Autologous Injectable Dermis: A Clinical and Histological Study

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Background: No perfect solution yet exists for dermal fillers. The authors hypothesized that autologous dermis can be processed in an operator-friendly manner and adopted in selected patients as a filler, following the principle of replacing "like with like."
Methods: The authors designed a prototype "cutting chamber" to morsel dermis into an injectable form. Autologous injectable dermis grafting was performed in 16 patients who underwent lip or labionasal fold correction concomitant with abdominoplasty or cesarean scar correction; patient dermis was used for the donor graft. Furthermore, injectable dermis grafting was performed in the subcutaneous tissue of three patients undergoing multistage reconstructive procedures for obesity. The grafts were harvested and examined histologically at 3, 7, and 12 months.
Results: Dermis processing and injection proved feasible with limited effort. All 16 patients presented good volume maintenance by 12 months. Two reported transient palpable firmness for the first 6 months, which subsequently resolved. Histological examination of processed and injected dermis showed volume maintenance over time, effective revascularization of the mass, and structural reorganization with collagen bundles and nested fibroblasts reminiscent of reticular dermis. A transient inflammatory reaction was observed, consistent with the expected healing events.
Conclusions: Use of autologous dermis as a filler substance for both aesthetic and reconstructive procedures appears to be a feasible option. It could be advised for patients requiring filler correction who undergo concomitant procedures involving excision of potential donor dermis. (*Plast. Reconstr. Surg.* 131: 589e, 2013.)
CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, IV.

Despite an ever-increasing variety of dermal fillers used in cosmetic and reconstructive surgery, no perfect solution exists. Over the past 20 years, advances in medical-grade processing, enzymatic digestion, micronization, and lyophilization have enabled manufacturers to market an array of single-component products found naturally in the dermis. Soft-tissue augmentation using acellular allograft dermis has also been described[1] for facial volume reconstruction. Autologous dermal strips or sheets used in cosmetic or reconstructive facial volume rejuvenation have been described for nearly a century,[2,3] but they have had a limited role due to donor-site morbidity, graft bulk, and the need for open-access incisions. Theoretically, dermis grafts seem appealing because they replace "like with like," but to this day, it has not been possible to efficiently process injectable dermis. The current investigation sought to examine three areas of interest with regard to autologous injectable dermis: (1) the practical feasibility of processing injectable dermis as an efficient intraoperative technique; (2) volume maintenance of injected dermis when grafted in the face; and (3) the histological structure of grafted dermis that has been placed subcutaneously.

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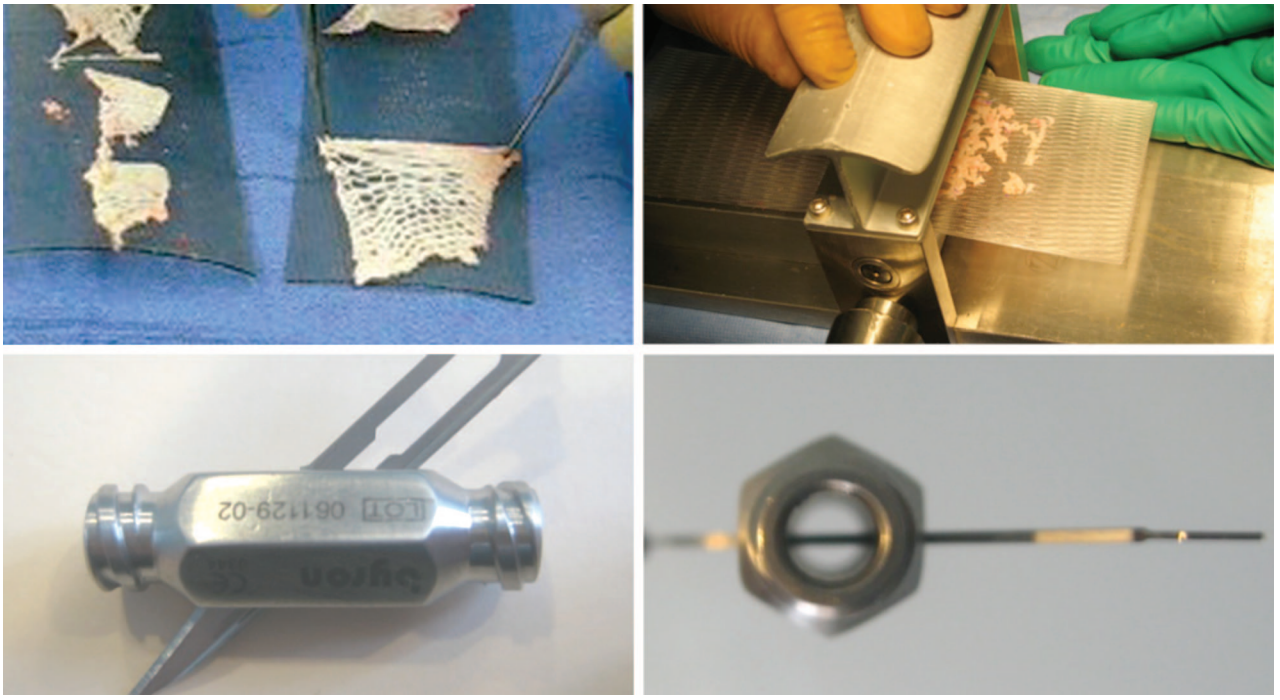

