



Artificial dermal substitutes for tissue regeneration: comparison of the clinical outcomes and histological findings of two templates

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Abstract

Objective: Artificial dermal substitutes (DSs) are fundamental in physiological wound healing to ensure consistent and enduring wound closure and provide a suitable scaffold to repair tissue. We compared the clinical and histological features of two DSs, Pelnac and Integra, in the treatment of traumatic and iatrogenic skin defects.

Methods: This prospective observational study involved 71 randomly selected patients from our hospital. Wound healing was analyzed using the Wound Surface Area Assessment, the Vancouver Scar Scale, and a visual analog scale. Histological and immunohistochemical evaluations were also performed.

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Results: At 2 weeks, greater regeneration with respect to proliferation of the epidermis and renewal of the dermis was observed with Pelnac than with Integra. At 4 weeks, the dermis had regenerated with both DSs. Both templates induced renewed collagen and revascularization. Differences in the Vancouver Scar Scale score were statistically significant at 4 weeks and 1 year. Pelnac produced a significant increase in contraction at 2 weeks with increasing effectiveness at 4 weeks. Integra produced a higher percentage reduction in the wound surface area and a shorter healing time than Pelnac for wounds >1.5 cm deep.

Conclusion: Our observational data indicate that both DSs are effective and applicable in different clinical contexts.

Keywords

Dermal substitutes, skin substitutes, Integra, Pelnac, tissue regeneration, clinical study, histological study

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Introduction

The skin is an important organ in the defense against microorganisms, conservation of body temperature, and detection of external sensory stimuli. Acute trauma, chronic wounds, or surgical interventions may cause skin loss and interfere with epithelial regeneration by inducing full-thickness wounds; skin recovery then mainly occurs from the wound edge with poor cosmetic or functional outcomes.^{1,2} The healing process involves three stages of achieving tissue integrity and homeostasis: inflammation, tissue formation, and tissue remodeling.³ Wound healing and tissue generation in dermal injuries involve functional and cosmetic processes that are akin to the mechanisms occurring in unwounded dermis processes with a complex organization among multiple types of cells, bioactive molecules, and extracellular matrix (ECM) components.⁴ Notwithstanding the potential of full-thickness skin wounds exceeding 1 cm in diameter to self-epithelialize, adequate wound care is certainly fundamental in the

form of moist dressings, infection control, and offloading or compression bandaging, depending on the wound type. Moreover, the wound healing process may result in substantial scarring with subsequent limited mobility and significant deformation, thus requiring full-thickness skin grafting.^{5,6} Plastic surgeons may use bioengineering replacement techniques, free flaps, and autologous skin grafts to cover such injuries. Skin grafting is certainly efficient and widely used to close large wounds as well as to repair a variety of acute and chronic wound discrepancies. However, insufficient availability of healthy skin and clinical complications related to donor-site deformation and morbidity are important consequences of skin grafting. Successful grafting will undoubtedly offer satisfactory outcomes regarding functionality and cosmesis, but poor grafting may result in the formation of ulcers, contractions, hypertrophy, and pigmentation. Encouraging early results have spurred researchers to widely investigate adequate graft beds containing well-vascularized solid granulation tissue.

Scientists and surgeons have recently considered various bioengineering and synthetic approaches to regenerate skin injuries. Tissue-engineered skin bio-constructs have been confirmed as valid options to manage donor-site anomalies in extensive burn injuries as well as protect the wound surface and generate ECM, thus providing efficient healing potential in the recipient site. Skin grafts contribute to decreasing contraction and scar formation by eliciting ECM remodeling and regeneration.^{7,8} Numerous tissue-engineered skin bio-constructs are currently accessible as dermoepidermal, epidermal, and dermal replacements.^{9–11} Dermal substitutes (DSs) are important skin replacements and play a major role in repairing full-thickness skin defects, improving long-term functional and cosmetic outcomes, and providing satisfactory scar quality.¹² DSs are mass-producible, effective, and easily stored and handled; thus, they may be considered optimal skin replacements. These substitutes can be further divided into acellular and synthetic materials, with the latter offering a lower risk of human viral disease contagion. Some DSs, such as Integra (Integra LifeSciences, Princeton, NJ, USA) and Pelnac (Gunze Corp., Osaka, Japan), have a three-dimensional collagen structure and contain a superficial silicone layer placed within the defect area that protects against dehydration, microorganisms, and toxins. The collagen layer slowly integrates into the wound, and natural recovery processes are promoted via localized inflammation; infiltration of neutrophils, macrophages, fibroblasts, and keratinocytes; and neovascularization of the scaffold.¹³ Differences in DSs are likely to affect multiple wound-healing mechanisms, including fibroplasia, because the ECM provides porosity, elasticity, and biocompatibility and may strongly influence cellular migration, proliferation, and differentiation during recovery.^{14–16} DSs physically differ based on the specific

manufacturing methods used.¹⁷ Biomaterials have been extensively investigated for use in skin replacement, and acellular DSs are fundamental to prepare the wound bed and enhance graft integration with the resultant recipient surface. Moreover, DSs are able to repair full-thickness cutaneous wounds and elicit effective wound healing,¹⁸ and they are recommended in reconstructive surgery^{19–22} because they provide adequate scaffolding to interpose fibroblasts and endothelial cells toward neodermis formation.^{23–26}

We conducted a randomized study to compare Pelnac and Integra, the two above-mentioned collagen acellular bilayer substitutes used to treat a variety of skin wounds. We have experience with these synthetic DSs in the treatment of various skin defects such as those caused by trauma-related injuries, tumor removal or nevus excision, contracture release, and full-thickness wounds. Although no specific DSs have yet been defined for different types of wounds, we performed the present study to investigate various post-treatment skin graft contractions affecting patients and herein report our *in vitro* analysis results. This study was performed to compare Pelnac and Integra from clinical, histological, and immunohistochemical perspectives for a period of 1 year.

Materials and methods

Patients

This prospective observational study was conducted for a 1-year period and involved patients presenting with partial- and full-thickness post-traumatic wounds and post-iatrogenic cutaneous defects. The investigation was performed in compliance with the Declaration of Helsinki and the Guidelines for Good Clinical Practice. All participants provided written informed consent to undergo surgery, follow-up, and biopsies.

The inclusion criteria were Caucasian patients of either sex, age of >18 to <85 years, wounds with surface dimensions of 2×1 to 35×20 cm, negative culture swab before surgery, maximum wound depth of 2.5 cm, negative traditional markers of systemic inflammation (C-reactive protein, erythrocyte sedimentation rate, and procalcitonin), post-traumatic or iatrogenic wounds, and wounds presenting anywhere on the entire exterior surface of the body with the exception of the face and neck.

The exclusion criteria were chronic liver disease, coagulopathy and/or anticoagulant therapy, treatment with immunosuppressive drugs and corticosteroids, smoking habit, type 1 and 2 diabetes mellitus, oncological disease in progression or remission, immunodeficiency (congenital, acquired, or metabolic), loss to follow-up, and incomplete medical/nursing records.

Following enrollment, the patients were randomly assigned to two groups (those treated with Pelnac and those treated with Integra) (Table 1(a), (b)) on a computer-generated list open to only one investigator who was blinded to assessment and treatment. Allocation was occasionally revealed to surgeons but on no occasion to assessors.

When the DS stimulated dermal growth to approximately the epidermal level, the participants in both groups underwent subsequent autologous dermoepidermal grafting. We assessed the wound contracture with respect to wound depth. Several patients in each group who had deep wounds (>1.5 cm) did not undergo autografting so that we could evaluate the different wound contraction capacities with respect to the depth of the lesion.

DSs

Integra is widely used in the treatment of full-thickness injuries. It consists of a bovine type 1 collagen bilayer attached to shark chondroitin 6-sulfate glycosaminoglycan

bonded to a temporary epidermal substitute layer of pseudoepidermis in silicone. Pelnac is a bilaminar membrane with a superficial silicone film layer and a porcine collagen sponge layer derived from pig tendon with a pore diameter ranging from 60 to 110 μm . Details are provided in Table 2.

