Autologous Collagen Dispersion (Autologen) as a Dermal Filler

Clinical Observations and Histologic Findings

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Objective: To assess the histologic behavior and clinical efficacy of autologous collagen dispersion (Autologen) in augmenting human dermis.

Subjects: Adult patients of the Facial Plastic Surgery Clinic at The New York Eye and Ear Infirmary who were undergoing facial aesthetic surgery with skin excision.

Methods: Five patients were injected intradermally with Autologen in one postauricular area and bovine cross-linked collagen (Zyplast) on the contralateral side. Patients were examined clinically for signs of infection, skin necrosis, or implant rejection/allergy 2, 4, and 12 weeks postinjection. Impressions and photographs of all implant sites were taken at all follow-up visits. Biopsy specimens of each implant were taken 4 and 12 weeks after injection and examined histologically for signs of integration, rejection, and resorption.

Results: All implants were well tolerated. No identifiable differences were noted in the clinical persistence of Zyplast vs Autologen. Histologically, there was more variability in the degree of fibroblast infiltration of Autologen vs Zyplast deposits.

Conclusions: Our trial suggests that autologous collagen dispersion may represent a viable alternative to bovine collagen. Clinical persistence and histologic behavior of Autologen appear to be at least as favorable as those of Zyplast, and Autologen obviates the need for allergy testing and eliminates the possibility of disease transmission.

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Surgeons have long recognized the need for a reliable material for soft-tissue replacement. Historically, surgical reconstruction was limited to the most severe cases, in which the preoperative state was so deformed that it warranted the risks of surgery and anesthesia and almost any intervention would achieve some degree of improvement. As anesthetic and surgical techniques have advanced, so have the indications for facial reconstruction. It is no longer acceptable to cover or fill a tissue void with any tissue. Surgeons now strive to replace the missing or defective tissue with analogous material, restoring structural support and/or soft-tissue volume and texture.

The science of soft-tissue fillers has advanced from the indiscriminate use of various viscous materials at the end of the 19th century to careful development of biological materials normally found in the tissue being replaced. Over the past 100 years, progress was hampered by the ill-conceived use of materials such as parafin and adulterated silicone; the science of soft-tissue augmentation is only beginning to emerge from the shadow of these early misadventures.

The current focus of soft-tissue augmentation is loosely divided into 2 philosophies: one approach espouses the use of synthetic polymers. These are known to be relatively permanent, but their physical characteristics are not always ideal, and they remain as foreign bodies. The other approach stresses the use of biological materials either to augment directly or to elicit a reparative process that will deposit enough new tissue to fill the defect.

Autologous collagen dispersion (Autologen; Collagenesis Inc, Beverly, Mass) is derived from the patient’s own skin and consists of a suspension of autologous tissue matrix predominantly composed of intact collagen fibrils. We have investigated the use of Autologen in augmenting facial soft tissue. This study examines the clinical behavior of Autologen as well as histologic findings and technical issues related to its use, with direct comparison with bovine collagen.
SUBJECTS, MATERIALS, AND METHODS

Adult patients undergoing skin resection surgery at the Facial Plastic and Reconstructive Surgery service of The New York Eye and Ear Infirmary between July 1997 and February 1998 were given the opportunity to participate in this study.

Consenting patients were tested for allergic reaction to bovine collagen in the standard fashion. Bovine collagen (0.10 mL) (Zyderm; Collagen Corp, Palo Alto, Calif) was placed intradermally with a 30-gauge needle on the volar forearm and observed for any adverse reaction for at least 30 days. Only patients who had previously been treated with bovine collagen without adverse reaction or who had negative Zyderm skin test results were allowed to participate.

At the time of surgery, hair was trimmed from the resected skin, which was then rinsed with sterile saline and frozen overnight in sterile, normal saline. The frozen specimens were then shipped by express mail to Collagenesis Inc for preparation of Autologen.

During processing of the skin, the epidermis is mechanically removed, and the dermis is minced. The resultant product is mechanically processed to generate a suspension of dermal tissue matrix, which is predominantly fibrillar, non-denatured collagen.

When the Autologen was received from the company, it was immediately refrigerated, and patients were injected within 1 week. Participating patients were anesthetized with a topical anesthetic agent (EMLA; AstraZeneca LP, Wayne, Pa) over both postauricular areas for a minimum of 20 minutes. Patients were then injected intradermally in 2 sites with Autologen (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 an area no larger than a circle 10 mm in diameter; care was taken to avoid migration of the material into the skin directly in the postauricular crease. If less than 1.0 mL of Autologen was provided, the injections were divided into 2 equal aliquots. Zyplast was injected in a similar fashion on the opposite side.

