Biophysical and Microscopic Analysis of Homologous Dermal and Fascial Materials for Facial Aesthetic and Reconstructive Uses

Anthony P. Sclafani, MD; Steven A. McCormick, MD; Rubina Cocker, MD

Objectives: To evaluate the microscopic structure and physical properties of homologous tissue grafts commonly used in aesthetic and reconstructive facial plastic surgery in order to determine specific properties of these materials that may affect their performance in vivo.

Methods: Two decellularized dermal materials (AlloDerm and DuraDerm) and 2 fascia lata tissue grafts (Tutoplast and cadaveric fascia lata) were examined by light microscopy (hematoxylin-eosin and Movat staining) and scanning electron microscopy. The physical properties of these materials were also examined for thickness, maximum sustainable load, strain, conformability, and elasticity.

Results: Significant differences in microscopic appearance existed between the 2 dermal materials and the 2 fascial materials. AlloDerm and Tutoplast fascia lata retained architecture closer to that of untreated tissue than did DuraDerm and cadaveric fascia lata, respectively. Tutoplast fascia lata and AlloDerm were also stronger than cadaveric fascia lata and DuraDerm, respectively. AlloDerm retained significantly more elasticity than DuraDerm.

Conclusions: AlloDerm and Tutoplast fascia lata retain more natural architecture and physical properties than do DuraDerm and cadaveric fascia lata, respectively. These differences clearly show the effect of the specific processing of these materials. The alteration in architecture and the degradation of the physical properties of DuraDerm and cadaveric fascia lata may hinder the performance of these grafts in vivo. Further studies on these materials in humans are currently under way.

Arch Facial Plast Surg. 2002;4:164-171
MATERIALS AND METHODS

MATERIAL PREPARATION

Dermis Derivatives

An acellular dermal graft, AlloDerm (LifeCell Corp, Branchburg, NJ), and DuraDerm (Collagenesis, Inc, Beverly, Mass) are both derived from skin obtained from tissue banks accredited by the American Association of Tissue Banks and then steriley processed. In each, the epidermis is removed and the dermis is treated with detergents to remove the cellular elements. DuraDerm is further treated with 3000- to 3900-rad (3- to 3.9-kGy) gamma irradiation. The resultant acellular dermal mass is then freeze-dried to produce stable, sterile materials with significant shelf lives. DuraDerm is identical to Dermaplant (Collagenesis, Inc) in its processing and was used in this study because Dermaplant had not yet become commercially available.

Fascia Lata Derivatives

Homologous fascia lata (Tutoplast; Tutogen Medical, Inc, Alachua, Fla) is treated with a patented chemical process to remove all cells and is then freeze-dried after irradiation. Cadaveric fascia lata (Community Tissue Services, Ft Worth, Tex) is gamma-irradiated with cobalt 60 at 15000 to 25000 rad (15-25 kGy) for sterilization and subsequently freeze-dried.

RECONSTITUTION

All materials were processed per the manufacturers’ or suppliers’ recommendations under sterile conditions before evaluation. AlloDerm sheets were rehydrated in 2 separate baths (5 minutes each) of isotonic sodium chloride solution. DuraDerm was rehydrated in a single isotonic sodium chloride solution bath for 5 minutes. Tutoplast fascia lata was rehydrated in isotonic sodium chloride solution for 10 minutes until soft. Cadaveric fascia lata was rehydrated in isotonic sodium chloride solution for 30 minutes.

MICROSCOPY

All specimens were divided after rehydration into portions for micrometry, light microscopy, and scanning and transmission electron microscopy. For evaluation of material thickness, formalin-fixed specimens were mounted on glass slides perpendicularly. Specimens were then examined under ×4 magnification and the material thickness was measured with an objective-mounted micrometer. Specimens were batch-processed for hematoxylin-eosin, Movat pentachrome, and anti-type IV collagen staining to ensure uniformity of staining. The materials were sectioned at 5 µm.

