Evaluation of Host Tissue Integration, Revascularization, and Cellular Infiltration Within Various Dermal Substrates

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Abstract: Acellular dermal matrices are used in a variety of reconstructive and cosmetic procedures. There seems to be host tissue integration, revascularization, and recellularization into these products, but the exact timing and differences among these remain unknown. The purpose of this study is to determine and compare these properties of 4 different acellular dermal matrices (AlloDerm, DermACELL, DermaMatrix, and Integra) in an in vivo rat model. Tissue specimens were obtained at various time points. Histology and immunohistologic assays were used to quantify the extent of cellular infiltration and revascularization within the various matrices. A bimodal cellular response was observed in all products except for DermACELL. Cellular infiltration was highest in DermACELL and lowest in AlloDerm, and angiogenesis was evident by day 7. There were clear differences within the various products. It is undetermined whether these differences are advantageous or clinically significant. Future work is needed to define the specific roles for each.

Key Words: acellular dermal matrix, AlloDerm, DermACELL, DermaMatrix, Integra, revascularization, dermal substrates

During the past 15 years, an increasing level of interest has evolved for the clinical use of acellular dermal matrices (ADMs). ADMs have been used for a number of applications, first with burns, but more recently with cosmetic and reconstructive procedures. Notably breast reconstruction. Generically, the utility of ADMs lies in their ability to incorporate into host tissues and function as an effective scaffold for cellular and vascular ingrowth. The ideal biomaterial for such applications would have a rapid incorporation of host tissue with minimal biologic inflammatory response and would be resistant to infection.

Although the list of available ADMs has increased significantly in the past few years, the prototypical ADM with the longest history of use is AlloDerm (LifeCell Corp., Branchburg, NJ). Most applications described in the literature have used this allogeneic decellularized human product. Other products have also been introduced based on the perceived shortcomings of AlloDerm. These include DermaMatrix (Synthes CMF, West Chester, PA) and DermACELL (LifeNet Health, Virginia Beach, VA). They are decellularized in a proprietary manner like AlloDerm, but differ in that they are provided as sterile—not aseptic—end products. DermACELL also differs from the other products in that it is gamma irradiated, and it is not freeze dried. None of these 3 products are cross-linked, and all are used for similar clinical applications.

Another class of ADMs includes engineered synthetic dermal substitutes, the best known of which is Integra Dermal Regeneration Template (Integra LifeSciences Corporation, Plainsboro, NJ). This is a cross-linked bovine type I collagen template with an optimized and random porosity (mean pore diameter, 30–120 μm) and degradation rate indicated for the treatment of life-threatening burn injuries and the repair of scar contractures. It is supplied as a 2-layer product, consisting of an outer thin layer made of silicone to prevent dehydration and protect the inner porous collagen-chondroitin-6-sulfate layer. Published applications of this technology range from primary and secondary burn reconstruction to limb salvage and genital reconstruction.

Although the clinical applications of these various matrices overlap, the unique differences in their processing and preparation likely lead to differing degrees of cellular and vascular ingrowth. These putative differences have not been thoroughly investigated and compared. In this study, each of these representative matrices was implanted around a vascular pedicle in a rodent model and evaluated both histologically and immunohistochemically for cellular density, cellular migration, and vascular ingrowth.

MATERIALS AND METHODS

Implantation of ADMs Around a Vascular Pedicle

Procedures were performed with approval of the University of Virginia Animal Care and Use Committee. Forty-eight adult retired breeder Sprague-Dawley rats (Charles River) were randomly assigned for implantation with different matrices. Induction was initiated with weight-based intramuscular injection of a ketamine-xylazine mixture. The surgical site was then shaved and prepped in sterile manner. Bilateral inguinal incisions were made, and microscopic dissection was performed to identify and isolate the superficial inferior epigastric pedicle. Next, the pedicle was circumferentially wrapped with a 10 × 10 mm piece of designated matrix. Each animal had the same type of matrix implanted bilaterally. The 4 resulting groups consisted of animals receiving (a) 1.04- to 2.28-mm-thick implantable AlloDerm, (b) implantable 0.8- to 1.7-mm-thick DermaMatrix, (c) implantable 0.76- to 1.25-mm-thick DermACELL, or (d) Integra. The dermal side of each product faced the pedicle. The construct was then circumferentially wrapped by a larger piece of 0.15-mm-thick Sil-Tec silicone sheeting (Technical Products, Inc, Lawenenceville, GA) and secured with 6–0 nylon interrupted sutures. This prevented the ingrowth of cells from the surrounding environment and maintained the pedicle as the only source of host ingrowth. The outer sheet of Sil-Tec sheeting was added to all groups, except for Integra that already contained its own silicone protective layer.