Clinical and surgical protocols

We assessed all patients for comorbidities and examined their wounds according to our protocol, which involved a swab culture, instrumental evaluation, and photographs. Instrumental laser Doppler measurements for vascular pathology were not performed. A short-term follow-up was performed at 2 and 4 weeks after DS implantation, and a long-term follow-up was performed at 1 year. The silicone layer was removed after 2 weeks following Pelnac implantation and after 4 weeks following Integra implantation as suggested by the manufacturers. In both groups, the dermoepidermal grafts were applied within 1 week after silicone removal. During follow-up, photographs were taken before and after application of the dermoepidermal grafts. We treated traumatic wounds by preparing the wound bed via debridement, bacterial balance, and exudate management. A swab culture was performed to check for the presence of microbiological infections and identify relevant antibiotic treatment if required. DS implants require an effective wound bed site to ensure optimal reception. We conducted these procedures under locoregional anesthesia or sedation in strictly aseptic conditions. We first debrided the damaged tissues and then applied the DS; the subsequent split-thickness dermoepidermal grafts were applied 21 or 30 days later, after removing the silicone layer. In patients with tumors, we performed wide excision of the neoplasia including healthy perilesional tissue and extending to the underlying fascia or

Table I(a). Pelnac group.

Patient	Age (years)	Pathology	Wound localization	Wound area (cm)
1	23	Post-traumatic	Finger	4 × 2
2	56	Iatrogenic	Right shoulder	13 × 9
3	45	Iatrogenic	Forearm	9 × 5
4	40	Iatrogenic	Finger	3 × 1
5	21	Post-traumatic	Right foot	10 × 4
6	60	Post-traumatic	Left leg	15 × 9
7	79	Post-traumatic	Left leg	6 × 4
8	75	Iatrogenic	Forearm	7 × 4
9	35	Post-traumatic	Left thigh	28 × 15
10	38	Iatrogenic	Right foot	9 × 4
11	49	Post-traumatic	Finger	3 × 2
12	57	Iatrogenic	Left hypogastrium	15 × 12
13	51	Post-traumatic	Left leg	14 × 7
14	65	Post-traumatic	Elbow	4 × 4
15	61	Post-traumatic	Finger	2 × 2
16	48	Iatrogenic	Left leg	12 × 9
17	45	Iatrogenic	Right leg	12 × 6
18	83	Post-traumatic	Left leg	6 × 5
19	40	Iatrogenic	Right foot	8 × 7
20	47	Iatrogenic	Hand	7 × 6
21	60	Iatrogenic	Right foot	8 × 5
22	67	Post-traumatic	Forearm	12 × 4
23	58	Iatrogenic	Hand	6 × 6
24	69	Iatrogenic	Chest	13 × 10
25	61	Post-traumatic	Forearm	7 × 4
26	82	Iatrogenic	Scalp	17 × 12
27	35	Post-traumatic	Right ankle	8 × 3
28	67	Iatrogenic	Abdomen	9 × 3
29	66	Iatrogenic	Left ankle	6 × 2
30	28	Iatrogenic	Scalp	7 × 7
31	50	Post-traumatic	Right leg	4 × 3
32	53	Iatrogenic	Left knee	4 × 3
33	76	Iatrogenic	Scalp	5 × 3
34	60	Post-traumatic	Left leg	17 × 7
35	40	Iatrogenic	Right arm	13 × 10

muscle if necessary. We then covered the substance loss with the Pelnac or Integra DS and proceeded with application of split-thickness dermoepidermal grafts 21 or 30 days later, respectively, after removing the silicone layer. We performed classic dermatome skin grafting using thin split-thickness skin grafts, meshing the grafts at a 1:2 ratio. The grafts were applied to the wounds with 3/0 nylon sutures or metallic

staples. The wound was covered with a moulage compressive dressing and sterile gauze.

Clinical criteria evaluation during treatment and follow-up

The clinical assessment was based on the healing time after dermoepidermal graft application, the Wound Surface Area

Table 1(b). Integra group.

Patient	Age (years)	Pathology	Wound localization	Wound area (cm)
1	67	latrogenic	Hand	4 × 4
2	56	latrogenic	Right shoulder	13 × 9
3	86	latrogenic	Abdomen	10 × 6
4	29	Post-traumatic	Finger	4 × 2
5	37	Post-traumatic	Left leg	13 × 6
6	74	latrogenic	Forearm	7 × 3
7	56	latrogenic	Abdomen	10 × 8
8	59	Post-traumatic	Right foot	8 × 6
9	35	Post-traumatic	Abdomen	29 × 14
10	39	Post-traumatic	Finger	2 × 2
11	78	latrogenic	Right leg	12 × 5
12	72	latrogenic	Left thigh	20 × 10
13	65	latrogenic	Hand	5 × 5
14	55	Post-traumatic	Forearm	7 × 4
15	59	latrogenic	Hand	4 × 4
16	51	Post-traumatic	Left leg	9 × 7
17	47	Post-traumatic	Right leg	5 × 3
18	32	Post-traumatic	Right foot	8 × 7
19	45	latrogenic	Finger	2 × 2
20	73	latrogenic	Forearm	12 × 4
21	47	Post-traumatic	Right leg	9 × 6
22	50	Post-traumatic	Right leg	12 × 6
23	61	latrogenic	Hand	5 × 4
24	42	Post-traumatic	Finger	2 × 2
25	40	Post-traumatic	Hand	6 × 6
26	48	Post-traumatic	Left leg	4 × 2
27	45	latrogenic	Trunk	36 × 16
28	55	latrogenic	Left thigh	7 × 4
29	40	Post-traumatic	Right foot	4 × 2
30	19	Post-traumatic	Right leg	8 × 3
31	51	Post-traumatic	Right foot	10 × 9
32	42	Post-traumatic	Foot	10 × 20
33	83	latrogenic	Scalp	9 × 10
34	48	latrogenic	Forearm	12 × 7
35	65	Post-traumatic	Left leg	6 × 5
36	55	Post-traumatic	Left leg	9 × 4

Assessment, the Vancouver Scar Scale (VSS), and a visual analog scale (VAS) for pain. Moreover, the color shift of the collagen layer in both DSs was analyzed to examine the graft and collagen maturation, appropriate disposition of the DS, and adequate reception of the dermoepidermal grafting. In all patients, we monitored the wound surface for contraction at 1 week

and every week until complete healing. We traced the wound edges via digital photography. Computer-based planimetry was used to assess wound contraction, measuring the reduction percentage of the initial wound surface area. At each follow-up, we recorded adverse effects and complications and assessed pain following DS implantation via the VAS. Patients were required to

Table 2. Details of Integra and Pelnac groups.

INTEGRA	PELNAC
<p><i>Integra</i> (Integra LifeSciences, Princeton, NJ, USA) is a double-layer membrane system in which the deep layer is composed of bovine type I collagen fibers linked to shark chondroitin 6-sulfate glycosaminoglycan linked to a silicone replacement layer.</p> <p><i>Epidermis:</i> Silicone</p> <p><i>Dermis/Matrix:</i> Three-dimensional porous matrix of cross-linked collagen and glycosaminoglycan</p> <p><i>Composition:</i> Bovine collagen with chondroitin 6-sulfate</p> <p><i>Type of collagen:</i> Collagen types I and III</p> <p><i>Extraction:</i> Bovine tendon</p> <p><i>Cost (our hospital):</i> 1008 €/100 cm²</p>	<p><i>Pelnac</i> (Gunze Corp., Osaka, Japan) is a bilaminar membrane with a surface layer of silicone film and a layer of porcine collagen sponge derived from the pork tendon.</p> <p><i>Epidermis:</i> Silicone</p> <p><i>Dermis/Matrix:</i> Atelocollagen sponge not cross-linked and without glycosaminoglycan</p> <p><i>Composition:</i> Porcine collagen</p> <p><i>Type of collagen:</i> Collagen type I</p> <p><i>Extraction:</i> Porcine tendon</p> <p><i>Cost (our hospital):</i> 753 €/100 cm²</p>

complete the VAS by placing a line perpendicular to the VAS line corresponding to their pain intensity. We recorded the result on a scale ranging from 0 to 10 in which 0 indicated no pain and 10 indicated intense pain. The functional and aesthetic outcomes of re-epithelialization (healing time) were assessed by a blinded plastic surgeon according to the VSS (height, pliability, vascularization, and scar pigmentation) at the time of healing and at the 1-year follow-up.

The patients self-estimated their functional and aesthetic outcomes. Results were graded from 1 to 4 (1, very disappointed; 2, disappointed; 3, satisfied; and 4, very satisfied).