ELECTRON MICROSCOPY

Each biomaterial sample was fixed in 4% glutaraldehyde in cacodylate buffer in phosphate-buffered saline. The samples...
were rinsed and dehydrated in graded ethanols; they were then placed for 1 hour in 50% hexamethyldisilazane (Electron Microscopy Sciences, Fort Washington, Pa) followed by 100% hexamethyldisilazane. After vacuum air-drying, the specimens were sputter-coated with gold-palladium alloy and examined in a scanning electron microscope (5-kV accelerating voltage) (JEOL 6400; JEOL Ltd, Tokyo, Japan) at ×3 to ×20 magnification.

BIOPHYSICAL TESTING

Tissue samples of AlloDerm, DuraDerm, cadaveric fascia lata, and Tutoplast fascia lata were obtained and rehydrated in room-temperature phosphate-buffered saline as described. Strips of tissue measuring 1 × 4 cm were then cut from each group. In the case of the fascia lata materials, where fiber orientation could be determined, strips were cut along the long axis of fiber orientation.

Tensile Testing

The 10 × 40-mm pieces were cut down by means of a dumbbell-shaped jig to a 3 × 15-mm test area with a 10 × 12.5-mm area on each end for gripping. Sample pieces were measured for thickness by means of a vernier micrometer (Mitutoyo Corp, Kyoto, Japan). Measurements were taken at 3 points along the test portion of tissue and averaged. Samples were then placed into a tensile tester (Chatillon LRX; AMETEK Test and Calibration Instruments Division, Largo, Fla) with the test portion centered between the grips. A 0.5-N preload was applied to all samples. Samples were then pulled to failure with the use of a 500-N load cell at a rate of 12.7 mm/min. All samples were noted to fail along the necked-down test portion of the tissue.

Maximum load, maximum stress, and elastic modulus (in the range of 0-2 N) were calculated by means of Chatillon Dapmat software (AMETEK Test and Calibration Instruments Division).

Three-Point Bend Load Testing

The tensile tester was used to calculate 3-point bend load. Rehydrated pieces of each material measuring 10 × 40 mm were carefully centered on a 15 × 45-mm piece of wax paper. Care was taken not to introduce moisture to the backside of the tissue-tissue combination. Samples were then centered across a 1-cm span and supported on either side by a 1-cm-wide block. A load was applied to the center of the tissue. The force required to deform the tissue over a vertical travel of 8 mm was recorded by means of a 50-N load cell. Rate of travel of the load cell arm was 12.7 mm/min. Thickness for the samples was determined by means of a vernier micrometer, as described above.

For analysis, the load vs extension curve was plotted, and a third-degree best-fit trend curve was applied. The maximum deformation load applied was graphically determined from the trend line. Three-point bend values were computed as follows: \( \frac{3.0 \times \text{maximum load (N)/span (mm)}}{2 \times \text{width (mm)} \times \text{thickness (mm)}} \).

Figure 2. A, Surface of DuraDerm dermal graft with compressed collagen bundles and nearly intact capillaries (hematoxylin-eosin, original magnification ×10). B, Full-thickness cut of DuraDerm also showing a preserved upper dermal border (Movat stain, original magnification ×25).
ness with normal orientation. No elastic fibers were seen. Scattered accumulations of nuclear remnants were seen throughout the specimen. The Tutoplast fascia lata specimen varied in thickness from 800 to 1000 µm.

The appearance of cadaveric fascia lata (Figure 4) differed little from that of Tutoplast fascia lata. Again, regularly oriented collagen fibers 10 to 25 µm in thickness were seen. Significantly more deposits of nuclear debris were seen in the cadaveric specimen than in the Tutoplast-processed fascia. The cadaveric fascia lata examined uniformly measured 500 µm in thickness.

SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy (Figure 5) demonstrated a dense arrangement of collagen fibers interspersed with small clefts in AlloDerm. By contrast, DuraDerm had a similar arrangement of collagen fibers; however, there was a substantially greater degree of clefting and a number of “pores.”

Tutoplast fascia lata demonstrated uniform, discrete, and parallel collagen bundles on scanning electron microscopy, in contrast to cadaveric fascia lata, which appeared to be a more uniform surface without distinct collagen fibers.