A total of 96 procedures were performed on 48 animals by a single surgeon in an identical manner. Three animals from each group were randomly designated for analysis at each of 4 time points (7, 14, 28, and 42 days), yielding 6 specimens of a given product for each evaluation point. Postoperative pain control was provided by...
CD 31 QUANTITATIVE ASSESSMENT

FIGURE 1. Vessel count schematic: CD31 cells were indentified under 40×, and the cells that formed luminal structures were counted within 3 separate random high-powered fields for each product over the 4 time points.

then analyzed using Image Pro Plus 7.0 to calculate the distance (in mm) from the inner margin of the matrix through which cells had infiltrated. Statistical analysis (ANOVA) was performed using SPSS 19.0 (mentioned in Fig. 4).

**RESULTS**

**Explantation/Gross Appearance of ADMs**

Removal of tissue specimens at the predetermined times demonstrated no loss of implanted ADMs to wound dehiscence or infection. The gross appearance of the matrices was of tissue with a reddish tinge. There were no signs of fluid accumulation or seroma, and the implanted scaffolds had become adherent to the pedicle. There was a defined smooth capsule surrounding all the tissue constructs protecting it from the external environment. The outer layer of silicone sheeting remained intact for all specimens.

**Histologic Evaluation of Matrix: Cell Density**

A bimodal pattern of cellular density was observed in all products except DermACELL at all 3 distances (300, 600, and 900 μm) measured. Cell density peaked at day 14 and subsequently decreased (day 21) before rising again. DermACELL had the most cells at all distances over all time points except for day 42 when Integra demonstrated the highest cell density. There were only a few cells in AlloDerm at 900 μm at all time points. There were statistically significant differences among the products at various time points over the varying distances evaluated (mentioned in Figs. 5–7).

**Histologic Evaluation of Matrix: Cellular Infiltration**

A statistically significant increase in the degree of cellular infiltration/migration was noted for DermACELL and Integra over time (P < 0.001 and P < 0.001 respectively). In fact, there was a

**Harvesting and Processing of Tissues**

At the predetermined time points, animals were euthanized per institutional guidelines. An infusion of 10% formalin was used intravascularly to fix the tissue constructs that were subsequently excised and placed into formalin for 1 day. Tissues were then transferred to saline and placed into 70% ethanol. Tissues were embedded in paraffin through a graded series of ethanol and xylenedehydration steps. Each “construct” was bisected at its midpoint perpendicular to the axis of the pedicle vessels, and sectioned from this cut edge toward the respective ends. Five micron sections were placed onto gelatin-coated slides and dried in place.

**Histology and Immunohistochemistry**

Sections previously cut were rehydrated and stained, one set with hematoxylin and eosin, and second set with hematoxylin only.

CD31 staining was performed using goat polyclonal antibody IgG/plaeteletendothelial cell adhesion molecule—1 (M—20; catalogue no: SC 1406, Santa Cruz Biotechnology Inc., CA). Slides were deparaffinized with xylene and rehydrated through a graded ethanol series, and then incubated for 30 minutes in 30% H2O2 prepared in methanol to destroy endogenous peroxidase activity. A heat-mediated antigen retrieval technique that included a 20-minutes boil in a microwave using unmasking solution (Vector labs) was done. Slides were incubated for 30 minutes in 10% Avidin blocking solution consisting of 10% normal rabbit serum and 0.5% gelatin from cold water fish skin (FSGO) for 1 hour to inhibit nonspecific binding of primary antibody. Following this, overnight incubation with primary antibody for 1:500 dilution was performed in a humidified chamber at 4°C. The primary antibody was washed with 0.5% FSGO/PBS, and a biotinylated rabbit anti-goat IgG at 1:200 dilution was used as secondary antibody (Vectastain ABC kit, Vector labs) in a humidified chamber for 1 hours. After washing off secondary antibody, slides were incubated in Vectastain ABC solution (Vector labs) for 30 minutes. A DAB detection kit SIGMA FAST 3, 3-diaminobenzidine tab (Sigma-Aldrich Biotechnology) was used for 2 to 5 minutes according to manufacturer’s instructions. The slides were dehydrated with absolute ethanol followed by xylene before coverslipping with xylene-based mounting media.

