the mesh tube.

The structural features of the device measured using electron microscopy (Figure 1) included the outer diameter, inner diameter of the hollow portion, and through-hole diameter.

Rat Model

Twelve female Sprague-Dawley rats, each weighing 400 g, were purchased from Orient Bio (Gyunggi-do, South Korea). A scaffold was nonsurgically implanted under the panniculus carnosus of the dorsal skin of each rat under isoflurane anesthesia. The distance between the entry and exit points of the scaffold was 50 mm. Three rats were sacrificed at different time points (3 days and 1, 2, 4, and 12 weeks) after scaffold implantation. Skin biopsy specimens, including the scaffold and surrounding tissue, were obtained and subjected to histologic evaluation. Samples were fixed in 10% formalin, embedded in paraffin, processed routinely, sectioned transversely to the thread axis at 4-μm thickness, and stained with hematoxylin and eosin. Replicate sections were stained with Masson's trichrome to examine all types of collagen and Herovici stain to differentiate Types I (purple) and III (blue) collagen. All experimental procedures and protocols were approved by and performed in accordance with the Institutional Animal Care and Use Committee of Aestura Corporation.

Mini-Pig Model

A single 5-month-old female PWG mini-pig (Medi Kinetics, Pyeongtaek, South Korea) weighing approximately 20 kg was used in this part of the experiment.

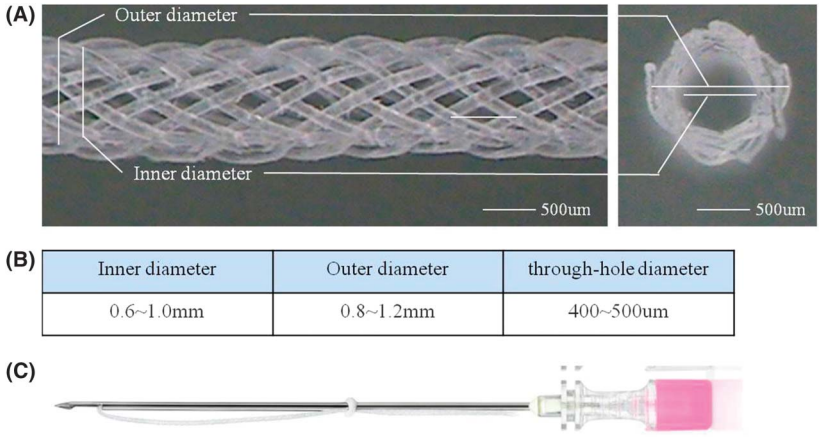

Figure 1. Structure of the stent-shaped hollow scaffold device. (A) Electron microscopic image of the stent-shaped hollow scaffold device. Bar indicates 500 μm. (B) Braided morphological pattern of the scaffold device. (C) Implantation of the scaffold device.

Scaffolds were implanted subcutaneously in the dorsal skin. The distance between the entry and exit points of the scaffold was 50 mm. Skin biopsy specimens were collected at 1, 2, 4, 8, and 12 weeks prior to sacrifice and processed as described above for rats.

Histologic Analysis

Histopathologic analysis was performed using a BX53 calibrated microscope, equipped with a DP72 digital camera and cellSens imaging software (Olympus, Tokyo, Japan). Borders were manually traced for the area of newly generated tissue. Histomorphometric analysis for a given scaffold was conducted by calculating the measured area of collagen neogenesis minus the thread area. Measurements were made on 5 cross-sections, including the proximal and distal ends and the midpoints of each implantation site.

Results

Scaffold Device Measurements

The outer diameter of the scaffold device was approximately 0.8 to 1.2 mm; the inner diameter of the hollow portion was 0.6 to 1.0 mm, and the through-hole diameter ranged between 400 and 500 μm.