The patients returned for follow-up visits at 2, 4, and 12 weeks after the initial injection. At each visit, the implant sites were inspected, and the patients were questioned regarding pain, fever, swelling, redness, and any other local or systemic symptom or sign that had developed since the injections. One implant of each type was removed (along with overlying skin) at both the 4- and 12-week visits under local anesthesia, and the wound was closed with interrupted 4-0 chromic sutures.

Standardized lateral and posterior digital photographs of the implants (with a ruler for calibration) were taken at each follow-up visit. The surface area and lateral projection of the implant were measured directly using proprietary software (Mirror 2000; Virtual Eyes Inc, Kirkland, Wash). In addition, a mold of the injection sites was fabricated with a dental epoxy (Resoprisl; 3M Products, St Paul, Minn). The volumes of the implants were measured by carefully filling the depressions created by the implants in the mold with water; this was then aspirated with a 1.0-mL syringe and measured. The average of 3 measurements was recorded as the volume. Implant volume persistence was calculated as the apparent volume present (determined either by photography or molds) divided by the original volume of injection.

Pathologic specimens were fixed in formalin, sectioned, and stained with hematoxylin-eosin and with Movat stain. The sections were examined by light microscopy and were inspected for implant location and persistence, fibroblast infiltration of the implant, acute and chronic inflammation, and foreign-body inflammatory reaction. Fibroblastic activity was described as none, peri-implant, or intra-implant, based on the presence and location of the fibroblasts. Inflammatory activity was described as either none, peri-implant, or intra-implant, based on the presence and location of the inflammatory cells.

RESULTS

Ten patients were initially enrolled. Skin excised from 8 patients during surgery (2 rhytidectomies and 6 upper blepharoplasties) was shipped to the manufacturer. The skin provided was insufficient in volume to produce enough Autologen for quality testing and administration in 2 patients (both bilateral upper eyelid specimens). The dimensions of the resected specimens did not differ significantly between those patients who did not provide enough skin for Autologen and the patients providing adequate specimens. Specimen adequacy appeared to be more related to the thickness of the resected eyelid skin than to its surface area. One patient voluntarily withdrew from the study prior to injection with Autologen.

Autologen was injected in 5 patients. The volumes administered varied from 0.2 to 1.0 mL. Impressions and clinical photographs were taken for all patients at the 2- and 4-week visits (Figure 1); however, 2 patients were unable to complete the 12-week impressions and photography. These same 2 patients were unable to undergo the 12-week biopsy.

Data from the 2-, 4-, and available 12-week photographs (Figure 2) and impressions reveal similar patterns of persistence for Autologen and Zyplast. The mean percentages of material still remaining (by photography) at 2, 4, and 12 weeks for Autologen were 81.4%, 60.8%, and 42.5%, respectively. The mean percentages of material remaining (by photography) at 2, 4, and 12 weeks for Zyplast were 78.6%, 51.9%, and 31.1%, respectively. The mean percentages of material remaining (by impressions) at 2, 4, and 12 weeks for Autologen were 34.0%, 29.7%, and 15.0%, respectively. The mean percentages of material remaining (by impressions) at 2, 4, and 12 weeks for Zyplast were 42.0%, 37.7%, and 27.4%, respectively (Table 1). No statistically significant differences between Autologen and Zyplast were noted at any time.

Specimens were scrutinized histologically for fibroblastic and inflammatory activity (Figure 3 and Figure 4). Both Autologen and Zyplast demonstrated a
fibroblastic reaction at the periphery of the implant. Fibroblasts invaded the core of both types of implant, although subjectively this ingrowth appeared to be greater in Autologen samples. The only foreign-body giant cell reactions were noted in 1 Zyplast implant at 4 weeks (Table 2).

COMMENT

Surgical treatment of tissue voids was reported as early as 600 BC and has been described over the past 2500 years for treatment of penal or wartime injuries. Early surgeons used large soft-tissue pedicle flaps to cover defects. However, in the 19th century, surgeons began to look for less invasive methods of treatment for soft-tissue deficits. Since structural support was not needed, the concept of an injectable substance to fill the soft-tissue void became a focus of scientific interest. Many workers have used a staggering number and variety of materials, often ill conceived and without any scientific grounding. These initial investigations (and frequent misadventures) have prejudiced surgeons against implantable biomaterials.