**Analysis of CD31 Images**

Three pictures (40×) were taken using an Olympus high magnification microscope for each tissue in areas where CD31 positive luminal structures could be identified. A blinded investigator subsequently colored these luminal structures on the images using ADOBE Photoshop CS5 Extended 12.0. Next, these colored images were analyzed using Image Pro Plus 7.0 for total count of luminal structures and total cross-sectional area. Statistical analysis (analysis of variance [ANOVA]) was performed using SPSS 19.0 (mentioned in Fig. 1).

**Semiquantitative Analysis of Cell Density**

Hematoxylin-stained sections were scanned with Aperio scanscope. A blinded investigator counted cells in each of 3 randomly selected high power fields (40X) located at 300 μm, 600 μm, and 900 μm from the graft-blood vessel interface using Image Pro Plus 7.0. Statistical analysis (ANOVA) was performed using SPSS 19.0 (mentioned in Figs. 2, 3).

**Cell Infiltration Measurement**

Hematoxylin and eosin-stained slides were scanned with Aperio scanscope at 40× magnification. A blinded investigator circumscribed the area through which cells had infiltrated using ADOBE Photoshop CS5 Extended 12.0. These marked images were

local anesthetic and intramuscular ketoprofen. All animals received oral antibiotics for 5 days after the operation.

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Cell density measurement at 300 μm, 600 μm, and 900 μm from the tissue interface

Assessment of Vascular Ingrowth

Evidence of vessel formation existed in all 3 of the human dermal matrices as early as day 7. There were statistically significant (P < 0.001) differences among products at day 7, with DermaCELL implants demonstrating almost double the number of vessels compared with the other products. There were no detectable differences at day 14 or 21. On day 42 (P = 0.02), however, both DermaCELL and AlloDerm demonstrated statistically notable numbers of vessels compared with the other materials. There was sparse vessel formation in Integra until day 14 (Fig. 9).

DISCUSSION

Novel allograft and synthetic materials for the regeneration or replacement of damaged tissue have been discovered and used for multiple tissues. However, each material that has been introduced to the marketplace is different in either the processing, procurement, construction, or in some cases, the basic components. Clinical reports of the use of these various materials have defined the limitations and sometimes indications for their use. AlloDerm is the first and classic example of a decellularized dermal matrix. It has been used for a variety of clinical applications, including lip augmentation, septal perforation repair, and breast reconstruction. 15-18

Although the starting material (human dermis) is the same for many of the scaffold products available to the clinician, the proprietary decellularization and processing methods may differ significantly between products. AlloDerm is a product of LifeCell Corporation and has been used the longest of all decellularized matrices. Its benefits are that it has an extensive and broad history of clinical use, and it is known to be safe and relatively immunologically inert.
FIGURE 5. Cell density at 300 μM: Bimodal distribution was seen for all products except DermACELL with increasing numbers to day 14, decreasing to day 21, and then rising to day 42. There was statistically significant differences among products at all time points except day 42 (P < 0.05).

FIGURE 6. Cell density at 600 μM: Fewer cells observed in all products at this distance compared with 300 μM. Bimodal distribution is still apparent with significant differences at all time points (P < 0.05).

FIGURE 7. Cell density at 900 μM: the fewest number of cells were observed at this distance comparatively among all products. AlloDerm had the fewest numbers of cells at this distance at all time points, and Integra had the most at day 42. Statistically significant differences were seen at days 7, 21, and 42 (P < 0.05).

FIGURE 8. Cellular infiltration: cellular infiltration distance increased steadily over time for all groups with statistically significant differences at all time points (P < 0.05). DermACELL was associated with the furthest cell migration and AlloDerm the least.

Its integration into host tissues over the course of years has been demonstrated clinically.21 Integra is the only artificially engineered dermal matrix present within this study. It is composed of a bovine type I collagen-glycosaminoglycan matrix of a random pore size and degradation rate. Its inclusion in this study was to compare a matrix that has been approved for more than 20 years and differs in that it does not have a structural microvascular skeleton. It is used in a similar manner to AlloDerm to provide soft-tissue coverage, especially in the setting of burn reconstruction.

The 2 additional scaffolds used in this study were DermACELL and DermaMatrix. They are different from AlloDerm in that they are marketed as being terminally sterile, which passes the United States Pharmacopeia Standard 71, while AlloDerm is bacteriologically clean. DermaMatrix is marketed as an off the shelf (stored at room temperature) product with minimal need for rehydration prior to clinical use. DermACELL is gamma irradiated and does not require rehydration.

