A 3D printed PCL-collagen hybrid mesh (TissueDerm™) for breast reconstruction after mastectomy in a pig model

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Abstract

Background: Implant supporting materials are currently used in breast reconstruction. However, when used in humans, they are associated with several problems. To address these issues, a new mesh called TissueDerm was created by combining a collagen sponge with a 3D printed polycaprolactone (PCL) mesh. It has shown promising results in pig experiments and could potentially replace the most commonly used acellular dermal matrix (ADM) for breast reconstruction.

Methods: Four 12-month-old minipigs were used in this experiment. Silicone implants were wrapped with ADM or TissueDerm, and the breast tissue was excised and implanted along with the wrapped implants. Three months later, the minipigs were sacrificed and the skin and mammary gland tissue surrounding the implants were harvested for further analysis. Histological analyses and immunostaining were performed.

Results: Although there was no significant difference in capsule thickness between the ADM and TissueDerm groups, collagen was more involved in TissueDerm, leading to better tissue regeneration. TissueDerm also induced lower levels of inflammatory markers TNF-α and IL-6 compared to ADM. However, capsules induced with ADM had significantly higher collagen fiber alignment and alpha-smooth muscle actin (α-SMA) positive immunoreactivity, suggesting that TissueDerm may be less likely to cause spherical contractures in the porcine model compared to ADM.

Methods

1. Preparation of PCL mesh and TissueDerm.
- TissueDerm was supplied by PLCOskin Co., Ltd. (Seoul, Korea).
- The PCL mesh was designed identically to the TissueDerm using the Fusion 360 computer-aided design (CAD) software (Autodesk, San Rafael, CA, USA) and fabricated using 3D printing techniques.

2. Characterization of PCL and TissueDerm.
- Photographs were captured to assess the appearance of the fabricated PCL mesh and TissueDerm. Scanning electron microscopy (SEM) of the collagen sponge, PCL mesh, and TissueDerm was conducted using a Merlin microscope (Merlin Carl Zeiss, Oberkochen, Germany) in high vacuum mode.
- The tensile properties of all specimens during loading were recorded. The load displacement was measured using a 50 kgf load cell at a constant crosshead speed of 100 mm/min.
- For tearing strength, 20mm is cut based on the middle part of one side of PCL mesh, collagen sheet and tissuederm. Set the distance between the two grips to 30 mm and set the test speed to 100 mm/min to measure until the sample is completely torn.
- Suture pull-out strength is measured by passing the surgical suture 15 mm from the end of the sample and winding the suture around the strand. Adjust the tension to be symmetrical so that it is evenly distributed over the entire cross-sectional area. Set the distance between the two grips to 80 mm and the test speed to 100 mm/min. Measure the maximum load at the moment the sample is ruptured.

3. Implantation of TissueDerm-enveloped silicone implant
- Four 12-month-old minipigs were purchased from APURES Co., Ltd. (Pyeongtaek, Republic of Korea).
- 200–250 ml of mammary tissue was excised from two sites on the minipig into and an ADM-enveloped silicone implant and a TissuDerm-enveloped silicone implant were implanted.
- Three months after implantation, the minipigs were sacrificed by KCL injection following anesthesia.

4. Histological analyses
- hematoxylin and eosin (H&E) staining.
- All samples were sectioned by 4 μm thickness after paraffin-embedding.

5. Immunocytochemistry
- Primary antibodies: anti-collagen type 1 (1:200), anti-collagen type 3 primary antibody (1:200), anti-perilipin-1(1:200) and alpha-smooth muscle actin antibody (1:200)
- Secondary antibodies: goat anti-rabbit IgG H&L Alexa Fluor 488 (1:200) and Alexa Fluor 555(1:200)
- Counter staining: DAPI nuclear staining

6. Evaluation of inflammation and microcalcification after implantation
- Primary antibodies: anti-tumor necrosis factor alpha (TNF-α) and mouse anti-interleukin 6 (IL-6) antibody
- VECTASTAIN® Elite® ABC Kit
- Counter staining: hematoxylin nuclear staining
- Alizarin red S staining: 2% Alizarin red S(pH4.2), 10 min