Changes in Histologic Findings After Implantation of the Multifilament PDO Scaffold in Rats

On day 3 postimplantation, the structure of the scaffold was almost unchanged. No significant newly formed tissue was noted, but some fibrin and inflammatory cells were seen inside the scaffold. Further, a small amount of Type III collagen was noted around each thread. At 1 week postimplantation, fibroblasts with high cellularity and a moderate amount of collagen (mostly Type III) were observed inside the scaffold. The inner area of the scaffold was 5 times larger than the area on day 3, and the space between threads had widened. After 2 weeks, the collagen density had increased but fibroblast cellularity had decreased. The inner area of the scaffold was 40% less than that at 1 week but 3 times larger than that on day 3 post-implantation. Both Types I and III collagen were observed, and many blood microvessels were seen inside the scaffold with accumulation of newly formed tissue. At 4 weeks postimplantation, the number of fibroblasts had decreased and the mature collagen bundle was thick and organized. The ratio of Type I to Type III collagen had increased, with the central regenerated area comprising mostly Type I collagen and the periphery comprising mostly Type III collagen. The number of microvessels had decreased from that at 2 weeks. After 12 weeks, collagen fibers had formed a dense structure and the amount of Type I collagen in the scaffold had increased significantly. Detailed histologic results are presented in Figure 2.

Changes in Histologic Findings After Implantation of the Multifilament PDO Scaffold in Mini-Pig

At 1 week postimplantation, thick layers of infiltrates comprising inflammatory cells and fibroblasts were present around the scaffold, but no particular structure was seen inside the scaffold. At 2 weeks, the collagen that filled up the interior of the scaffold was entirely of Type III, and fibroblast cellularity seemed low. At 4 weeks, the proportion of Type I collagen had increased while that of Type III collagen had decreased inside the scaffold. Then, at 8 weeks postimplantation, blood microvessels were seen inside the scaffold, and the number of fibroblasts had decreased. Further, the collagen bundles were thicker and more organized than before. The ratio of Type I to Type III collagen had increased, with the central area comprising Type I collagen and the peripheral single filament comprising Type III collagen. Twelve weeks after implantation, the collagen fibers formed a dense structure, accompanied by phenotypic replacement of Type III collagen by Type I collagen. Additionally, stent degradation was observed within the irregularly shaped scaffold structure. Detailed histologic results are presented in Figure 3.

Discussion

Cutaneous aging is a process caused by intrinsic and extrinsic factors associated with many pathologic changes, including gradual reduction of dermal extracellular components, dermal cell functions, and skin elasticity. All these processes ultimately lead to facial ptosis and wrinkles. During the last decade,