Fig. 1. (*Above, left*) The meshed dermal sheet was turned 90 degrees on a carrier before the second pass. (*Above, right*) Cubed dermal elements are shown emerging from the mesher after a second pass. (*Below*) Prototype of the "cutting chamber" Luer-Lock connector used to morsel dermis.

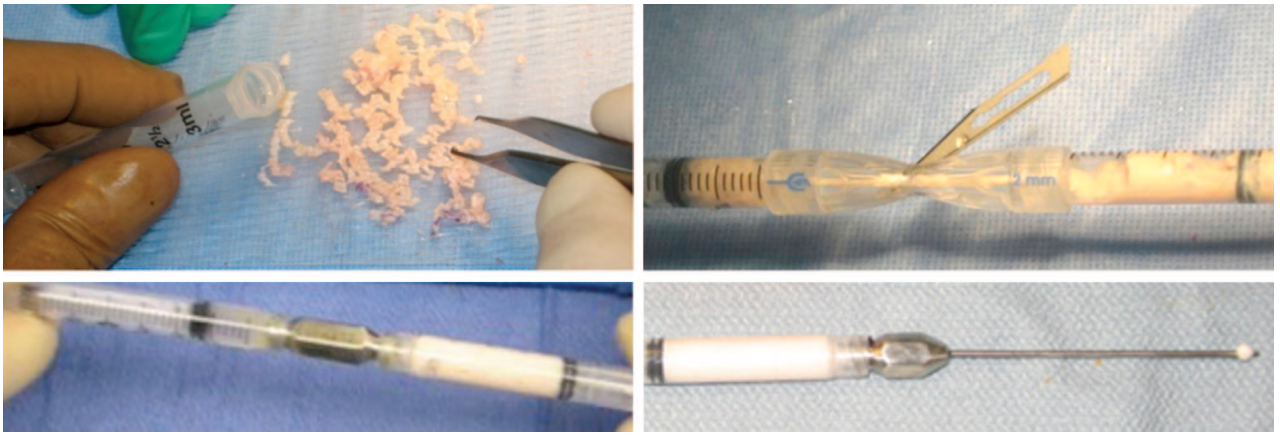

Fig. 2. (*Above, left*) Cubed dermis is placed into syringe plungers. (*Above, right*) The "cutting chamber" demonstrates the to-and-fro motion of dermis passing through the blade. Note the paste-like consistency of the injectable dermis in the syringe on the right. (*Below, left*) Once the graft is morselized, saline can be used as a lubricant to improve flow. (*Below, right*) A syringe of autologous dermis is shown loaded on 16-gauge side-hole needles.