Results

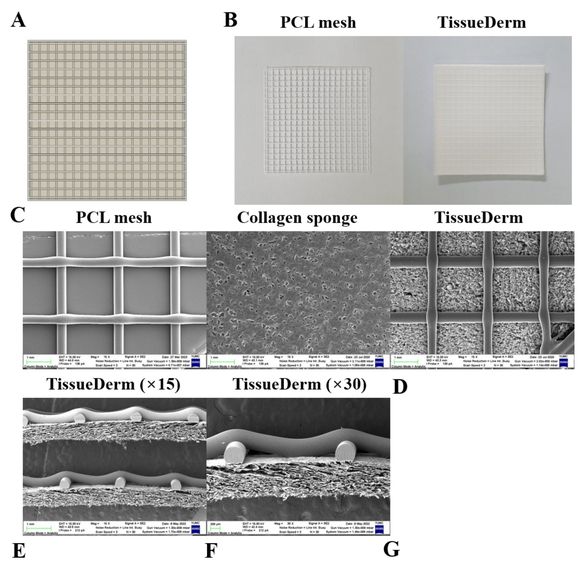

Figure 1. Characterization of PCL mesh and TissueDerm. A CAD designed PCL mesh B Photograph of 3D printed PCL mesh and TissueDerm C SEM image of PCL mesh, Collagen sponge, TissueDerm and Cross sectional TissueDerm. D Tensile strength, E Tear strength, F Bursting strength, and G Suture pull-out strength of PCL mesh, Collagen sponge and TissueDerm.

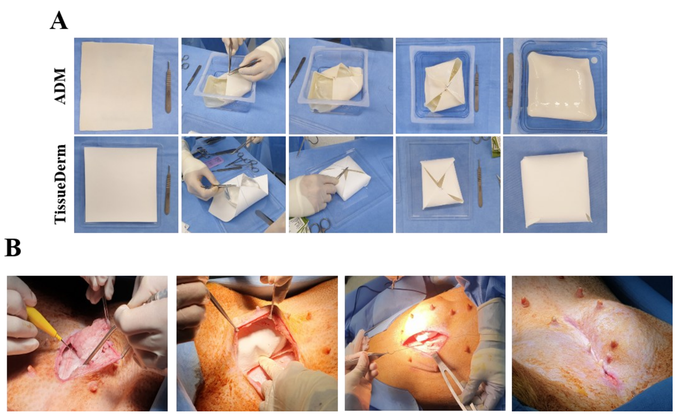

Figure 2. Silicone implantation followed by TissueDerm envelopment in minipig. A Surgical procedure of envelopment of ADM and TissueDerm for silicone implantation. B Silicone implantation after mammary tissue excision in minipig

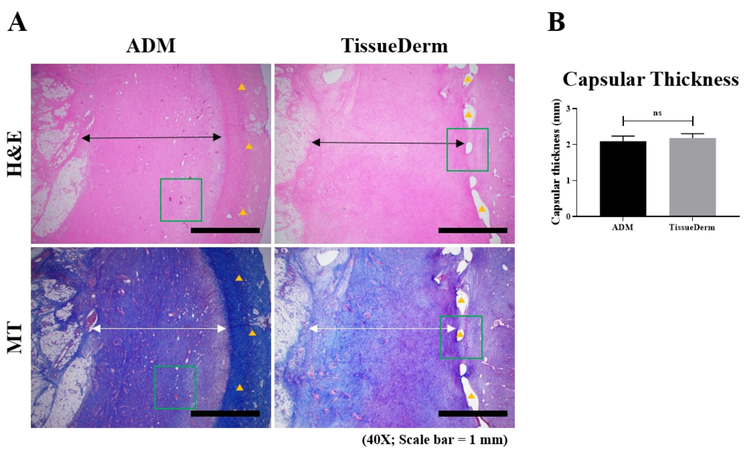

Figure 3. Histological analysis of capsule after silicone implantation followed by ADM and TissueDerm envelopment. A Representative images of the H&E and MT staining images (A; ×40; scale bar = 1 mm; yellow triangle = implant side, black & white arrow = capsule). B Quantitative analysis of capsular thickness (ns; p > 0.05 *; p ≤ 0.05, **; p ≤ 0.01, *; p ≤ 0.001, ****; p ≤ 0.0001).**

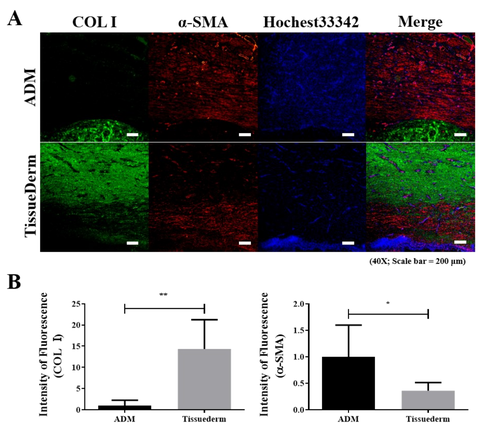

Figure 4. Immunofluorescence analysis of capsule after silicone implantation followed by ADM and TissueDerm envelopment. A Representative immunofluorescence analysis image, enlarged of the green square line part of A, of COL Ⅰ (green), α-SMA (red), and DAPI (blue) in the capsule after silicone implantation followed by ADM and TissueDerm envelopment (B; ×40; scale bar = 200 μm). B Quantitative analysis of expression of COL Ⅰ and α-SMA in the capsule after silicone implantation followed by ADM and TissueDerm envelopment (ns; p > 0.05 *; p ≤ 0.05, **; p ≤ 0.01, *; p ≤ 0.001, ****; p ≤ 0.0001).**