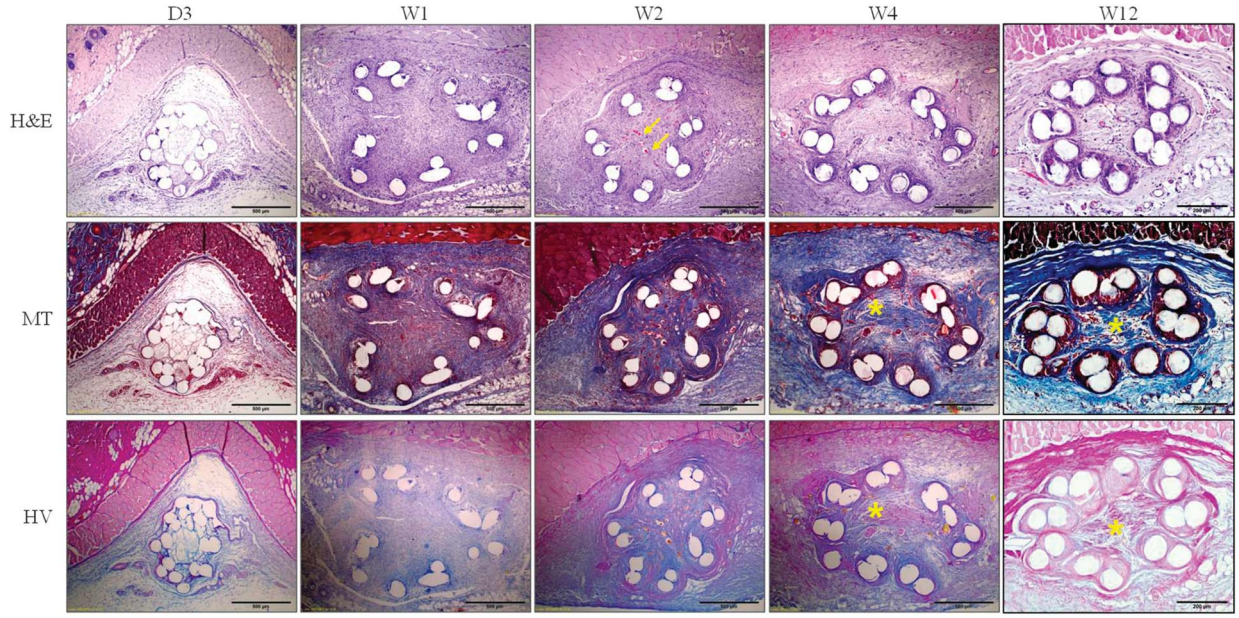

Figure 2. Serial histologic changes in the tissue surrounding the scaffold embedded in the rat dorsal skin. Formation of micro blood vessels (arrow) and bundles of collagen (asterisk) can be seen. Replicate sections of the samples were used for Masson trichrome (MT) staining for all types of collagen and Herovici (HV) staining to differentiate Types I (purple) and III (blue) collagen.

various dermal implants and cosmetic procedures have been developed for wrinkle removal and skin rejuvenation. These include plastic surgical approaches (such as facelifts) as well as nonsurgical and minimally invasive methods (such as botulinum toxin injection, laser therapy, and implantation of lifting threads and soft tissue augmentation fillers).[5–7] These methods are designed to restore diminished skin volume and encourage tissue regeneration via *de novo* collagen synthesis.