BIOPHYSICAL TESTING

Sample thickness as measured by physical micrometry correlated well with measurements by microscopy. AlloDerm was significantly thicker than DuraDerm, and both of these were thicker than either cadaveric or Tutoplast fascia lata (1.89 vs 1.33 vs 0.77 vs 0.77 mm; both comparisons \( P = .03 \)) (Figure 6). Maximum load to breaking was significantly lower in DuraDerm than in either Tutoplast fascia lata or AlloDerm; similar findings were noted with cadaveric fascia lata (Figure 7). Maximum stress and modulus of elasticity were significantly higher in cadaveric and Tutoplast fascia lata than in either DuraDerm or AlloDerm; these were also higher in DuraDerm than in AlloDerm (Figure 8 and Figure 9). Three-point bend testing showed that conformability was greatest in AlloDerm and least in cadaveric fascia lata (Figure 10).

COMMENT

Homologous soft tissue grafts are useful for a variety of cosmetic and reconstructive applications in the face, head, and neck. They have been used for repair of congenital ptosis; in static sling procedures for facial paralysis; as...
scaffolding in the repair of nasal septal perforations; for obliteration of soft tissue defects such as depressed scars; and for augmentation of facial areas such as the nasal dorsum, nasolabial folds, and lips. These proteinaceous materials of human origin are all resorbed to some degree after implantation, but some fibrous invasion does occur. The ultimate success of procedures that use these materials is affected by their initial bulk and structural integrity, as well as the biological processes that affect them after implantation.

The materials examined in this study are derived from either dermis or fascia. AlloDerm and DuraDerm are both derived from dermis and are subsequently de-cellularized and freeze-dried by patented processes. Tutoplast fascia lata and cadaveric fascia lata are also processed and treated to remove donor cells and major histocompatibility antigens. The processing that homologous tissues require to eliminate antigenicity may lead to structural degradation of the protein network. Damage to these (predominantly) collagen fibers, as well as incomplete removal of degradation products, may compromise the integrity of these materials, as well as promote a graft-vs-host reaction by exposing new collagen epitopes to host inflammatory cells.

In this study, we have examined the gross, microscopic, and ultrascopic morphologic characteristics of 4 readily available homologous soft tissue materials. Dermis-based materials like AlloDerm and DuraDerm show col-
lagent (and elastin) fiber networks in a heterogeneous pattern. These acellular proteinaceous materials differ, however, in more subtle ways. AlloDerm appeared to be more organized than DuraDerm, with a more consistent surface texture as seen on scanning electron microscopy. AlloDerm appeared much more compact and coherent than did DuraDerm; the interstices between collagen bundles were markedly larger in DuraDerm and appeared to be expanded by a dilute mucopolysaccharide. When used for soft tissue augmentation or replacement, fluid flux across a DuraDerm implant may lead to a situation in which accurate assessment of the required material is difficult, as the apparent volume of the ex vivo material is difficult, as the apparent volume of the ex vivo implant may be significantly different from its true volume once it is implanted and the mucopolysaccharide is resorbed.

Both fascia lata–based materials demonstrated bland, uniform acellular bands of collagen bundles. However, cadaveric fascia lata showed fine separations between collagen fibers and large gaps between collagen bundles; collagen fibers in Tutoplast fascia lata were much more tightly compacted. Interestingly, significant amounts of presumed nuclear remnants were present in both specimens, more so in the cadaveric fascia lata than in the Tutoplast fascia lata. This material potentially could elicit an inflammatory response, leading to enhanced graft resorption.

The ex vivo features of homologous soft tissue materials will have a direct impact on their utility and long-term success, which will also be affected by host factors. Boyce et al1 and Muldashev et al2 believed that replacement tissues fare better when they more closely resemble the tissues of the host bed. The absence of a healthy vascular bed will ultimately lead to severe degeneration and resorption of the allograft. Das et al3 working with autologous fascia, concluded that the success of a fascial graft was determined by the thickness of the graft and the vascular quality of the recipient bed. A vascular bed provides a reserve of inflammatory tissue that can invade, repopulate, replace, or resorb an allograft, and any compromise of the host tissue bed may delay the incorporation of the graft. Ibrahim et al4 noted complete, albeit delayed, fibroblast repopulation and neovascularization of AlloDerm grafts in the setting of early postoperative radiation therapy.