Histology and immunohistochemistry procedures

Excised tissues were fixed in 10% formalin solution for 24 hours. Tissues were embedded in paraffin wax, sectioned at 7- μ m thickness, assembled on glass slides, deparaffinized, then stained with hematoxylin-eosin and Van Gieson (Bio-Optica, Milan, Italy) for collagen analysis.

Immunohistochemistry samples were fixed in 40 g/L formaldehyde and processed

by paraffin embedding using standard methods. Sections were cut to 7- μ m thickness, placed on polylysine-coated microscope slides, and processed for collagen type I, collagen type III, vascular endothelial growth factor (VEGF), CD68, and intracellular adhesion molecule 1 (ICAM-1). The sections were deparaffinized in xylene, rehydrated, and placed in a microwave oven at 800 W for three 5-minute cycles in 0.01 mol/L citrate buffer (pH 6) to maximize antigen retrieval. Endogenous peroxidase activity was interrupted by slide incubation in 30 mL/L hydrogen peroxide (3% in methanol) for 30 minutes. The slides were rinsed in phosphate-buffered saline and then incubated with blocking solution (3 mL/L Triton X-100, 10 g/L bovine serum albumin, and 10 mL/L normal swine or rabbit serum); the solution served as an antibody dilution. The sections were then placed in an incubator with the following primary antibodies overnight at 4°C: rabbit anti-collagen type I, dilution 1:500 (GTX41286; GeneTex, Irvine, CA, USA); mouse anti-collagen type III, dilution 1:1000 (GTX26310; GeneTex); mouse anti-VEGF, dilution 1:100 (GTX83426; GeneTex); mouse anti-CD68, dilution 1:200 (M0876; Dako,

Glostrup, Denmark); and rabbit anti-ICAM-1, dilution 1:100 (AD062401; eBioscience, San Diego, CA, USA). The sections were then reacted with biotinylated secondary antibody (anti-rabbit antibody diluted 1:400 for collagen type I and ICAM-1; anti-mouse antibody diluted 1:400 for collagen type III, VEGF, and CD68) for 1 hour at room temperature. The immunoreaction was identified using a VECTASTAIN Elite ABC kit (Vector Laboratories, Burlingame, CA, USA) and then marked with 3,3'-diaminobenzidine tetrahydrochloride (Dako) for 5 to 10 minutes. Finally, the sections were mounted in Entellan medium (Merck, Kenilworth, NJ, USA). Processed sections void of primary antibody were used as negative controls, while healthy human skin specimens were used as positive controls to validate the antibodies.

Cell culture

Human fibroblasts were cultured in Dulbecco's modified Eagle medium with 10% fetal bovine serum and 1% penicillin/streptomycin 1:1 in 25-cm² plates and then incubated at 37°C in humidified air with 5% carbon dioxide. The medium, serum, and antibiotic mix were purchased from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). When confluence was reached, the cells were treated with trypsin-EDTA 1% (Gibco), harvested, and then centrifuged at 1200 rpm for 5 minutes. The supernatant was removed and the cell pellet was resuspended in 1 mL of complete medium, positioned in 75-cm² plates, and incubated at 37°C and 5% carbon dioxide until 80% confluence was reached.

The Pelnac and Integra scaffolds were pre-incubated in 24 plastic wells with culture medium at 37°C in a 5% carbon dioxide humidified atmosphere. After 24 hours, 10⁴ human fibroblasts cells were seeded in the wells containing the scaffolds for 7 days.

Scanning electron microscopy

Samples of scaffolds were set with 2% glutaraldehyde in 0.1 M phosphate buffered saline for 4 hours, then set in 1% osmium tetroxide in the same buffer for 1 hour, dehydrated in gradient ethanol, dried to critical point (CPD 030; Bal-Tec AG, Balzers, Liechtenstein), fixed to stubs with colloidal silver, sprayed with gold using an MED 010 coater (Bal-Tec AG), and then analyzed with an FEI XL30 scanning electron microscope (FEI, Hillsboro, OR, USA).

Statistical analysis

The Kolmogorov–Smirnov test revealed non-normally distributed data; therefore, all statistical analyses were carried out according to a non-parametric approach. To investigate the effectiveness of Pelnac versus Integra, we calculated the improvement in the average VAS score, Likert score, and VSS score for each group at each follow-up as well as the corresponding median values and 95% confidence intervals. The median values were then compared using the Mann–Whitney U test.

The threshold for statistical significance was set at $p < 0.05$. Repeatability is represented as a standard deviation to calculate the differences between measurements using SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA).

Ethics statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Our institutional ethics committee (Comitato Etico Regionale Marche C.E. R.M., Ancona, Italy) approved the study design (protocol number: 2019-0275 OR).

Results

Clinical results

Seventy-one patients were included in this study. The two study groups (Pelnac, $n = 35$; Integra, $n = 36$) (Table 3(a), (b)) were homogenous in terms of age (Pelnac, 54.3 ± 16.11 years vs. Integra, 52.9 ± 15.36 years) and lesion size (Pelnac, 67.3 ± 80.66 cm² vs. Integra, 75.4 ± 114.23 cm²). In both groups, the dressing was changed every 2 days, but the silicone film was maintained until suture removal (2 weeks after Pelnac implantation and up to 4 weeks after Integra implantation). Two weeks following surgery, there was evidence of substantial wound exudate in the Pelnac grafts, causing detachment of the silicone film most likely due to early degradation of the collagen sponge, which was not cross-linked.²⁷ In comparison, the wounds in the Integra group remained largely dry and the silicone film remained in place until removal by the surgeon after 4 weeks. Nevertheless, we observed temporary wound coverage in all patients treated with Integra, with all dermal templates fully engrafted in the wound bed.

Clinically, all patients achieved satisfactory recovery with no health-related issues.

All adherent bandages spontaneously detached 5 days postoperatively in both groups with the bandages remaining largely dry and easily removable. The dermal matrix and/or skin graft was well-unified in most sites at the end of treatment (Figure 1).

The resultant biointegration allowed for staged, definitive, and durable coverage of the soft tissue. Evaluation of the collagen color shift (from red to yellow) was feasible because of the transparent silicone layer in each DS; the color shifts indicated the different collagen maturation stages (Figure 2).²⁸ However, the patients treated with Pelnac did not show the same clinical results, especially in cases of extremely deep wounds. Patients treated with both Integra and Pelnac who underwent autografting showed appropriate and rapid engraftment without rejection (Figure 3).

The differences in contraction were evaluated after measuring the size of the artificial dermis. We assessed the wound contracture by measuring the wound site diameter when the silicone film was removed. Pelnac showed significantly greater contraction than Integra after 2 weeks, reaching $79.4\% \pm 20.16\%$ of the initial area at week 4. Contraction subsequently reached a plateau phase (Table 4(a)).

Table 3(a). Sample distribution by age.

Treatment	n	Minimum age (years)	Maximum age (years)	Average age (years)	SD
Pelnac	35	21	83	54.3	16.11
Integra	36	19	86	52.9	15.36
Total	71	19	86	53.5	15.63

Table 3(b). Sample distribution by wound area.

Treatment	n	Minimum WA	Maximum WA	Average WA	SD
Pelnac	35	3	420	67.3	80.66
Integra	36	4	576	75.4	114.23
Total	71	3	576	71.4	98.49

SD, standard deviation; WA, wound area (cm²).

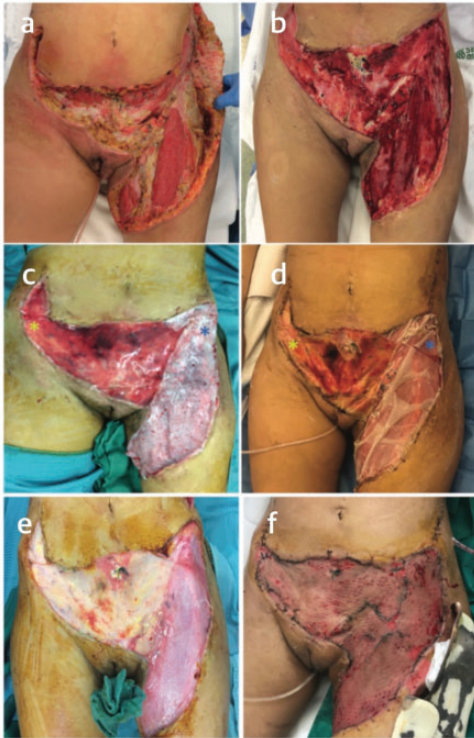


Figure 1. Representative case of necrotizing postpartum fasciitis treated with both dermal substitutes. (a, b) Outcome of necrotizing fasciitis after several surgical debridement procedures. (c) Immediate postoperative view after positioning of dermal substitutes (yellow asterisk, Integra; blue asterisk, Pelnac). (d) Two weeks following surgery, there was evidence of substantial wound exudate in the Pelnac grafts, causing detachment of the silicone film in this group. In comparison, the wounds in the Integra group remained largely dry and the silicone film remained in place until removal by the surgeon after 4 weeks. (e) The dermal matrix was well unified in most sites at the end of treatment. (f) The skin graft was well unified in most sites at the end of treatment.

