Evaluation of Acellular Dermal Graft in Sheet (AlloDerm) and Injectable (Micronized AlloDerm) Forms for Soft Tissue Augmentation

Clinical Observations and Histological Analysis

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Objectives: To evaluate the histological and clinical properties of (1) subdermally implanted acellular dermal graft (AlloDerm) sheets vs intradermal bovine collagen and (2) subdermally or intradermally injected micronized AlloDerm vs type I bovine collagen cross-linked with glutaraldehyde (Zyplast).

Patients: Twenty-five adult patients testing nonallergic to bovine collagen.

Methods: (1) Stacked disks of AlloDerm were implanted subdermally behind one ear, and bovine collagen was injected intradermally behind the other. The soft tissue augmentation caused by the implants was measured by digital photography at 1, 4, and 12 weeks, and biopsy specimens of each implant type were examined at 3 months after implantation. (2) Micronized AlloDerm was injected intradermally and subdermally in 2 different locations behind one ear, and bovine collagen was injected in the same manner behind the other. The soft tissue augmentation caused by the implants was measured by digital photography at the time of implantation and at 1 and 4 weeks after implantation. All implants were examined 1 month after implantation.

Results: All patients tolerated both implants well. (1) AlloDerm implants retained a higher percentage of the original implant volume than Zyplast at 1 and 3 months after implantation. Histologically, AlloDerm implants were extensively invaded by host fibroblasts without any foreign body reaction. (2) Intradermally injected micronized AlloDerm implants retained a higher percentage of the original implant volume at 1 month after implantation than intradermal Zyplast. Histologically, micronized AlloDerm implants were extensively invaded by host fibroblasts without any foreign body reaction. No significant differences were noted between subdermally injected micronized AlloDerm and Zyplast.

Conclusions: The macroscopic and microscopic behavior of subdermally implanted AlloDerm sheets and subdermally and intradermally injected micronized AlloDerm was compared with intradermally injected Zyplast. AlloDerm sheet volume persisted to a significantly ($P<.001$) greater degree than bovine collagen during the first 3 months after placement. Clinically, intradermally injected micronized AlloDerm volume persisted to a significantly ($P=.01$, $.04$, and $.01$, respectively) greater degree than intradermal Zyplast or subdermal micronized AlloDerm or Zyplast. Histologically, micronized AlloDerm and AlloDerm are well tolerated at 1 and 3 months, respectively. Host tissue incorporation with fibroblast ingrowth and collagen deposition is seen in both materials. AlloDerm and micronized AlloDerm hold promise for use in facial soft tissue augmentation.

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Materials available for soft tissue augmentation of facial contour defects can be classified broadly as autologous (dermis and fat) or foreign (homo- grafts and synthetic implants). The ideal material should match the surrounding tissue in texture, pliability, and color; be inert with respect to the patient’s own tissues; neither transmit nor cause any disease in the recipient; and persist and ultimately be integrated into the host tissues. These materials are either surgically implanted or injected. Expanded polytetrafluoroethylene (Gore-Tex) is a synthetic material that is commonly used for facial augmentation. While generally well tolerated, expanded polytetrafluoroethylene is firmer than most facial tissues, requiring deep placement. In addition, as a foreign body, a significant drawback of this material is its nonbiological nature, predisposing the implant to infection and extrusion.
PATIENTS AND METHODS

PATIENTS

Adult patients seen at The New York Eye and Ear Infirmary, New York, between November 1, 1997, and February 28, 1999, were given the opportunity to participate in this study. Patients were tested for an allergic reaction to bovine collagen in the standard fashion and observed for any adverse reaction for at least 30 days. Only patients who had previously been treated with bovine collagen without reaction or those who had negative Zyderm skin test results were allowed to participate.

PREPARATION OF ALLODERM SHEETS AND MICRONIZED ALLODERM

AlloDerm Sheets

AlloDerm sheets are provided in a sterile, freeze-dried form. The material is rehydrated in 2 separate baths of 0.9% isotonic sodium chloride solution for 5 minutes each. Once rehydrated, the AlloDerm sheets are soft and pliable.