Previous studies of ADMs have focused on the rates of revascularization and recellularization. For instance, in 1 such study, vascularized AlloDerm was used to repair a rat hernia model. Rats were divided into 4 groups, and a prefabricated flap of vascularized AlloDerm was used to repair a surgically created hernia. This prefabricated revascularization occurred during the course of 3 weeks, and animals treated with it demonstrated no hernia recurrence.22 Similarly, in a rabbit hernia model using AlloDerm, vascularization was seen after 30 days by evaluation with fluorescent staining.23

Although some models may show revascularization is possible, conflicting evidence is present. In an in vitro model of endothelial cell growth, AlloDerm was shown to inhibit endothelial cell growth.24 This study compared AlloDerm with and without platelet poor plasma, and although mechanistically the experiment shows that platelet poor plasma is helpful in allowing endothelial cells to attach, it does not completely correspond with the in vivo situation.
Even though revascularization is important, integration into host tissue also involves recellularization. This occurs first with infiltration of migratory inflammatory cells into the ADM, then matrix remodeling, and eventual revascularization. The limit of recellularization is likely the limit of diffusion of nutrients into the substance of the ADM. A prior study using a similar model demonstrated recellularization within the first 7 days with an increase in myofibroblasts and CD31-positive endothelial cells. The present study is qualitatively similar in that the number of cells present does increase in the first 14 days.

The prior study was terminated at 14 days with the demonstration of Prox-1+ cells, likely lymphatic endothelial cells. The current study continued to 42 days to better model the clinical scenario of long-term implantation. There was a bimodal pattern of cellular density in 3 of the 4 dermal matrices with steady ingrowth of cells to day 14 with a sudden drop at day 21. The cell density then steadily increased to day 42 in all samples except for DermACELL. This bimodal distribution might be explained by the changing of the inflammatory cells present within the matrix from acute inflammatory cells to chronic remodeling ones. Clinically, this is the period when drains can usually be removed from a decrease in output. It is unknown whether a higher cellular density influx could lead clinically toward seroma formation or faster integration rates which may reduce the incidence of infection.

Nonspecific cellular infiltration was variable but statistically significant between the products. There was a steady rise in cell migration in the DermACELL and Integra products. DermACELL had the most cellular migration, with Alloderm among the least. The clinical implication, if any, of cellular infiltration is unknown. Rapid and complete cellular migration would theoretically lessen the risk of infection, as the matrix is rapidly incorporated with the host's cells; however, certain cell population migration (eg, myofibroblast) may have some detrimental effects in regards to capsular contracture.

The types of differences seen in this study are not the first reported in the literature. Orenstein et al. showed in vitro that the levels of interleukin-1β expression of peripheral blood monocytes/macrophages differ when they are plated on AlloDerm versus FlexHD and AlloMax. These are 2 other ADMs processed under differing conditions. Interestingly, AlloDerm induced interleukin-1β activation to a much smaller degree than did the other 2 ADMs. These differences could result in varying intensity of inflammation and wound healing. Histology from this study shows CD31 positivity that migrates from the vessel to the matrix. Although some of this may be endothelial cells, CD31 is also known to stain monocytes and macrophages; some of these cells are likely phagocytic and degradative cells that are part of the wound-healing process. CD31 positivity is less for the Alloderm group than for the DermaMatrix or DermACELL groups for the first 3 weeks and then dramatically increases by day 42. The bimodal pattern was also seen for the CD31.
slides in all products except DermACELL. All 3 human dermal matrices had evidence of vessel formation by day 7 with sparse CD31 positivity in Integra. Integra has been shown to be revascularized by day 14, which is similar to our findings.27 The decellularized human products still have an intact microvascular skeleton within the extracellular matrix, which could provide a pathway for new endothelial cells initially and allow for early revascularization28 (mentioned in Fig. 10).

Although this study is the first to directly and simultaneously compare 4 regenerative products, it is not without limitations. The cellular density counts are based on all nucleated cells stained by H&E and specific cell types were not identified; therefore, the reasoning for the bimodal pattern of infiltration can only be speculated. An animal model was used and the healing/regenerative process of humans could differ.

In conclusion, this is the one of the first studies in which a significant difference between various ADMs has been histologically and immunohistologically shown. Although company literature emphasizes that newer second-generation ADMs are similar to AlloDerm, it is very likely that their clinically biologically relevant activity may be very dissimilar. Each product causes a variable cellular reaction that may lead to quicker host tissue incorporation or potentially to foreign body reaction and encapsulation. Ongoing work aims to further characterize the cellular and remodeling process associated with each matrix, which could lead to rational choices in use of materials for differing clinical applications.

REFERENCES