Gersuny\(^1\) reported using paraffin injections to simulate a testicular prosthesis in 1899. Other workers quickly followed, using paraffin as a facial soft-tissue filler. Within 3 years, reports of paraffin pulmonary emboli and foreign-body granulomas had been published. Most Western surgeons abandoned paraffin injections, but paraffin continued to be used as late as the 1950s in the Far East.\(^2\)

Silicone refers to a class of polydimethylsiloxanes that vary in their length and side chains. Silicones are odorless, tasteless, and colorless and have varying viscosities. The history of injectable silicone has been clouded by a variety of factors: silicones differing in their precise structure, manufacturing impurities, postproduction adulteration, poor experimental design, and occasionally inappropriate use. Under optimal conditions, silicone injection leads to a low-grade chronic inflammatory response. Phagocytosis, silicone migration, and recovery from distant organs have been reported with large-volume injections. Granulomas, silicone-induced pneumonitis, hepatitis, and chronic local inflammatory reactions have been reported up to several years after injection. The validity of silicone-induced collagen vascular diseases will continue to be debated for decades to come.

The most common soft-tissue alloplast currently used is polytetrafluoroethylene (Gore-Tex; W. L. Gore & Associates, Flagstaff, Ariz). Gore-Tex has been used successfully for the past 7 to 8 years for facial soft-tissue deficit repair and for the treatment of lip augmentation with limited complications. However, the possibility of infection and extrusion affects the use of any synthetic implant, and no implant currently in use accurately approximates the pliancy, elasticity, and density of skin. More physiological solutions to the problem of soft-tissue defects are currently in use. However, all of these are limited by the one feature for which nonbiologic materials are indisputably superior: implant permanence.

Biologic fillers attempt to replace or augment dermis or subdermal tissue. Autologous fat has been advocated as an easily obtained material for augmentation. However, persistence of injected fat is widely variable,\(^3\) from 20% to 80%, and may be better if injected into muscle.\(^4\) Lipocytic dermal augmentation, described by Coleman et al,\(^5\) theoretically uses the postwounding reparative response to gen-
erate soft-tissue augmentation. Fibrel (Mentor Corp, Goleta, Calif) adds the patient’s plasma (as a source of fibrinogen) to porcine gelatin (as a carrier to localize the inflammatory reaction) and e-aminocaproic acid (as an inhibitor of fibrinolysis). The ensuing coagulation cascade is thought to lead to fibrin and later collagen deposition. Isologen (Isologen Technologies, Metuchen, NJ) also relies on a competent inflammatory and reparative response to wound ing to generate sufficient collagen to achieve adequate augmentation. Isologen is a logical continuation of the subcision technique described by Orentreich, whereby lysis of subdermal scar tissue creates an environment in which the body’s natural reparative process generates sufficient scar tissue to fill a tissue void. Isologen directly adds cultured fibroblasts to conduct this process. However, long-term studies on Isologen persistence have not yet been pub-

<table>
<thead>
<tr>
<th>Time After Injection, wk</th>
<th>Persistence by Photography, %</th>
<th>Persistence by Molds, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Autologen</td>
<td>Zyplast</td>
</tr>
<tr>
<td>2</td>
<td>81.4</td>
<td>78.6</td>
</tr>
<tr>
<td>4</td>
<td>60.8</td>
<td>51.9</td>
</tr>
<tr>
<td>12</td>
<td>42.5</td>
<td>31.1</td>
</tr>
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Table 1. Persistence of Initial Injection of Autologous Collagen Dispersion (Autologen) and Bovine Cross-Linked Collagen (Zyplast) Over 12 Weeks as Measured by Photography and Molds

Table 2. Histologic Findings in Autologous Collagen Dispersion (Autologen) and Bovine Cross-Linked Collagen (Zyplast) Specimens 4 and 12 Weeks After Injection

<table>
<thead>
<tr>
<th>Patient</th>
<th>Fibroblast Location</th>
<th>Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 wk</td>
<td>12 wk</td>
</tr>
<tr>
<td>Autologen</td>
<td>Periphery</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>Periphery</td>
<td>Periphery</td>
</tr>
<tr>
<td>3</td>
<td>Periphery</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>Core</td>
<td>Periphery</td>
</tr>
<tr>
<td>5</td>
<td>Core</td>
<td>Periphery</td>
</tr>
<tr>
<td>Zyplast</td>
<td>Periphery</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>Periphery</td>
<td>Periphery</td>
</tr>
<tr>
<td>2</td>
<td>Core</td>
<td>Periphery</td>
</tr>
<tr>
<td>3</td>
<td>Core</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>Periphery</td>
<td>Periphery</td>
</tr>
<tr>
<td>5</td>
<td>Periphery</td>
<td>Core</td>
</tr>
</tbody>
</table>

*Ellipses indicate data not available.