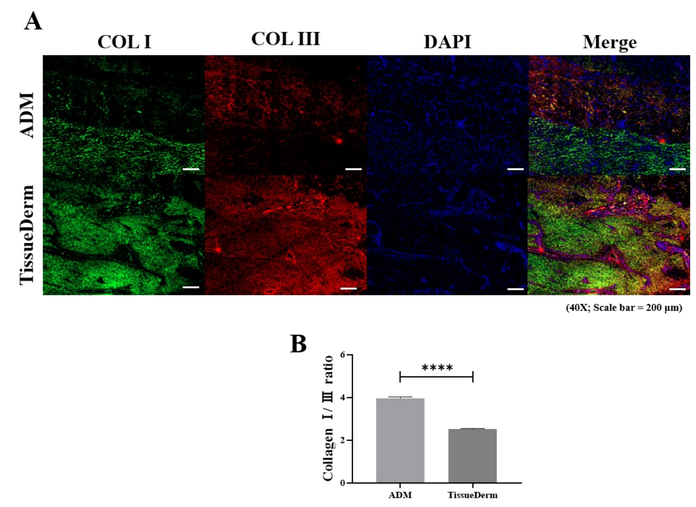

Figure 4. Immunofluorescence analysis confirms the distribution of collagen types I and III. A Representative immunofluorescence analysis image of COL Ⅰ (green), COL III (red), and DAPI (blue) in the capsule after silicone implantation followed by ADM and TissueDerm envelopment (A; ×40; scale bar = 200 μm). C COL Ⅰ/III rato of the dermis surrounding implanted tissue in each group (ns; p > 0.05 *; p ≤ 0.05, **; p ≤ 0.01, *; p ≤ 0.001, ****; p ≤ 0.0001).**

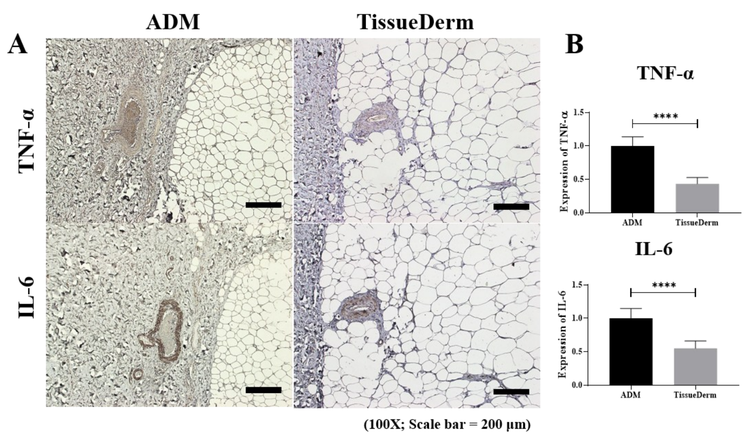

Figure 5. Immunohistochemical (IHC) analysis of dermis after silicone implantation followed by ADM and TissueDerm envelopment. A Representative image of IHC staining of TNF-α and IL-6 (A; ×100; scale bar =200 μm). B Representative image, enlarged of the green square line part of A. B Quantitative analysis of IHC for TNF-α and IL-6 in each group after normalization from ADM (ns; p > 0.05 *; p ≤ 0.05, **; p ≤ 0.01, *; p ≤ 0.001, ****; p ≤ 0.0001).**

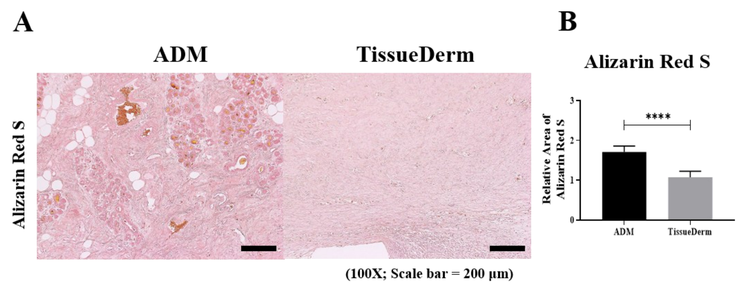

Figure 6. Alizarin red S staining of dermis after silicone implantation followed by ADM and TissueDerm envelopment. A Microcalcification analysis image in each group by Alizarin red S staining (A; ×100; scale bar = 200 μm). B Quantitative analysis of microcalcification area in each group (ns; p > 0.05 *; p ≤ 0.05, **; p ≤ 0.01, *; p ≤ 0.001, ****; p ≤ 0.0001).**

Conclusion

The study found that TissueDerm has advantages over ADM in terms of easier tissue invasion and reduced spheroidization in a porcine model. The results showed that TissueDerm is a promising new mesh for implant-based breast reconstruction (IBBR) and could potentially replace ADM.

Acknowledgements

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