Five patients in the Integra group and five patients in the Pelnac group had deep wounds (>1.5 cm) and did not undergo autografting so that we could evaluate the different wound contraction capacities with respect to the depth of the lesion. All 10 of

these patients achieved rapid colonization of the neoderm by keratinocytes with stable and trophic scar formation after 40 days with no pathological hypertrophy. We observed differences in the Wound Surface Area Assessment among these patients with respect to wound depth. The mean lesion size was similar between the two groups (Pelnac, $25.3 \pm 3.04 \text{ cm}^2$ vs. Integra, $31.2 \pm 5.12 \text{ cm}^2$). Table 4(b) shows that for wounds measuring >1.5 cm, Integra produced a greater percentage reduction and a shorter healing time than Pelnac. At the short-term follow-up, 60% of the patients had completely recovered with Integra compared with 0% of the patients with Pelnac; at the long-term follow-up, 100% of the patients had completely recovered with Integra compared with 0% of the patients with Pelnac. Assessment was not possible in the Integra group because macroscopic observation of the ex vivo grafts confirmed no occurrence of contracture, and all grafts had the same diameter as in the initial transplantation.

The difference in the healing time between the groups was not statistically significant. The VAS pain scores were also similar between the two groups (Pelnac, 1.5 ± 1.04 vs. Integra, 1.8 ± 1.33) at both the short- and long-term follow-ups (Table 5(a)). Significant differences were observed in patient self-estimation of complete recovery at 4 weeks (short-term follow-up) (Pelnac, 3.1 ± 0.24 vs. Integra, 2.8 ± 0.48 ; $p = 0.003$); however, no significant differences were observed at 1 year (long-term follow-up) (Pelnac, 3.6 ± 0.48 vs. Integra, 3.4 ± 0.51) (Table 5(b)). At both the short- and long-term follow-ups, statistically significant differences in the VSS score were observed between the groups and are shown in Table 6.

Significant differences were also noted in the VSS scores ($p < 0.05$) as shown in the box-and-whiskers plots in Figure 4(a) to (c).



Figure 2. (a–c) Visualization of the collagen color shift (from red to yellow) was feasible because of the transparent silicone layer in each dermal substitute, indicating the different collagen maturation stages. Patients treated with either Integra or Pelnac who underwent autografting showed appropriate and rapid engraftment without rejection. (d) Thirty days after engraftment. (e) Sixty days after engraftment. (f) Ninety days after engraftment.

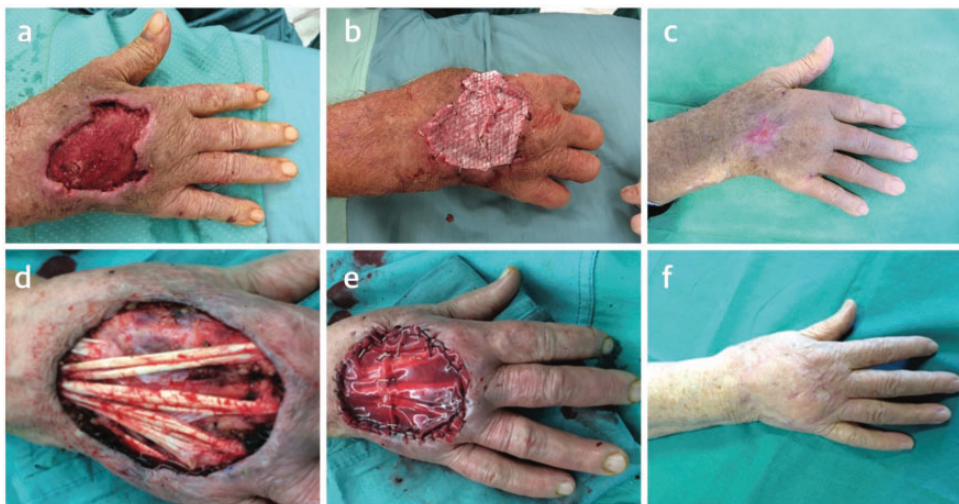


Figure 3. Patients who did not undergo autografting achieved rapid colonization of the neoderm by keratinocytes with stable and trophic scar formation. (a–c) Hand trauma treated with Pelnac. (d–f) Hand trauma treated with Integra.

Table 4(a). Differences in scar contraction.

	Pelnac		Integra	
	Median	SD	Median	SD
Wound area (cm ²)				
Pre-op	67.3	80.66	75.4	114.23
2 weeks	47.8	52.14	66.7	90.78
4 weeks	15.5	26.36	43.6	72.32
1 year	5.1	14.24	22.1	54.97
Wound area contraction (%)				
Pre-op vs. 2 weeks	-24.9%	18.05%	-0.1%	0.14%
Pre-op vs. 4 weeks	-79.4%	20.16%	-34.2%	33.33%
Pre-op vs. 1 year	-64.7%	8.06%	-74.7%	22.46%

For the two groups, the averages of the treated areas were calculated and the percentages of wound contraction were calculated. Pre-op, preoperatively; SD, standard deviation.

Table 4(b). Differences in scar contraction.

Treatment	Wound depth	4 weeks		1 year	
		n	% Reduction	n	% Reduction
Pelnac	1.5 cm	3	-39%	3	-89%
Integra		2	-100%		
Pelnac	2.0 cm	2	-70%	2	-86%
Integra		2	-89%	2	-100%
Pelnac	2.5 cm	0			
Integra		1	-100%		

For the two groups, the percentages of wound reduction based on depth were calculated.

Table 5(a). Differences in pain-related VAS scores at short- and long-term follow up.

Treatment	n	Min	Max	Average	SD
Integra					
VAS score at 2 weeks	36	0	6	3.0	1.66
VAS score at 4 weeks	36	0	5	1.8	1.33
VAS score at 1 year	36	0	1	0.1	0.35
Pelnac					
VAS score at 2 weeks	35	0	5	2.9	1.50
VAS score at 4 weeks	35	0	4	1.5	1.04
VAS score at 1 year	35	0	1	0.1	0.35

Histological and immunohistochemical analyses

Figure 5 shows histological sections of skin wounds treated with an Integra or Pelnac membrane stained with hematoxylin and

eosin 15 days after the treatment. The images show a clear distinction between the DS and the resident tissue.

In the tissue treated with Integra (Figure 5(a)), partial and initial integration

Table 5(b). Differences in patient self-estimation on complete recovery scores at short and long-term follow up.

Treatment	n	Min	Max	Average	SD
Integra					
Satisfied at 2 weeks	36	2	4	2.6	0.69
Satisfied at 4 weeks	36	2	4	2.8	0.48
Satisfied at 1 year	36	3	4	3.4	0.50
Pelnac					
Satisfied at 2 weeks	34	2	4	2.8	0.50
Satisfied at 4 weeks	34	3	4	3.1	0.24
Satisfied at 1 year	34	3	4	3.6	0.48

VAS, visual analog scale; Min, minimum; Max, maximum; SD, standard deviation

Table 6. Differences in Vancouver Scar Scale score at short- and long-term follow-up.