Micronized AlloDerm

Micronized AlloDerm was produced under sterile conditions. AlloDerm sheets were cut into 2.0-cm × 1.2-mm pieces with a No. 15 blade. The strips were homogenized (LaTech Macromhomogenizer, fitted with a 20-mm generator probe; OMNI International, Warrenton, Va) in liquid nitrogen to produce microfractures rather than shredding the AlloDerm ultrastructure. The particulate matrix was freeze dried overnight and vacuum sealed in samples of approximately 160 mg.

At the time of use, the micronized AlloDerm was rehydrated with 1 mL of isotonic sodium chloride solution. Part (0.5 mL) of this suspension was withdrawn and used as unwashed micronized AlloDerm. Isotonic sodium chloride solution (3 mL) was then added to the remaining suspension. This was centrifuged at 3000 rpm for 3.5 minutes. The supernatant was aspirated, and the residual pellet of micronized AlloDerm was resuspended with isotonic sodium chloride solution to a final volume of 0.5 mL. This suspension was used as washed micronized AlloDerm. Approximately 6% to 7% of the weight of micronized AlloDerm is lost in this rehydration process, yielding final concentrations of approximately 130 mg/mL. Suspended particles of micronized AlloDerm range from 1.3 to more than 1000 µm; the median particle size was 123 µm, with 68% ranging from 58 to 593 µm.

TREATMENT

AlloDerm Sheets

Patients receiving AlloDerm sheet implants were anesthetized with a topical anesthetic agent (lidocaine and prilocaine hydrochloride in an emulsion base; Astra, Westborough, Mass) over both postauricular areas for a minimum of 20 minutes. Zyplast was then injected intradermally in the patients at 2 sites (0.50 mL each) in the skin overlying the mastoid (just posterior to the postauricular crease), separated by at least 2 cm. The material was injected in small volumes serially, and occupied no larger than a 10-mm-diameter circle; care was taken to avoid migration of the injectant into the skin directly in the postauricular crease. AlloDerm was placed behind the opposite ear. After infiltration of 1% lidocaine, a 1.5-cm incision was made and subcutaneous pockets were developed superiorly and inferiorly. AlloDerm sheets (approximately 1 mm thick) were steriley cut into circular pieces with an 8-mm-diameter dermal punch. A stack of five 8-mm AlloDerm disks was placed into each pocket, separated by at least 2 cm. The wound was closed with 5-0 nylon sutures, which were removed on postoperative day 7.

The patients returned for follow-up visits at 1 week, 1 month, and 3 months after the initial injection. At each visit, patients were evaluated for any evidence of allergic reactions or other complications. A material that attempts to eliminate the problems, such as implant rigidity, necessary preharvesting of the soft tissue defects. Autologen is composed of autologous collagen extracted from skin procured from the patient during previous elective surgery. Dermalogen is similar, except the skin is obtained from tissue banks and processed routinely. The tissue is modified to remove all cellular elements of the dermis, and a suspension of dermal protein fibers is produced. Disruption of the normal relation of the dermal matrix may have negative effects on the performance of collagen. Autologen and Dermalogen do not cause an immune response because the collagen is human in origin. The major drawbacks of these products are the requirement of a previous surgical procedure to acquire skin and a processing time of 3 to 4 weeks (Autologen) and the theoretical risk of disease transmission (Dermalogen). Other materials (lipocytic dermal augmentation products and Isolagen) have been introduced that attempt to induce or replicate a dermal reparative process, thereby producing autologous tissue for augmentation. To our knowledge, reproducible, long-term data are not yet available for these materials.

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visit, the implant sites were inspected and the patients questioned regarding pain, fever, swelling, redness, and any other local or systemic sign or symptom that had developed since the injections. Standardized lateral and posterior digital photographs were taken at 1 and 3 months after implantation with a digital imaging system (Mirror 2000; Virtual Eyes, Kirkland, Wash). This system allowed for measurement of the surface area and lateral projection of the implant. These 2 data points were used to calculate the volume of the implant by assuming a cylindrical form of the implant and multiplying the surface area by the lateral projection. The percentage of implant persistence at 1 and 3 months after implantation was obtained by dividing the calculated volume of the implant by the originally implanted volume. One implant of each type was removed at the 3-month visit under local anesthesia, and the wound was closed with interrupted 4-0 chromic sutures. The remaining implants were left in situ and will be used to assess long-term persistence.