Testing of the fascial and dermal materials showed differences in biophysical properties. As expected, dermal materials were notably thicker than the fascia lata. Fascial materials were stiffer and less elastic, in general, than dermal derivatives. AlloDerm, however, was slightly more elastic than DuraDerm, and the maximum load to breaking was twice as great in AlloDerm than in DuraDerm. Our results correlate well with the data presented by Lemer et al6 (differences in absolute values between these 2 studies are related to differences in technique). Changes to the protein matrix during the processing of DuraDerm may adversely affect the elasticity and strength of the material. Tutoplast fascia lata and AlloDerm sustained greater loads before breaking than did either cadaveric fascia lata or DuraDerm. These data suggest that variations in processing, despite similar source tissues, can have significant effects on the physical characteristics of these tissues. This finding would suggest, for example, that DuraDerm is, to some degree, degraded more from natural dermis than is AlloDerm, as AlloDerm is able to tolerate a greater load but retains greater elasticity than DuraDerm.

A number of investigators have confirmed a dynamic inflammatory response to allograft implantation, which appears to correlate with clinical observations. Muldashev et al2 observed that, after placement of dermis, fascia, or tendon allografts, a severe lymphocytic and neutrophilic reaction was seen around the allograft 20 days after graft placement, with subsequent decline in severity; these workers found that the postimplantation inflammatory reaction could be reduced if the glucosaminoglycans were extracted from the collagen fibers.
before implantation. Merritt et al\textsuperscript{7} documented an increase in local collagen production after transplantation of fascia lata autografts and allografts, and initially postulated that the clinically observed decrease in fascial strength was related to a synchronous enhancement of collagen degradation. In a subsequent article, however, Merritt et al\textsuperscript{7} found that the transplanted fascia was not the mediator of the increased collagen production, which was viewed as part of the recipient wounding response. FitzGerald et al\textsuperscript{8} noted repopulation of freeze-dried fascia lata by host fibroblasts, with varying degrees of cellularity and organization and inflammatory cells in a specimen removed 7 months after insertion. These authors postulated a process of initial degeneration of the collagen fibers, followed by repopulation by host blood vessels and fibroblasts. This repopulation is believed to stabilize the biomechanical qualities of the graft, after an early period of decreasing strength. Broughton et al\textsuperscript{9} noted a 20\% early (first 3 months postoperatively) failure rate. Curtis et al\textsuperscript{10} found a period of weakness between 6 and 12 weeks that later stabilized in dogs undergoing anterior cruciate ligament reconstruction with freeze-dried fascia lata. Graft integrity appears to stabilize by 3 to 6 months after implantation. Aebi et al\textsuperscript{11} described transplanted autogenous fascia lata in monkeys and found degeneration of collagen fibers at 3 months, with subsequent fibrosis by 6 months. Orlando et al\textsuperscript{12} harvested fascia lata 42 years after transplantation and noted indistinct margins, with incorporation of adjacent fat muscle and nerve into the fascia.

Factors acting on the graft will also affect long-term survival and performance. Evaluating irradiated fascia, Cutz et al\textsuperscript{13} noted a decrease in tensile strength in tissue irradiated with 4.0 Mrad compared with 2.7 Mrad. Thomas et al\textsuperscript{14} found that fascial stiffness could be enhanced and strain reduced when the material was tubularized. However, rolling or stacking fascia creates an internal volume of graft that is not in direct contact with the host vascular bed. This area of relatively starved tissue will show a decrease in viable fibroblasts, even while gross volume may remain unchanged.\textsuperscript{1} Burres\textsuperscript{15} advocated the use of diced, freeze-dried fascia lata for lip augmentation, claiming 6 to 12 months of persistence. By implanting small pieces of material, host incorporation of the material presumably may be enhanced; however, this process may also subject the material to greater resorptive forces.