PATIENTS AND METHODS

Processing Injectable Dermis

Over a 2-year period, patients who presented for abdominoplasty or for revision of a cesarean delivery scar ($n = 16$) were selected for autologous dermal grafting to the nasolabial folds or lips using injectable dermis. These patients desired a concomitant increase in volume in their lips or nasolabial folds and expressed a desire for a more natural solution than commercially available products. Augmentation using autologous dermal tissue was proposed and carried out, after patients provided informed consent for dermis grafting in addition to that given for the primary procedure.

During the primary procedure, the following workflow was incorporated to process injectable dermis: *1. Harvest.* Before excision of the abdominal ellipse, 10 to 20 cc of a standard tumescent

solution (30 cc of 1% lidocaine with epinephrine 1:100,000 per liter of normal saline) was injected intradermally. A Padgett dermatome was then used with a thickness of 15/1000 of an inch to de-epithelialize the area to be excised. Once the underlying dermis was exposed, one or two additional passages were made with the dermatome at a thickness of 25/1000 of an inch, resulting in pure dermal sheets of uniform thickness. *2. Cubing.* Dermis sheets were placed on a 1.5/1 carrier and meshed using a Tanner Vanderput mesher (Fig. 1, *above, left*). After one pass through the mesher, the sheet was rotated 90 degrees on the carrier and the sheet was remeshed. By remeshing 90 degrees to the initial mesh orientation, the dermis was cubed (Fig. 1, *above, right*). Additional scissor cutting was performed as necessary for areas in the dermal sheets that failed to cube. *3. Morselization.* Once in a cubed form, the dermis was loaded into 3-cc syringes. A female-female luer-lock hub connector was customized to house a disposable 11 blade in the cross-sectional lumen of the connector and was used as a "cutting chamber" (Fig. 1, *below*). Using the cutting chamber connector between two 3-cc syringes filled with cubed dermis (Fig. 2, *above, left*), the to-and-fro motion of the plungers pushed material from one syringe to the other, pithing the dermis on the blade and further morselizing the graft (Fig. 2, *above, right*). The processed dermis was then mixed with 1 to 2 cc of injectable saline acting as lubricant (Fig. 2, *below, left*). Graft material was then cooled by placing the syringes over sterile ice before grafting (Fig. 2, *below, right*).

Clinical Facial Grafting

After completion of the primary procedure, injection of the nasolabial folds, lips, or both was performed using a blunt, 16-gauge, side-hole needle. Injection was performed in all cases through the oral commissures. No percutaneous injections were performed. Care was taken to inject dermis upon withdrawal of the needle in a methodical manner to avoid bolus injections. Any sensation of flow obstruction in the plunger was addressed not by increased digital plunger pressure but by disconnecting the needle from the syringe and searching with microsurgical forceps for the obstruction in the system. These rare obstructions were most often caused by noncubed fibrous strands of dermis, which were discarded. Because there was no prior clinical experience with the volume yield predictability of processed injectable dermis, it was felt prudent to slightly undergraft all patients and to avoid any overcorrection. Patients were followed up at regular intervals and were photographed at 6 and 12 months.

Histological Study

Patients presenting after massive weight loss at a university plastic surgery unit (University of Padova) were selected for this portion of the study. The patients gave informed consent to the procedure, and the study met the requirements of the Declaration of Helsinki. Massive weight loss patients in this unit are routinely staged for multiple corrective procedures requiring skin excision. Three patients in this arm of the study, aged 48, 51, and 45 years, were selected and gave informed consent to participate. All were scheduled to undergo abdominoplasty as a first stage of a body contouring plan, to be followed at a second stage by thighplasty or brachioplasty.

At the time of the abdominoplasty procedure, the dermis of the excised abdominal ellipse was processed into an injectable form, as outlined above. The dermis was then injected into the thigh or arm regions for which surgical correction had been planned. Injection was performed at the subcutaneous level in multiple parallel linear patterns, all within the area of the future planned skin excision. Nylon 5-0 sutures were placed at the polar ends of each linear subcutaneous injection to further aid in identifying the implant at the time of future harvest.