**Micronized AlloDerm**

The patients receiving micronized AlloDerm were also anesthetized with a topical anesthetic agent (lidocaine and prilocaine hydrochloride in an emulsion base, Astra) over both postauricular areas for a minimum of 20 minutes. Zyplast (0.50 mL) was then injected intradermally in the skin overlying the mastoid, and another 0.50 mL was injected subdermally, just posterior to the postauricular crease; these 2 injections were separated by at least 2 cm. Micronized AlloDerm was injected through a 26-gauge needle behind the opposite ear in a similar fashion, with a total volume of 0.5 mL injected at each site. Each patient had 1 injection of washed and 1 injection of unwashed micronized AlloDerm, randomly placed in either a subdermal or intradermal location.

The patients returned for follow-up visits at 1 week and 1 month after the initial injection. At each visit, the implant sites were inspected and the patients questioned regarding pain, fever, swelling, redness, and any other local or systemic sign or symptom that had developed since the injections. Standardized lateral and posterior digital photographs were taken immediately after injection and at 1 week and 1 month after implantation. Again, the apparent surface area and lateral projection caused by the implant was measured, the implant shape approximated to a cylinder, and the volume of the implant calculated. The percentage of implant persistence at 1 week and 1 month after implantation was obtained by dividing the calculated volume of the implant by the originally implanted volume. The augmentation volume of the injection at the time of implantation was also calculated by dividing the calculated volume of the implant by the known amount injected. These percentages were then compared using χ² analysis.

All implants were removed at the 1-month visit under local anesthesia, and the wounds were closed with interrupted 4-0 chromic sutures.

**HISTOPATHOLOGIC FEATURES**

Pathological specimens were sectioned and stained with hematoxylin-eosin and with the Movat pentachrome stain. The sections were examined by light microscopy under low and high power, and were inspected for implant location and persistence, fibroblast infiltration of the implant, and short- or long-term inflammatory response.

Excised specimens were examined by light microscopy under low and high power after hematoxylin-eosin and Movat staining. Fibroblastic activity was described as either none or intraimplant, based on the presence and location of the fibroblasts. Inflammatory activity was described as none, peri-implant, or intraimplant, based on the presence and location of the inflammatory cells; the presence of giant cells was also noted.

This study was approved by The New York Eye and Ear Infirmary Institutional Review Board for the Protection of Human Subjects.

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RESULTS

CLINICAL RESULTS

AlloDerm Sheets

Ten patients were enrolled (5 men and 5 women; average age, 46 years; age range, 37-59 years). Because of variations in the thickness of AlloDerm sheets, the volume of implanted AlloDerm varied between 0.22 and 0.29 mL. No patients experienced any signs or symptoms of implant infection, rejection, allergic reaction, or extrusion.

AlloDerm persistence at all points was statistically greater than that for Zyplast. The mean percentage volume persistence of AlloDerm at 1 and 3 months was 82.8% and 48.3%, respectively, while the mean percentage volume persistence of Zyplast at 1 and 3 months was 26.6% and 8.0%, respectively ($P < .001$ at both times) \(\text{Figure 1}\).

Micronized AlloDerm

Fifteen patients were enrolled (5 men and 10 women; average age, 46 years; age range, 20-58 years). All patients attended their 4-week follow-up visits and biopsy appointments, but 4 were unable to attend the 1-week follow-up visit. No infections developed around the injection sites and no signs of allergic reaction to any implant were noted. Most patients experienced only minimal discomfort for 1 to 3 hours after injection.

The mean percentage volume persistence of washed micronized AlloDerm in any location was not significantly ($P = .08$) different from that of unwashed micronized AlloDerm at any point (80.1% vs 82.7%, 35.8% vs 26.5%, and 25.3% vs 23.9% at the time of injection, at 1 week after injection, and at 4 weeks after injection, respectively). In light of this and the histological results (vide infra), all micronized AlloDerm implants at a given location (intradermal or subdermal) were considered together for further analysis.

