level. However, in their study, PDO was inserted into the panniculus carnosus layer. Generally, the main insertion area for PDO thread during the lifting of the face using an absorbable thread is the subcutaneous fat layer or the SMAS layer. Amuso et al[5] performed a histological evaluation of bio-revitalization after PDO injection through human tissue biopsy. Upon collagen formation activation, the collagen fibers of the dermis thickened. At the same time, elastic punctiform fibers were prolonged. These changes in collagen persisted for up to 12 months, but the collagen returned to its original state at 18 months.

The above histological studies have shown that certain tissue changes occur in the skin and subcutaneous tissue after PDO injection. However, there is insufficient evidence to explain the positive changes that have been shown in clinical studies after PDO thread injection.

To address this research gap, animal experiments were performed to determine the causes of tissue changes, physiological responses, and the positive results after PDO thread injection.

2 | MATERIALS AND METHODS

2.1 | Experimental animal

Four White Yucatan Pigs (3 months old) were selected for the study of the histological changes after the insertion of the threads. The skin of the Yucatan pig is structurally similar to human skin and is a useful tissue for skin studies. Yucatan pigs were prepared, bred, and euthanized by the Optipharm (Cheongju, Korea), which has a specific pathogen-free facility and laboratory. The animal IRB number is MPK-2012-001.

2.2 | Experimental process

After the administration of Zoletil anesthesia via intramuscular injection in the sterile laboratory, the dorsal skin of pig was disinfected with alcohol. In the dorsal skin, 9-cm USP 4-0 non-barbed PDO threads in a 25-G needle were inserted into the subcutaneous layer parallel to the skin at 1-cm intervals. The insertion sites of PDO threads were tattooed on the skin to facilitate subsequent biopsy. The same procedure was performed in a total of four Yucatan pigs. Four weeks after the thread insertion procedure, the skin and subcutaneous tissue were removed under Zoletil anesthesia. Immediately after removal, the center section of the thread insertion area was taken. The sample was flattened and fixed onto a Styrofoam plate to prevent curling of the tissue using pins. The prepared sample was fixated using 10% formalin solution. The remaining three pigs underwent the same procedure 12 weeks, 24 weeks, and 12 months after the thread procedure, respectively.

2.3 | Histologic analysis

Paraffin blocks were created using previously fixated (10% formalin) tissue. The tissue was microtomed vertical to the longitudinal axis of the thread. The samples were stained using the hematoxylin and eosin staining, the Masson trichrome staining, and immunohistochemical staining using anti-alpha smooth muscle actin antibody (Abcam, Cambridge UK).

3 | RESULTS

3.1 | After 4 weeks

Upon initial visual inspection of the preserved tissue, there was no visible difference between the control site and the experimental site. The blue color of the PDO thread became transparent at the experimental site, but its physical form was maintained. The first histological finding in the experimental group was the observation of two circular empty spaces resembling owl eyes due to the inserted PDO threads because of the V-shaped appearance of the product, with half of the thread inserted inside the needle and the other half exposed outside of the needle (Figure 1). Second, abundant loose collagen fiber, eosinophils, and lymphocytes were visible inside the newly formed granulation tissue near the thread insertion site (Figure 1). After Masson trichrome staining, newly formed, rich collagen fibers (stained light blue) with concentric circular shapes were observed near the thread insertion site, which were clearly distinguishable from adipocytes, eosinophils, and lymphocytes (Figure 2). Third, the newly formed collagen fibers had connected and merged with the pre-existing fibrous connective tissue (Figure 3). Fourth, the anti-smooth muscle actin (SMA) immunohistochemical stain showed blue-stained fibroblasts and red-stained, cigar-shaped myofibroblasts inside the granulation tissue (Figure 4). Fifth, the capillary gross area was measured to confirm increased blood circulation. To avoid any bias, the capillary with the largest gross area from the control group

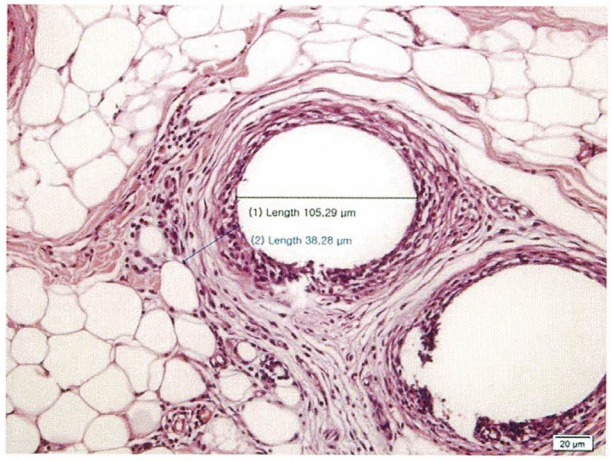

FIGURE 1 Two circular empty spaces resembling owl eyes are observed due to inserted V-shape banded PDO thread. Abundant loose collagen fibers, eosinophils, and lymphocytes are visible inside the newly formed granulation tissue. The degeneration of adipocytes near the granulation tissue is observed (H&E stain, magnification 400×). Four weeks after PDO thread insertion