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# Comparison of contraction among three dermal substitutes: Morphological differences in scaffolds

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ABSTRACT

Various kinds of synthetic dermal substitutes are produced and used in clinical application and contribute to wound bed preparation for subsequent skin grafting. Although their appearance and outcomes are different, the criteria for the selection of proper synthetic dermal substitutes is not well defined yet. In this study, we focused on the contraction of dermal substitutes and compared the morphological differences in scaffolds. A marked contraction was observed with Pelnac® compared to Integra® and Terudermis® in vitro. We also showed that the pore size of Pelnac® was smaller than that of Integra® and Terudermis®. The shape of the pore was oval in Pelnac®, whereas those in Integra® and Terudermis® were more circular. Differences in the morphological structure may have affected the contraction of the synthetic dermal substitutes.



## 1. Introduction

Skin grafting is the fundamental and principal procedure for large wound closure. Although good graft take leads to better functional and cosmetic outcomes, poor graft take results in ulceration, contraction, hypertrophic scaring, and pigmentation. Because wound bed preparation is imperative for good graft take, clinicians have invested a lot of effort in preparing an optimal graft bed with well-vascularized solid granulation tissue. Tissue-engineered skin bioconstructs were originally produced to resolve the limitation of donor site in extensively large burn wounds and have provided better quality of healing in recipient site by protecting wound surface and producing extracellular matrix. They reduced contraction and scar formation with improvement of extracellular matrix remodeling and elastin regeneration [1,2]. Many kinds of tissue-engineered skin bioconstructs have been developed and are commercially available these days. They are anatomically and structurally classified as dermo-epidermal skin substitutes, epidermal substitutes, and dermal substitutes. Dermal substitutes are major skin substitutes that are relatively easy to produce in large quantities with low cost, easy to store, and easy to handle. They are further classified as acellular and synthetic. Synthetic materials offer a lesser risk of human viral diseases transmission. It is much easier to obtain license for their clinical application, and they are the only approved materials for skin grafting in Japan. We have been using three different synthetic dermal substitutes for many kinds of wounds such as traumatic skin defect, skin defect after

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excision of tumor or nevus, skin defect after contracture release, and full-thickness injury. Although all these wounds look different, there is no concrete way to select dermal substitutes properly yet. We focused on the differences in contraction, which sometimes afflicts patients after skin grafting, and attempted to find some differences in vitro.

## 2. Materials and methods

The study was approved by the Ethic Committee of Tokyo Women's Medical University.

### 2.1. Artificial dermis

Three synthetic xenogeneic artificial dermis, which are approved by the Japanese Pharmaceutical Affairs Law and clinically available in Japan, were used for comparison:

- Integra® Dermal Regeneration Template (Integra® Life-Sciences Corp., Plainsboro, NJ, USA) consists of bovine tendon type 1 collagen and shark chondroitin-6-sulphate glycosaminoglycan that is bonded to a silicone pseudoepidermis [3].
- Terudermis® (Olympus Terumo Biomaterials Corp., Tokyo, Japan) is a lyophilized collagen sponge reconstituted form heat-denatured bovine dermal type 1 collagen that is crosslinked by dehydrothermal treatment [4].
- Pelnac® (Gunze Medical Materials Center, Kyoto, Japan) is a lyophilized collagen sponge that consists of pig tendon atelocollagen and is chemically crosslinked to prevent contraction by inhibiting degradation.

### 2.2. Culture of human fibroblasts

Skin surgical residues were collected from healthy volunteers who were operated for benign skin tumor or polydactyly at the department of plastic surgery. Fibroblasts were isolated and cultured from the skin as previously described with some modifications [4]. Briefly, the harvested skin was immersed in Dulbecco's Modified Eagle Medium (DMEM, low glucose; ThermoFisher scientific, Waltham, MA, USA) containing dispase (1000 U/ml) overnight at 4 °C. Then, the epidermis was separated from the dermis, and the dermis was cut into small pieces. The dermis cells were subjected to explant culture in DMEM with 10% fetal bovine serum (GibcoBRL). Cultures were grown in an incubator maintained at 37 °C with 5% $CO_2$. The obtained cultured cells were re-suspended in a cryoprotective medium consisting of RPMI medium 1640 (GibcoBRL), 10% fetal bovine serum (GibcoBRL), and 10% glycerol at a concentration of $1 \times 10^5$ cells/ml and stored at −150 °C until use.

Cells at third or fourth passage were used for experiments.

### 2.3. Artificial dermis contraction assay

Three different kinds of artificial dermis were prepared as follows: the silicone sheet was gently removed from the collagen layer of Integra®, and a single-layered collagen sponge that does not bond with the silicone sheet was used for Terudermis® and Pelnac®. Each artificial dermis was cut into a circle of 30-mm diameter.

Fibroblast-populated and nonpopulated groups were established for each artificial dermis, six groups in total. Fibroblasts suspended in medium were populated on each artificial dermis as previously described, with some modifications [4,5]. Briefly, each disk of the artificial dermis was placed in 6-well plate and covered with a ring. Fibroblast suspended in medium, $1.75 \times 10^5$ cells/ml, were populated inside the ring and incubated at 37 °C with 5% $CO_2$ overnight. Sterile medium was added instead of fibroblast suspension for nonpopulated groups as control. Subsequently, the ring was removed, and 3 ml of DMEM with 10% fetal bovine serum was added and incubation was continued. Photographs were obtained at day 3, 7, 14, 21, and 35, and the contraction was quantified by measuring the size of the artificial dermis using digital planimetry (Image J, NIH). Contraction of the artificial dermis was compared with the percent original area.

### 2.4. Histological analysis

The cultured artificial dermis of days 0 and 35 were fixed in 10% formalin and embedded in paraffin, and sections (5 μm thick) were stained with hematoxylin and eosin (H&E). For the staining of myofibroblast α-smooth muscle actin (α-SMA), sections were incubated overnight with monoclonal mouse anti-human SMA at 4 °C (DaKo, Glostrup, Denmark) and then visualized with EnVision DualLink (DaKo). H&E sections were viewed under a microscope, and photographs were obtained for digital planimetry (Image J, NIH) to quantify the pore size, lengths of long and short axes, and ratio of long to short axis. Measurements were obtained in five high-power light microscope fields for each sample. Morphological changes in the collagen scaffold were also observed.

### 2.5. Statistical analysis

Experiments were performed in quintuple for each group. Data are expressed as mean ± SD. Statistical analysis of the data was performed using ANOVA and Tukey's honestly significant difference test. $^*P < 0.05$, $^{**}P < 0.001$.

## 3. Results

### 3.1. Contraction of fibroblast-nonpopulated artificial dermis

We observed the differences in contraction by measuring the size of the artificial dermis. Pelnac® showed significant contraction compared to Integra® and Terudermis® from day 7 and became 47.4 ± 7.1% of the original area at day 28, after which the contraction plateaued. Integra® and Terudermis® did not show any contraction throughout the experiment (Fig. 1).

### 3.2. Contraction of fibroblast-populated artificial dermis

We observed drastic contraction with fibroblast-populated Pelnac®, which showed significant differences compared to

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