The mean percentage volume persistence of all intradermally injected AlloDerm at implantation, at 1 week, and at 4 weeks was 81.4%, 31.2%, and 24.6%, respectively. The mean percentage volume persistence of intradermally injected Zyplast initially, at 1 week, and at 4 weeks was 56.6%, 32.2%, and 12.6%, respectively. The differences at implantation and at 4 weeks between intradermally injected micronized AlloDerm and Zyplast were statistically significant ($P = .004$ and $P = .01$, respectively) \(\text{Figure 2}\).

The mean percentage volume persistence of subdermally injected AlloDerm at implantation, at 1 week, and at 4 weeks was 58.7%, 30.5%, and 14.0%, respectively, vs that of subdermally injected Zyplast, which was 58.6%, 17.8%, and 14.2%, respectively. There was no statistically significant ($P = .80$) difference between the subdermally injected Zyplast and AlloDerm groups \(\text{Figure 3}\).

When intradermally injected AlloDerm was compared with subdermally injected AlloDerm, there was a statistically significant difference in the mean percentage volume persistence noted at implantation (81.4% vs 58.8%; $P = .01$) and at 1 month (24.6% vs 14.0%; $P = .04$). There was no statistically significant ($P = .18$) difference in the mean percentage volume persistence of intradermally vs subdermally injected Zyplast.

HISTOPATHOLOGIC FEATURES

Micronized AlloDerm was present in all 1-month biopsy specimens, and AlloDerm was seen in all 3-month biopsy specimens. Zyplast was observed in all 1- and 3-month specimens. Intraintplant fibroblast activity was noted in all of the specimens with AlloDerm and micronized AlloDerm but not in any of the

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**Figure 1.** Mean percentage volume persistence of acellular dermal graft (AlloDerm) vs type I bovine collagen cross-linked with glutaraldehyde (Zyplast).

**Figure 2.** Mean percentage volume persistence of intradermally injected micronized acellular dermal graft (AlloDerm) vs type I bovine collagen cross-linked with glutaraldehyde (Zyplast).
specimens with Zyplast (Figure 4 and Figure 5). Minimal peri-implant inflammation was observed around all specimens with AlloDerm, micronized AlloDerm, and Zyplast. No giant cell reaction was noted in any treatment group.

COMMENT

We have investigated the use of AlloDerm for surgical soft tissue augmentation. In addition, we examined a new material, micronized AlloDerm (an injectable acellular dermal graft), for the same purpose. Unlike bovine collagen, which is treated to remove the telopeptide units reducing antigenicity but causing loss of collagen fiber alignment, AlloDerm contains only human proteins and replaces dermal deficits with identical tissue, ie, an acellular matrix of dermal proteins. AlloDerm has been used to obliterate soft tissue deficits after tumor excision and for dorsal nasal augmentation, lip augmentation, and effacement of nasolabial folds and depressed scars.10-14 Most researchers note a variable degree of loss of correction immediately postoperatively, after which implant volume appears to stabilize.

The ultrastructure of the dermal matrix is maintained after AlloDerm sheets are homogenized to create the micronized product. The median particle size of micronized AlloDerm is 123 µm, with approximately two thirds of the particles measuring 59 to 593 µm. Twenty-seven percent of the particles, however, measure 52 µm or less and thus are subject to host phagocytosis (S. Griffey, PhD, LifeCell Corp, unpublished data, 1999).

Bovine collagen is the simplest and most commonly used biological filler material; because of this, it remains the criterion standard for injectable biological fillers. It is easily placed and generally well tolerated. However, 3% of patients will display allergic sensitivity to the collagen skin test. The most significant drawback of bovine collagen is its rapid (3-4 month) resorption by the body and loss of clinical effect. Bovine collagen enjoys its status as the most commonly used injectable filler because of its availability and ease in use. Bovine collagen acts as a foreign body and undergoes a slow resorption during a 3-month period, modulated by the inflammatory cells at the implant periphery.