Treatment	Pigmentation	Pliability	Height	Vascularity	Total score
2 weeks					
Pelnac	2.4 ± 0.74	2.4 ± 0.56	3.8 ± 0.96	2.0 ± 0.82	10.7 ± 2.10
Integra	2.5 ± 0.69	2.6 ± 0.55	3.9 ± 0.82	2.1 ± 0.93	11.1 ± 1.79
p	0.55	0.31	0.53	0.67	0.31
4 weeks					
Pelnac	1.4 ± 0.85	1.5 ± 0.50	1.8 ± 0.94	1.2 ± 0.63	6.0 ± 1.83
Integra	2.5 ± 0.65	2.1 ± 0.59	3.3 ± 0.74	1.6 ± 0.72	9.5 ± 1.69
p	<0.001*	<0.001*	<0.001*	0.001*	<0.001*
1 year					
Pelnac	0.6 ± 0.88	0.7 ± 0.44	0.8 ± 1.16	0.1 ± 0.35	2.3 ± 1.81
Integra	1.2 ± 0.87	1.5 ± 0.77	2.7 ± 1.03	0.9 ± 0.59	6.3 ± 1.66
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

Data are presented as mean ± standard deviation. Comparisons between the two groups were performed using the Mann-Whitney U test.

*p < 0.05 indicated statistical significance.

between the tissue and the DS was observed (Figure 5(a)). This area exhibited a massive and localized inflammatory reaction (Figure 5(c)) with the presence of granulation tissue. Conversely, inflammatory infiltration was visibly reduced at the wound edge, precisely where the DS had not yet grafted with the tissue (Figure 5(a)).

The tissue treated with Pelnac exhibited organic distribution of the collagen membrane with a more advanced engraftment than that in the Integra template. An inflammatory reaction was also evident in Pelnac-treated tissue (Figure 5(d)), and the

Pelnac-treated tissue displayed a moderate level of inflammatory infiltration 15 days after treatment. To confirm the presence of inflammatory infiltration, the samples underwent immunohistochemical analysis for CD68 and ICAM-1, two inflammatory markers. Figure 5(e) clearly shows the presence of CD68-positive inflammatory cells that were particularly close to the DS graft and around the vessels. Figure 5(f) shows the results of the immunohistochemical analysis for ICAM-1. Positive ICAM-1 labeling was found in proximity to the DS graft and around the vessels. Moreover, the

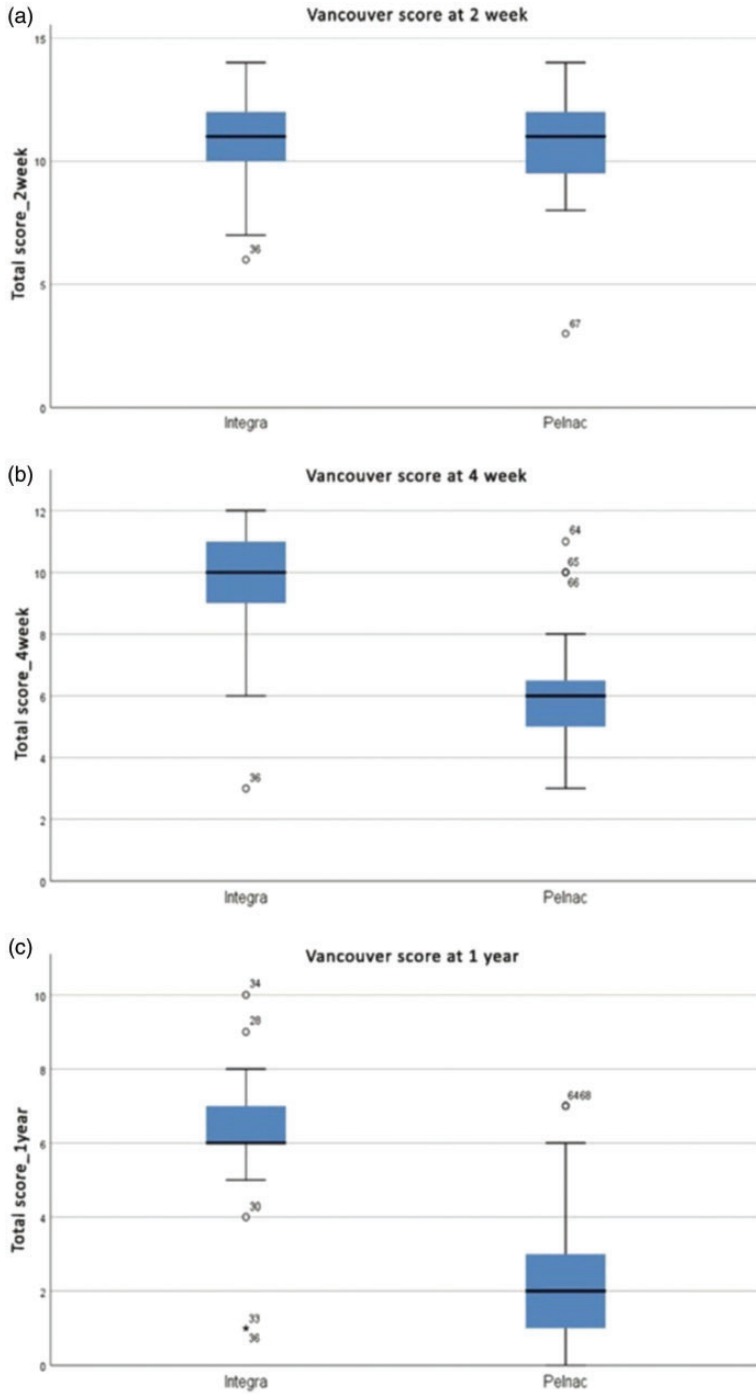


Figure 4. At both the short-term and long-term follow-ups, statistically significant differences in the Vancouver Scar Scale score were observed between the Pelnac and Integra groups. (a) Two weeks. (b) Four weeks. (c) One year.