Figure 3. Microscopic views of autologous collagen dispersion (Autologen) implant 12 weeks after injection (A: hematoxylin-eosin, original magnification ×10; B: Movat stain, original magnification ×40).

Figure 4. Microscopic view of Zyplast implant 12 weeks after injection (hematoxylin-eosin, original magnification ×10).

Bovine collagen is the simplest and most commonly used biologic filler material; because of this, it remains the criterion standard for injectable biologic fillers. It is readily available in large quantities, easily placed, and generally well tolerated. However, a small percentage (approximately 3%) of patients will display allergic sensitivity on the collagen skin test; a much smaller proportion of patients (approximately 1%) can develop allergic sensitivity during treatment. An association of bovine collagen administration with autoimmune disease has never been shown conclusively. However, the most significant drawback to bovine collagen is the relatively rapid (3-4 months) and inexorable resorption by the body and loss of clinical effect. Because of its ease in use and administration, bovine collagen currently enjoys its status as the most commonly used injectable filler.
We have investigated the use of injectable Autologen for soft-tissue augmentation. Autologen and its homologous counterpart (Dermalogen) are derived from human dermis. Autologen is a dispersion of dermal matrix shown to contain intact collagen of types I, III, and VI; elastic fibers; fibronectin; and glycosaminoglycans. This material is easily injected into the dermis using a 30-gauge needle in a fashion similar to bovine collagen. Unlike bovine collagen, which is treated to remove the telopeptide units (reducing immunogenicity and leading to loss of collagen fiber alignment), Autologen and Dermalogen contain only human, nonallergenic proteins; the tight alignment of the collagen fibers and the absence of any xenoproteins were believed to reduce the resultant inflammatory response to the implant and reduce subsequent resorption. The obvious advantage of Autologen is its autologous nature. There is no potential for allergic reaction or donor-to-recipient disease transmission, and the concept of Autologen is very attractive to patients undergoing skin-excision procedures. Those patients not undergoing facial lift, abdominoplasty, breast reduction, or other skin-excision procedures must submit themselves to a skin-harvesting procedure. In response to this issue, the manufacturer has introduced Dermalogen, which is derived from skin obtained from tissue banks. While eliminating the need for skin excision, this material is homologous and has features that are theoretically somewhere in the spectrum between homologous (bovine collagen) and autologous (Autologen) material.

Autologen has been used with good clinical results for treatment of vocal fold paralysis and facial rhytids, but long-term, objective studies have not been performed. In our study, we did not observe a statistically significant difference between Autologen and Zyplast in implant persistence over a 12-week period. However, when examining the implant survival curves, there appears to be a slight trend toward longer persistence of Autologen than Zyplast. Our small sample size and relatively short follow-up may have masked a clinically significant difference between these 2 materials. Larger studies with longer follow-up periods are in development.

In our study, digital photography appeared to be a better method than surface molds to measure the size of the implant. The molds required the measurement of a liquid (water) filling the depression created by the implant. The meniscus formed by this water made precise measurements impossible. Of course, both methods measure only a portion of the implant, which is a 3-dimensional structure with displacement of deep as well as superficial tissues. Laser scanners or ultrasonic devices may be better tools for measuring all surfaces of dermal implants, but high-resolution digital photography allowed us to monitor the clinically relevant (external) effect of the implant.

Histologically, there was no significant difference in the degree of fibroblast infiltration of inflammatory response. Because of the small sample size, no statistical conclusions can be drawn in regard to the clinical differences between Autologen and Zyplast. However, it is unclear whether intact collagen fibers in an altered milieu can avoid eliciting a resorptive process. The degree of protection offered by and the behavior elicited by the association of collagen with various ground substances certainly may affect the body’s response to injection of Autologen. Additionally, the qualities of the skin at the injection site also affect the subsequent behavior of the implant. Clinicians have long recognized that injectable fillers placed in sites subject to significant muscular activity (eg, perioral complex and melolabial folds) undergo a greater degree of resorption than those at other sites. Finally, the tensile and elastic characteristics of skin may also affect injectable implant behavior; replacement of volume in an area of chronologically aged skin and skin with less elasticity may be more successful than augmentation of younger, more elastic, skin.

CONCLUSIONS

Autologous dermal matrix dispersion (Autologen) can be readily produced from the skin that is usually discarded during skin-excision procedures. Autologen is very well tolerated and clinically persists at least as well as Zyplast. There is no need for pretreatment allergy testing, and there is no risk of disease transmission or induction. Further work on Autologen and its homologous counterpart Dermalogen will elucidate the role that these materials will play in replacing facial soft tissue.

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