Our investigation of AlloDerm has revealed it to have superior clinical persistence, based on volumetric analysis, having 6 times the percentage volume persistence of implanted material vs Zyplast at 3 months. Histologically, there was a significant difference in the degree of intraimplant fibroblasts, with all AlloDerm implants showing intraimplant proliferation of fibroblasts and Zyplast implants revealing no proliferation. There was no significant difference in the mild degree of peri-implant inflammation noted in both groups. It is clear from this small sample that AlloDerm supports tissue ingrowth and, therefore, volume persistence should continue, decreasing the need for subsequent

Figure 3. Mean percentage volume persistence of subdermally injected micronized acellular dermal graft (AlloDerm) vs type I bovine collagen cross-linked with glutaraldehyde (Zyplast).

Figure 4. Top, Acellular dermal graft (AlloDerm) implant explanted at 3 months. There is fibroblast and endothelial infiltration of the implant (entire lower half of the image) (Movat stain, original magnification ×10). Bottom, Type I bovine collagen cross-linked with glutaraldehyde (Zyplast) implant explanted at 3 months. The implant shows amorphous injected collagen (in the lower third of the image) but no new endothelial or fibroblast ingrowth (hematoxylin-eosin, original magnification ×4).
treatments, which is often the case with injectable bovine collagen. At 3 months, approximately 50% of the initial volume of the implant was clinically visible. Some resorption of the material may occur, or this volume loss may be due to consolidation of scar tissue. Based on these findings, some degree of clinical overcorrection appears to be necessary; the precise degree of overcorrection will depend on factors associated with a particular defect, but overcorrection by a factor of at least 2 may be necessary. Areas where lost tissue volume is being replaced with AlloDerm may be more forgiving that those sites undergoing augmentation (as in our experimental model). In addition, biological implants placed near areas of active muscular contraction and mechanical skin stress may be more prone to resorption of material.

Our investigation of micronized AlloDerm has revealed it to have superior clinical persistence, based on volumetric analysis, with twice the percentage volume persistence of intradermally injected material vs Zyplast at 1 month. Histologically, there was a significant difference in the degree of intramplant fibroblast ingrowth. All AlloDerm implants showed intramplant proliferation of fibroblasts, and Zyplast implants revealed no proliferation. There was no significant difference in the mild degree of peri-implant inflammation noted in both groups. It is clear from this small sample that micronized AlloDerm supports tissue ingrowth; this should continue to stabilize the implant, to improve persistence, and ultimately to decrease the need for subsequent treatments.

Obviously, the long-term benefits and ultimate fate of micronized AlloDerm are unknown. Possibilities include resorption of the micronized AlloDerm with or without replacement by reactive fibroplasia or incorporation of the material with cellular ingrowth. The fibroblasts observed in the interior of the micronized AlloDerm implants could mediate any of these possible outcomes. The clinical effect of subdermal injection of micronized AlloDerm was quickly lost and clearly inferior to intradermally injected micronized AlloDerm. Although injections were initially localized to a discrete area, the loose connective tissue in the subdermal plane in the postauricular area may have allowed rapid dispersion of the micronized AlloDerm. This is in distinct contrast to AlloDerm sheets, which do not disperse and have a broad, continuous surface that anchors quickly to surrounding tissues.

The long-term persistence and histological study of AlloDerm and Zyplast at 12 months after implantation is pending.

We compared the macroscopic and microscopic behavior of subdermally implanted AlloDerm sheets with that of intradermally injected Zyplast. Clinically, AlloDerm sheet volume persisted to a significantly greater degree than bovine collagen volume. Histologically, AlloDerm is well tolerated, supports host tissue ingrowth with fibroblast ingrowth and collagen deposition, and appears to provide improved and longer-lasting correction of soft tissue deficits at 1- and 3-month follow-up visits compared with Zyplast.

In addition, we compared the macroscopic and microscopic behavior of intradermally and subdermally injected micronized AlloDerm with that of intradermally and subdermally injected Zyplast. Clinically, the volume of intradermally injected micronized AlloDerm persisted to a significantly greater degree than the volume of bovine collagen. Histologically, micronized AlloDerm is well tolerated and supports host tissue ingrowth with fibroblast ingrowth and collagen deposition. Micronized AlloDerm holds great promise for use in correction of dermal tissue deficits and may hold significant advantages over injectable bovine collagen and even AlloDerm sheets.

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