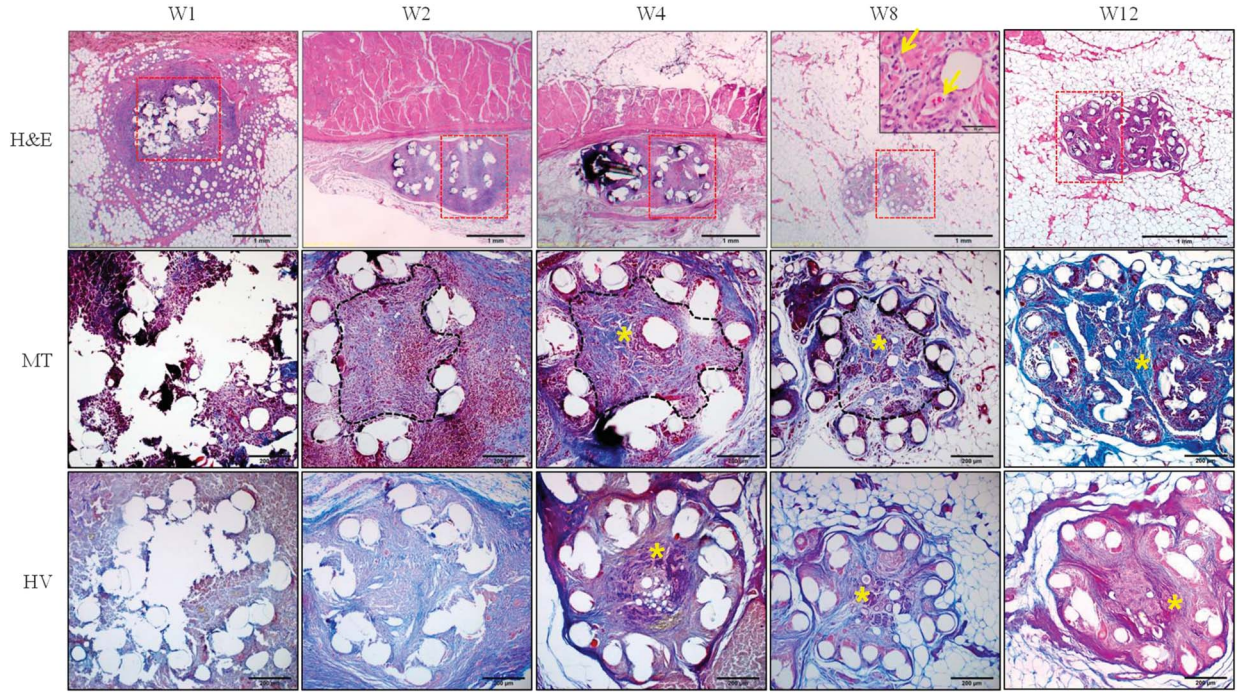

Figure 3. Serial histologic changes in the tissue surrounding the scaffold device implanted in mini-pig dorsal skin, showing formation of micro blood vessels (arrow) and bundles of collagen (asterisk) and an area of newly generated fibrous connective tissue (dotted line). Insets in red squares show MT and HV staining.

Ideal dermal implants should resist physical force and be chemically inert and biocompatible.[8,9] They should also be nonallergenic, nonimmunogenic, noncarcinogenic, nonpyrogenic, and noninflammatory. Metals, ceramics, and polymers have been used as implants in humans. The novel stent-shaped hollow scaffold used in the present study is composed of PDO monofilaments, which are stable and easy to process and induce only mild adverse reactions. PDO is a colorless, crystalline, bioabsorbable polymer that was developed specifically for wound closure sutures. It is derived from the monomer paradioxanone after ring-opening polymerization with heat and application of organometallic catalysts like zirconium acetylacetone, diethyl zinc, and zinc L-lactate ($ZnLac_2$) resulting in a poly(ether–ester).[10] It is typically manufactured as monofilaments for suturing purposes. As an absorbable suture, it has higher flexibility (irrespective of the diameter) and tensile strength, slow absorption rates, and lower inflammatory response rates than both polyglycolic acid (Vicryl; Ethicon, Somerville, NJ) and polylactic acid (Dexon; Covidien, Dublin, Ireland).[11,12] In vivo, PDO is slowly hydrolyzed to 2-hydroxyethoxyacetic monomer, the majority of which is excreted in urine with the rest being eliminated by digestion or exhaled as carbon dioxide in 6 to 8 months.[8]

Type I collagen is the main fibrillar protein responsible for the mechanical properties of newly formed tissue. Although Type III collagen is also a fibrillar collagen found in extensible connective tissues, it is mostly related to fast-growing tissues, particularly in the early stages of tissue regeneration. During remodeling, Type III collagen is replaced by the stronger and tougher Type I collagen, which provides the required strength to support load. In the present study, the ratio of Type I to Type III collagen gradually increased with time, in both the rat and mini-pig models, with Type I collagen eventually replacing Type III collagen entirely. Additionally, abundant newly formed fibrous tissue, including collagen fibers, accumulated inside the scaffold through the surface holes of its mesh-like structure. These findings indicate that the stent-shaped multifilament PDO scaffold used here may sufficiently support and accelerate fibroblast infiltration from the surrounding tissue and induce tissue regeneration in the scaffold interior. Further, the intrinsic mechanical properties of our novel stent-shaped hollow PDO scaffold include recoil force, that is, the scaffold is not deformed by any external force applied to the skin after it is inserted into the subcutaneous layer.

On the basis of our findings, we believe that this novel PDO scaffold could be applicable for various kinds of wrinkles, including deep furrows, by adjusting the diameters of the scaffold, hollow portion, and through-holes. Further, the wrinkle-diminishing effect of this scaffold may last semipermanently because it enables de novo formation of fibrous tissue. However, clinical studies are required to confirm the safety and long-term efficacy of this scaffold for wrinkle correction.

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