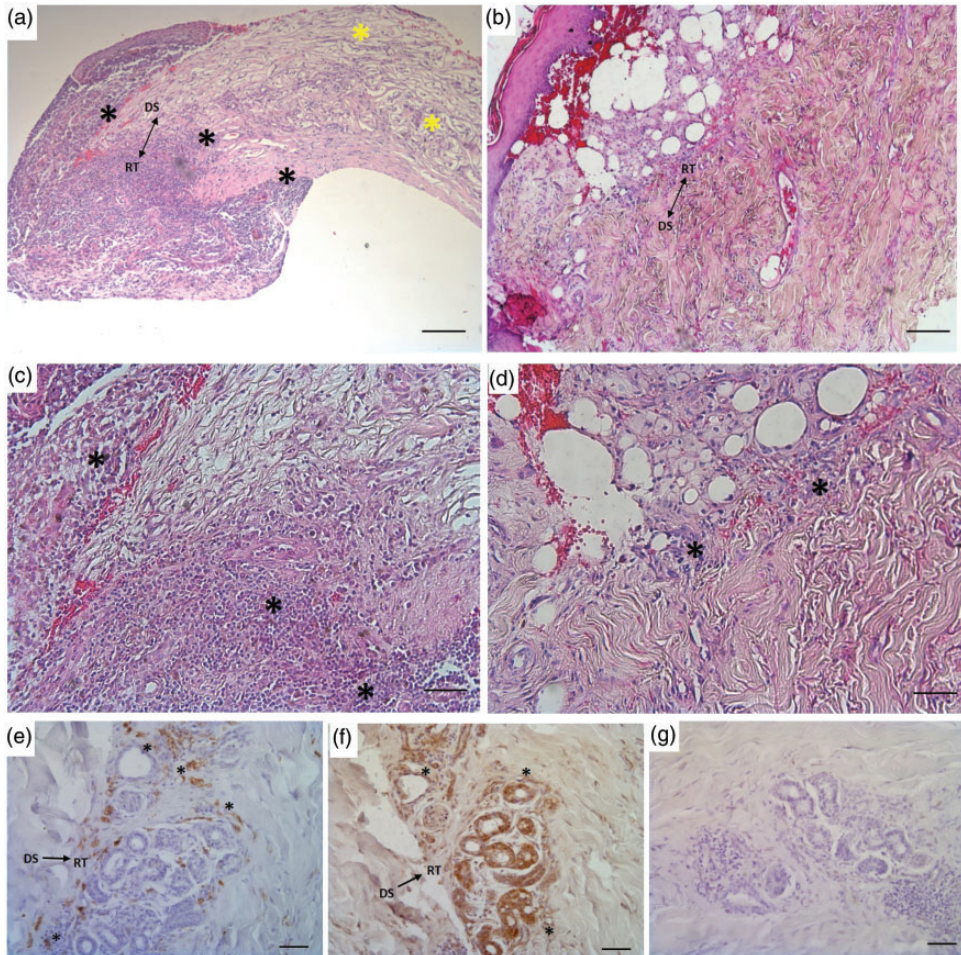


Figure 5. Histological sections of skin wounds treated with (a) Integra or (b) Pelnac membrane stained with hematoxylin and eosin 15 days after treatment. The images illustrate differentiation between the dermal substitute and the resident tissue. The black arrows indicate the graft area between the dermal substitute and the resident tissue (a, b; black arrows). The black asterisks indicate areas of partial integration between the tissue and the dermal substitute, and the yellow asterisks indicate areas in which the dermal substitute had not yet grafted with the tissue. (c, d) Higher magnification of (a) and (b), respectively; asterisks indicate a localized inflammatory reaction. (e) Immunohistochemical analysis of CD68, confirming the presence of inflammatory cells located close to the dermal substitute graft (arrows) and around the vessels (asterisk). (f) Immunohistochemical analysis of intracellular adhesion molecule 1. Positive labeling was found close to the dermal substitute graft (arrows), around the vessels (asterisk), and in the eccrine glands. (g) Negative control for immunostaining. (a, b: magnification $\times 4$, scale bar = 300 μm ; c–g: magnification $\times 10$, scale bar = 150 μm).

images showed positivity for ICAM-1 in the eccrine glands (both glands and ducts), but not positivity for CD68. Figure 5(g) shows the negative control for the

immunostaining. The histological section was stained using the Van Gieson technique and displayed evidence of newly formed collagen fibers in the dermis treated with

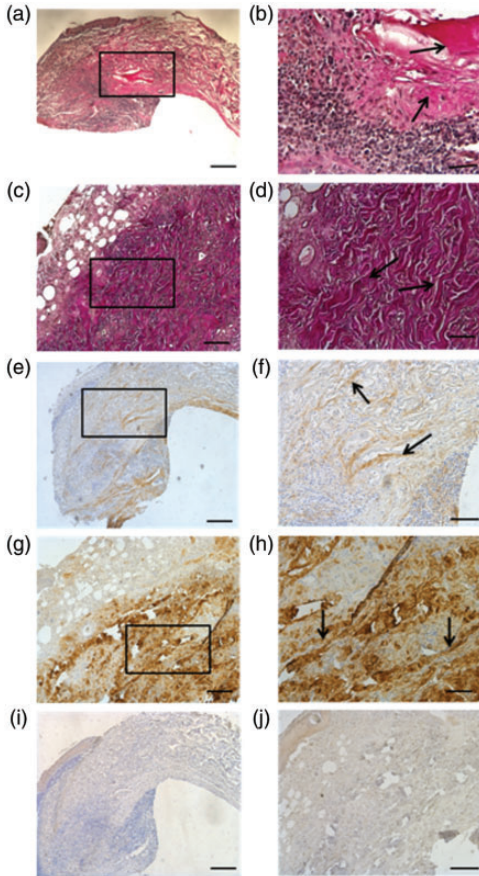


Figure 6. Newly formed collagen fibers in the dermis treated with (a, b) Integra and (c, d) Pelnac. Van Gieson staining showed that both dermal substitutes contributed to the formation of collagen fibers. (e–j) Immunohistochemical analysis of tissue. The samples appeared strongly positive for collagen type III (e, f: Integra; g, h: Pelnac). (f, h) Higher magnification of (e) and (g), respectively; arrows indicate the collagen type III fiber (f–h). (i, j) All samples were negative for collagen type I. (a, c, e, g, i, j: magnification $\times 4$, scale bar = 300 μm ; b, d, f: magnification $\times 10$, scale bar = 150 μm).

both Integra and Pelnac membranes (Figure 6(a)–(d)). Organic collagen fibers efficiently reconstructed the damaged structures. Moreover, immunohistochemical analysis for collagen was performed to investigate the effect of the DSs on dermal

remodeling fibers in the tissues (Figure 6 (e)). The immunohistochemical images confirmed the data obtained with Van Gieson staining: both DSs contributed to the formation of collagen fibers, and the tissue was strongly positive for collagen type III (Figure 6(f), (h)). Indeed, during the process of wound healing, collagen type III acts as a scaffold for fibroblast attachment, and high secretion of collagen type III is promoted by the DS graft.²⁹ In the Integra-treated tissue, we observed collagen type III fibers specifically localized in the tissue engraftment area with the DS, while in the Pelnac-treated tissue, the collagen type III fibers were homogeneously distributed throughout the dermis. All samples were negative for collagen type I, indicating that prominent mature collagen fibers (type I) were still not detectable 15 days after treatment (Figure 6(i), (j)).

Consistent with the regenerative response previously reported, both DSs exhibited substantial angiogenic potential and vascularization in the healing process, indicating the development of granulation tissue and the onset of the proliferative stage of wound healing. Figure 7 shows immunohistochemical analysis of VEGF for the Integra and Pelnac membranes. The images clearly demonstrate the formation of new vessels and microvessels distributed homogeneously in the tissue with significant positivity for VEGF (Figure 7 (b), (d)). The negative control for immunostaining analysis is shown in Figure 7(c) and (f) with a marker-negative sample. The epidermal area displayed a diffuse background due to the staining procedure and was not accounted for in the analysis of the results considering the nonspecific positivity for the marker.

Cell adhesion

A morphological study of human fibroblasts grown on the two DSs (Integra and

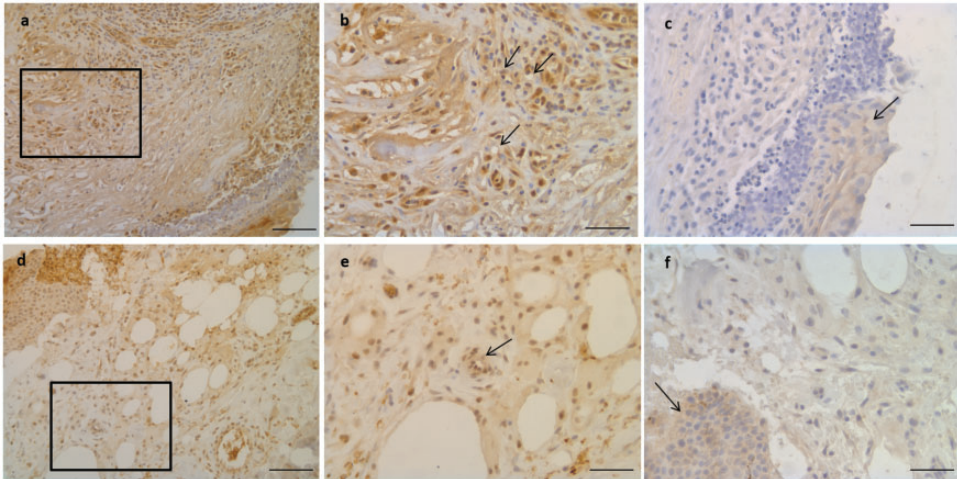


Figure 7. Both dermal substitutes exhibited significant angiogenic potential. The images show immunohistochemical analysis of vascular endothelial growth factor for (a, b) Integra and (d, e) Pelnac membrane. (c, f) Negative control for staining. (a–d: magnification $\times 10$, scale bar = 100 μm ; b–e: magnification $\times 20$, scale bar = 50 μm).

Pelnac) was performed by scanning electron microscopy after 7 days of cell culture. Figure 8 shows images of cell-free and cell-seeded scaffolding. We compared the two DSs without adherent cells (Figure 8 (a)–(c) and Figure 8(e), (f)) via scanning electron microscopy images and distinguished the bovine from porcine collagen fibers contained in the Integra and Pelnac scaffolds, respectively. In particular, the bovine fibers produced a less smooth and less wide-pored layer than did the porcine collagen fibers. After 7 days of incubation, cell attachment was visible between the collagen scaffolds and the human fibroblasts in both DSs. Moreover, scanning electron microscopy images of the Integra scaffold showed that the cells not only adhered to the surface of the scaffold but also migrated within, covering the existing pores (Figure 8 (b), (d)). The pore size for adhesion and growth of cells normally ranges from 10 to 100 μm .³⁰ The porosity in the Integra scaffold was around 20 μm , which may account for the adequate cell adhesion to the scaffold. Despite the smoother surface

of the Pelnac scaffold, the cells covered and infiltrated the scaffold with fibroblast-like fusiform morphology (Figure 8(f), (h)). No differences in cell growth or adhesion were observed between the two DSs.