The lower elasticity of fascia compared with dermis-derived grafts is expected and can be related to the microscopic findings. The tighter parallel organization of collagen fibers together with the absence of elastin fibers in fascia are similar to in vivo fascia. The elasticity of the dermal materials examined is similar, but AlloDerm had a significantly greater loading strength than did DuraDerm and was equivalent to that of Tutoplast fascia lata. Clearly, these features should be taken into consideration when different applications for these materials are considered. Different materials may be of varying utility in procedures where the grafts will be placed under considerable tension and are used for structural support. For example, Tutoplast fascia lata or AlloDerm may be a better choice for static sling procedures for facial paralysis than DuraDerm, given the latter’s lower breaking strength. AlloDerm may need to be pre-stretched before placement, given its greater elasticity than fascia lata, to avoid early “stretch-back” and loss of support. The higher elasticity in AlloDerm compared with fascial lata and DuraDerm may explain the suboptimal results found in the first 2 cases reported by Fisher and Frodel.\textsuperscript{16} If these materials are to be used purely for soft tissue replacement or augmentation, a more important feature may be the relative “purity” of the material from remnant cellular debris, such as nucleic acid breakdown products, which may act as antigenic stimuli. Also, dermal materials are considerably thicker than fascia-derived grafts and are better for adding bulk to soft tissues. The significantly greater protein density of AlloDerm compared with DuraDerm may also affect the final degree of augmentation produced with these 2 materials. Histologic and persistence studies of DuraDerm have not yet been published; unless significant host ingrowth and augmentation of the DuraDerm occur, this material may not yield adequate long-term results.

The most obvious concern about allograft materials is their safety. The potential for disease transmission exists when tissue is transplanted from one organism to another. Clarke\textsuperscript{17} described a case of human immunodeficiency virus (HIV) transmission after allograft skin was used for temporary wound coverage. Between 1985 and 1994, only 2 cases of HIV transmission from donor to recipient were documented, from more than 1 million tissue transplant operations.\textsuperscript{18} Simonds et al\textsuperscript{19} described transmission of HIV to 7 of 41 tested recipients of tissue from a donor who was subsequently found to be HIV positive. All of those who seroconverted received implants of either whole organs or unprocessed fresh-frozen bone. No patients receiving lyophilized, ethanol-treated, or gamma-irradiated tissues or marrow-evacuated fresh-frozen bone became HIV positive.

All of the materials examined in this study are processed in ways that reduce the potential for disease transmission. The fascia lata materials were terminally gamma-irradiated, while the dermal materials were chemically decellularized. In addition, one proprietary step in the processing of skin into AlloDerm has been shown to inactivate HIV in vitro. In light of this, acellular tissue grafts, with subsequent chemical or radiation treatment, are preferable to whole organ or cellular transplants in reducing the potential for HIV transmission.

CONCLUSIONS

The processing of homologous tissue is designed to remove immunogenic structures, but should allow the tissue remaining to maintain its normal structure. Radiation and lyophilization are known to degrade tissue to varying degrees, and the surgeon, when choosing an allograft, should consider how different homologous tissue are processed and how degraded they become. Of the fascial materials, Tutoplast fascia lata retains a greater degree of the microscopic structure of untreated fascia than does cadaveric fascia lata. In addition, its biophysical qualities are closer to native fascia than those of cadaveric fascia lata.
AlloDerm and DuraDerm are both derived from human skin, but the different manufacturing processes involved alter these tissues in different ways. On the basis of both the microscopic appearance and the biophysical qualities of these materials, AlloDerm appears to retain more dermal features and qualities than does DuraDerm. AlloDerm is stronger and more elastic and appears very similar to normal dermis microscopically. Theoretically, this should be associated with a higher persistence rate in vivo and more useful and predictable success in facial plastic and reconstructive surgery. Clinical trials are currently under way to test this hypothesis.

Accepted for publication January 11, 2001.

The costs of this study were underwritten by LifeCell Corp, Branchburg, NJ.

Biophysical testing was performed by Sy Griffey, PhD, of LifeCell Corp.

Corresponding author and reprints: Anthony P. Sclafani, MD, Division of Facial Plastic and Reconstructive Surgery, The New York Eye & Ear Infirmary, 310 E 14th St, Sixth Floor, New York, NY 10003 (e-mail: drsclafani@nyfacialsurgery.com).

REFERENCES