Discussion

Full-thickness skin defects resulting from trauma, war-related injuries, oncologic extirpation, burns, and chronic diseases such as diabetes mellitus present unique challenges to patients and reconstructive surgeons.³¹ Local wound care, aggressive debridement of infected/devitalized tissue, and the reconstructive ladder (consisting of the lower rung of skin grafting to more advanced flap transfers) are all mainstay therapies for full-thickness skin defects. However, complex wounds involving large surface areas in patients with limited donor tissue and/or significant metabolic comorbidities (e.g., uncontrolled diabetes, sepsis, acute/chronic soft tissue/bone infection) increase the risk of skin graft loss and failure. Wound healing involves a multicellular

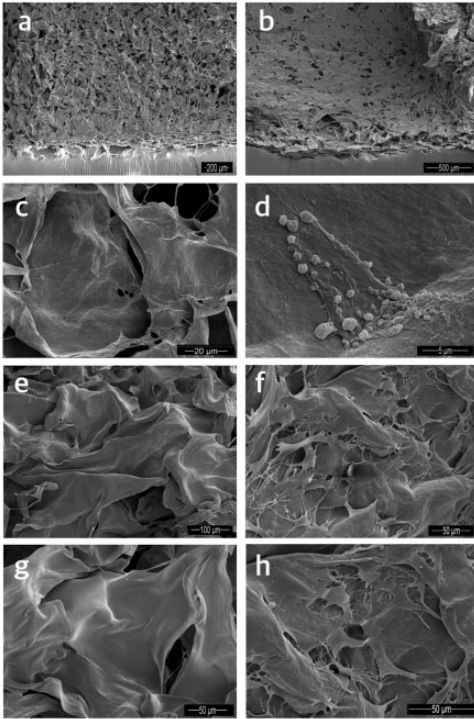


Figure 8. (a–d) Representative scanning electron microscopy images of the bovine dermal substitute Integra. (a, c) Integra scaffold (a: 200 μm , c: 50 μm). (b, d) Integra scaffold 7 days after incubation of 10^4 human fibroblasts (b: 200 μm , d: 5 μm). (e–h) Representative scanning electron microscopy images of the porcine dermal substitute Pelnac. (e, g) Pelnac scaffold without cells as control (e: 100 μm , g: 50 μm). (f, h) Pelnac scaffold 7 days after incubation with 10^4 human fibroblasts (f: 100 μm , h: 50 μm).

mechanism that includes coordination of different cell types such as keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets. These cells may migrate, infiltrate, proliferate, and differentiate to yield an inflammatory response that generates new tissue and promotes subsequent wound closure. Several authors have reported intricate signaling coordination within this complex process. Additionally, several biologic and synthetic dermal and epidermal regenerative modalities have

recently been investigated to treat full-thickness skin defects and investigate the fundamental characteristics of the biomaterial that closely resembles the natural features of tissue.^{3,4,15,16,24,29,30,32}

In the present study, we defined tissue-engineered skin bio-constructs as DSs that were acellular with xenogeneic synthetic scaffolding and biodegradable material. We observed differences in the composition, structure, and storage adequacy of the two DSs. Integra is moist, gelatinous, and translucent, while Pelnac is dry, spongy, and white. Suppliers of DSs are required to provide information regarding the origins of the DSs. Integra possesses a thickness and pore size of 2 mm and 70 to 200 μm , respectively, while these dimensions for Pelnac are 3 mm and 70 to 110 μm , respectively. Investigations have been conducted to better understand the most appropriate conditions for DSs. Yannas³² determined the average pore diameter and pore channel orientation. They found that cells are able to infiltrate the scaffold and bind to the ligands on the scaffold surface at an average pore diameter of 20 to 120 μm . Moreover, Hori et al.³³ illustrated the differing morphological structures of DSs, including the pore diameter and shape. Integra may prevent the wound from contracting and is efficient in releasing burn contracture and re-sectioning giant nevi, and it yields positive outcomes in extensive trauma and full-thickness burns. Pelnac may be indicated in wounds that are likely to shrink, such as small dermal malignant tumors and fingertip defects.

This observational study was performed to compare Pelnac and Integra, classified as dermal collagen matrices with identical indications for use but different structures, to understand their contribution in restoring and regenerating post-traumatic injuries. Wosgrau et al.²⁹ conducted similar comparative studies in mice. In each patient of our study, we observed positive effects of

the DS in terms of improving the quality and functionality of dermal reconstruction. We assessed patient self-estimation of recovery and pain on wound closure at the short-term follow-up as well as the clinical outcomes, particularly the healing time after 40 days, and found no statistically significant differences between the DSs. Moreover, no differences were observed between the two groups at the 1-year follow-up, indicating satisfactory and consistent clinical outcomes. However, the Pelnac group showed more favorable results in terms of the VSS score at the short-term (4 weeks) and long-term (1-year) follow-ups. Pelnac also resulted in more significant wound contracture. Interestingly, Integra provided a marked reduction in deeper wound injuries. Microscopic analysis of skin biopsy specimens revealed cellular debris and skin inflammatory infiltration with granulation tissue. The presence of inflammatory infiltrates confirmed our findings regarding early granulation tissue containing primarily type III collagen and little type I collagen. Conversely, we examined the effect of the Integra or Pelnac template on dermal remodeling and observed reactive epidermal hyperplasia and dermal granulation tissue as well as collagen fiber deposition and newly formed vessels. Indeed, type III collagen serves as scaffolding for fibroblast attachment during wound healing, and high secretion of collagen type III is promoted by the graft of the DS.²⁹ This histological assessment is in line with the distinct color shift of the collagen layer, examination of which was made possible by the transparent silicone layer in both the Pelnac and Integra DS, during the first 2 weeks following implantation. The biomaterial color shift indicates recellularization and new vessel formation. This allows for an efficient and fundamental two-step technique with subsequent skin grafting. The color shift begins in the first week following implantation,

revealing the diverse maturation phases of the collagen, which span from red to vanilla/yellow.²⁸ In fact, at this early stage, we observed a change in the color of Pelnac approximately 10 days before a change in the color of Integra, suggesting that Pelnac may become vascularized and recellularized before Integra.

Pelnac is a DS that allows rapid neoangiogenesis and tissue regeneration with neoformed tissue architecture closer to the true skin physiology. In contrast, Pelnac has limited efficiency in deep wounds with or without exposure to underlying structures such as bone and tendon tissue. Integra is more advantageous in deep wounds. No difference were observed in the healing time, but an important difference was noted in wound contraction, which was more marked in the injuries treated with Pelnac than Integra.

Based on our observational data, we propose a treatment algorithm that can serve as a guideline for appropriate use of these DSs (Figure 9). Both DSs are effective and applicable in different clinical contexts. Pelnac may be indicated in superficial wounds of any size and in any part of the body (with the exception of the face and sites of articulation). Pelnac is not indicated in burns, but it may be efficient in extensive oncological resections. Integra may be used for deep wounds of any size and in any part of the body, and it is applicable in burns and extensive oncological resections.

Limitations

This study has some limitations. Patients with vascular ulcers, patients with diabetic ulcers, and smokers were excluded. Furthermore, the ulcer dimensions differed between the study groups. Additionally, the patients had an extensive age range. Finally, the patient population was small, and the follow-up was limited to 12 months. Larger series with longer

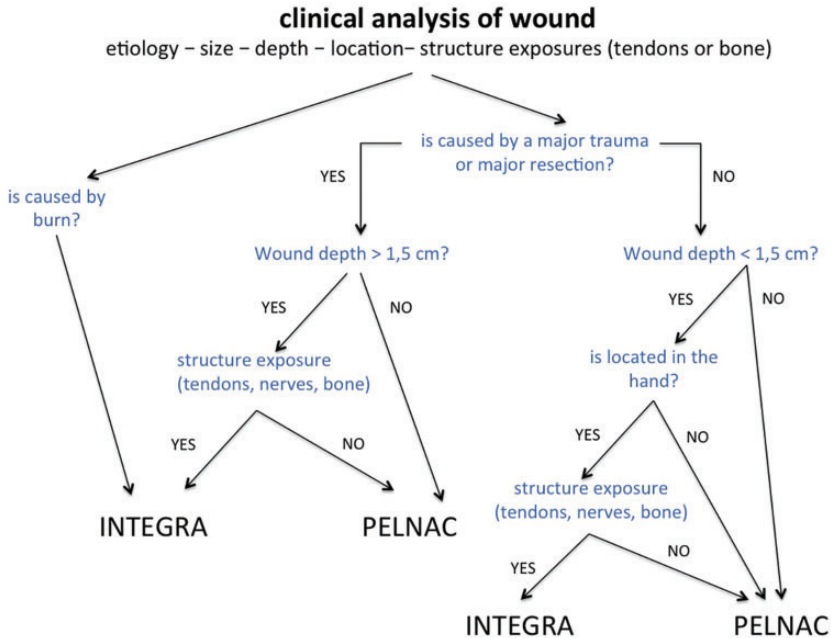


Figure 9. Decision and management algorithm flowchart.

follow-up periods are needed to validate the optimal DS.

Conclusions

The efficacy of Integra and Pelnac is comparable in post-traumatic and iatrogenic wounds based on the physiological similarities of these DSs in terms of lesion type, tissue regeneration, and healing processes. Both DSs yielded positive outcomes in hastening and improving the quality and functionality of skin reconstruction. Both Pelnac and Integra are clinically applicable, can be used successfully in surgery, and are efficient in terms of healing time.

This randomized study demonstrated differences between Integra and Pelnac from long-term clinical and histological perspectives in post-traumatic wounds, suggesting that biomaterials influence the wound microenvironment and tissue regeneration for a prolonged period after

implantation. Further studies are required to better understand the different mechanisms of action and impacts of DSs on clinical outcomes.

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Author contributions

FDF: conceptualization, investigation, methodology, and writing; AB: investigation and methodology; NZ: review and editing; SM: investigation and methodology; GC: data curation; FA: data curation; MDF: statistical analysis; FV: investigation; FM: investigation; VR: validation; LV: supervision; AS: resources and supervision; PCP: review and editing; MR:

conceptualization, funding acquisition, project administration, supervision, review, and editing.

Data availability statement

The clinical data used to support the findings of this study are included within the article.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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References

- Sherratt JA, Martin P, Murray JD, et al. Mathematical models of wound healing in embryonic and adult epidermis. *IMA J Math Appl Med Biol* 1992; 9: 177–196.
- Velnar T, Bailey T and Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. *J Int Med Res* 2009; 37: 1528–1542.
- Wang PH, Huang BS, Horng HC, et al. Wound healing. *J Chin Med Assoc* 2018; 81: 94–101.
- Eming SA, Wynn TA and Martin P. Inflammation and metabolism in tissue repair and regeneration. *Science* 2017; 356: 1026–1030.
- Herskovitz I, Hughes OB, Macquhae F, et al. Epidermal skin grafting. *Int Wound J* 2016; 3: 52–56.
- Lee G, Hunter-Smith DJ and Rozen WM. Autologous fat grafting in keloids and hypertrophic scars: a review. *Scars Burn Heal* 2017; 3: 2059513117700157.
- Negenborn VL, Groen JW, Smit JM, et al. The use of autologous fat grafting for treatment of scar tissue and scar-related conditions: a systematic review. *Plast Reconstr Surg* 2016; 137: 31e–43e.
- Lim JY, Choi BH, Lee S, et al. Regulation of wound healing by granulocyte-macrophage colony-stimulating injury. *PLoS One* 2013; 8: e54256.
- Halim AS, Khoo TL and MohdYusoff SJ. Biologic and synthetic skin substitutes: an overview. *Indian J Plast Surg* 2010; 43: S23–S28.
- Saba I, Jakubowska W, Bolduc S, et al. Engineering tissues without the use of a synthetic scaffold: a twenty-year history of the self-assembly method. *Biomed Res Int* 2018; 8: 5684679.
- Davison-Kotler E, Sharma V, Kang NV, et al. A universal classification system of skin substitutes inspired by factorial design. *Tissue Eng Part B Rev* 2018; 24: 279–288.
- Hodgkinson T and Bayat A. Dermal substitute-assisted healing: enhancing stem cell therapy with novel biomaterial design. *Arch Dermatol Res* 2011; 303: 301–315.
- Dickinson LE and Gerecht S. Engineered biopolymeric scaffolds for chronic wound healing. *Front Physiol* 2016; 5: 341.
- Philandrianos C, Andrac-Meyer L, Mordon S, et al. Comparison of five dermal substitutes in full-thickness skin wound healing in a porcine model. *Burns* 2012; 38: 820–829.
- De Angelis B, Orlandi F, Fernandes Lopes Morais D'aulilio M, et al. Long-term follow-up comparison of two different bi-layer dermal substitutes in tissue regeneration: clinical outcomes and histological findings. *Int Wound J* 2018; 15: 695–706.
- De Angelis B, Orlandi F, Morais D'aulilio MFL, et al. Vasculogenic chronic ulcers: tissue regeneration with an innovative dermal substitute. *J Clin Med* 2019; 8: 525.
- Nicholas MN, Jeschke MG and Amini-Nik S. Methodologies in creating skin substitutes. *Cell Mol Life Sci* 2016; 73: 3453–3472.
- Wu SC, Marston W and Armstrong DG. Wound care: the role of advanced wound healing technologies. *J Vasc Surg* 2010; 52: 59S–66S.
- Morozzo U, Villafane JH, Ieropoli G, et al. Soft tissue reconstructions with dermal substitutes versus alternative approaches in

- patients with traumatic complex wounds. *Indian J Surg* 2015; 77: 1180–1186.
20. Nicoletti G, Tresoldi MM, Malovini A, et al. Versatile use of dermal substitutes: a retrospective survey of 127 consecutive cases. *Indian J Plast Surg* 2018; 51: 46–53.
 21. Rehim SA, Singhal M and Chung KC. Dermal skin substitutes for upper limb reconstruction: current status, indications, and contraindications. *Hand Clin* 2014; 30: 239–252.
 22. Campagnari M, Jafelicci AS, Carneiro HA, et al. Dermal substitutes use in reconstructive surgery for skin tumors: a single-center experience. *Int J Surg Oncol* 2017; 2017: 9805980.
 23. Miyazaki H, Tsunoi Y, Akagi T, et al. A novel strategy to engineer pre-vascularized 3-dimensional skin substitutes to achieve efficient, functional engraftment. *Sci Rep* 2019; 9: 7797.
 24. Liu Y, Luo H, Wang X, et al. In vitro construction of scaffold-free bilayered tissue-engineered skin containing capillary networks. *Biomed Res Int* 2013; 2013: 561410.
 25. Vig K, Chaudhari A, Tripathi S, et al. Advances in skin regeneration using tissue engineering. *Int J Mol Sci* 2017; 18: 789.
 26. Cardoso L, Cleto MC, Barbo MLP, et al. Bioresorbable scaffold as a dermal substitute. *Int J Burns Trauma* 2017; 7: 34–46.
 27. Suzuki S, Matsuda K, Isshiki N, et al. Experimental study of a newly developed bilayer artificial skin. *Biomaterials* 1990; 11: 356–360.
 28. Szendroi M, Vajta G, Kovacs L, et al. Polarization colours of collagen fibres: a sign of collagen production activity in fibrotic processes. *Acta Morphol Hung* 1984; 32: 47–55.
 29. Wosgrau AC, Jeremias Tda S, Leonardi DF, et al. Comparative experimental study of wound healing in mice: Pelnac versus Integra. *PLoS One* 2015; 10: e0120322.
 30. Sadeghi-Avalshahr A, Nokhasteh S, Molavi AM, et al. Synthesis and characterization of collagen/PLGA biodegradable skin scaffold fibers. *Regen Biomater* 2017; 4: 309–314.
 31. Lucich EA, Rendon JL and Valerio IL. Advances in addressing full-thickness skin defects: a review of dermal and epidermal substitutes. *Regen Med* 2018; 13: 443–456.
 32. Yannas IV. Model of organ regeneration process induced by templates. *Ann N Y Acad Sci* 1997; 831: 280–293.
 33. Hori K, Osada A, Isago T, et al. Comparison of contraction among three dermal substitutes: morphological differences in scaffolds. *Burns* 2017; 43: 846–851.