The second-stage arm- or thighplasty was performed 3, 7, and 11 months after abdominoplasty, with removal of the arm or thigh skin ellipse and accompanying subcutaneous tissue containing dermal grafts. Full-thickness specimens of injected dermal grafts and normal dermis above them were sent for pathologic examination.

The specimens were fixed in 10% buffered formalin and processed according to the usual protocols for paraffin embedding. Specimens were cut into 4-μm-thick sections and stained with hematoxylin and eosin, van Gieson stain for elastic fibers, and periodic acid–Schiff stain.

In addition, an array of immunohistochemical analyses was performed, with staining for cytokeratin (MNF116, 1:400; Dako, Carpinteria, Calif.), smooth muscle actin (1:500; Dako), desmin (1:100; Dako), CD68 (1:500; Shandon, Pittsburgh, Pa.), CD45 leukocyte common antigen, CD3 T-cell marker (1:200; Dako), CD20 B-cell marker (1:200; Dako), carcinoembryonic antigen (1:100; Dako), epithelial membrane antigen (1:200; Dako), and finally CD31 for endothelial cells (1:25; Dako).

RESULTS

Processing Injectable Dermis

Total processing times, including dermal harvesting, cubing, and morselizing, averaged 33 minutes. All graft material was injected via a 16-gauge side-hole needle or via a 16-gauge hypodermic needle. The average volume of injected dermis used in each recipient site (a nasolabial fold, an upper lip, or a lower lip) was 1.5 cc. There were no infections, hematomas, or epithelial inclusion cysts in this patient group.

Clinical Facial Soft-Tissue Augmentation with Autologous Injectable Dermis

At 12 months, all patients demonstrated visible evidence of volume maintenance (Figs. 3 and 4). During the initial 2- to 6-month follow-up, two of the 16 patients complained of palpable firmness along their nasolabial folds (Fig. 5). Expectant treatment over the ensuing 2 to 4 months resulted in complete softening of these areas, without steroid injections or additional surgery. No patients who had lip grafting thought they could feel the grafted material.

Histological Study

Dermis injected into the subcutaneous region of the thigh or arm was harvested and examined 3, 7, or 12 months after grafting. At low magnification, the injected dermal graft was identifiable within the subcutaneous fat, approximately 50 μm from the dermal-subcutaneous junction (Fig. 6). As these grafted dermal elements resided in the subcutaneous tissue, well below the natural anatomic location, they could be readily identified and not confused with native dermis. Hematoxylin and eosin staining of the grafts revealed that they were populated by viable fibroblasts and consisted mainly of mature collagen fibers at all three time points (Fig. 7, *above*).

As early as 3 months and in all subsequent graft specimens at 7 and 12 months, there was CD31-specific antibody activity for endothelial cells in the graft, suggesting a homogenous neovascularization of the grafted material (Fig. 7, *below*). At 3 months, there was evidence of moderate giant cell activity at the periphery of all the grafts, surrounding the grafted material.

The 7- and 12-month graft specimens showed a persistence of volume with no evidence of absorption. Specimens at 7 months showed a diminution of perigraft giant cell activity, and by 12 months scant giant cells were found, located primarily in the periphery. No granulomatous reaction or infiltration of white blood cells was noted, nor were any epithelial cysts identified in the specimens. The pattern of the collagen bundles underwent progressive reorganization, and by 12 months was similar to that of the thick bundles of collagen fibers found in the reticular dermis, with fibroblasts distributed in an orderly fashion, unlike the coarse or hyalinized arrangement usually seen in hypertrophic scarring or keloids.

DISCUSSION

An injectable "cocktail" of cubed dermis, fascia, and fat has been described for soft-tissue facial augmentation.[4] However, the technique has been questioned with regard to the method of processing[5] and the lack of documentation of the histological fate of the grafted material in humans.

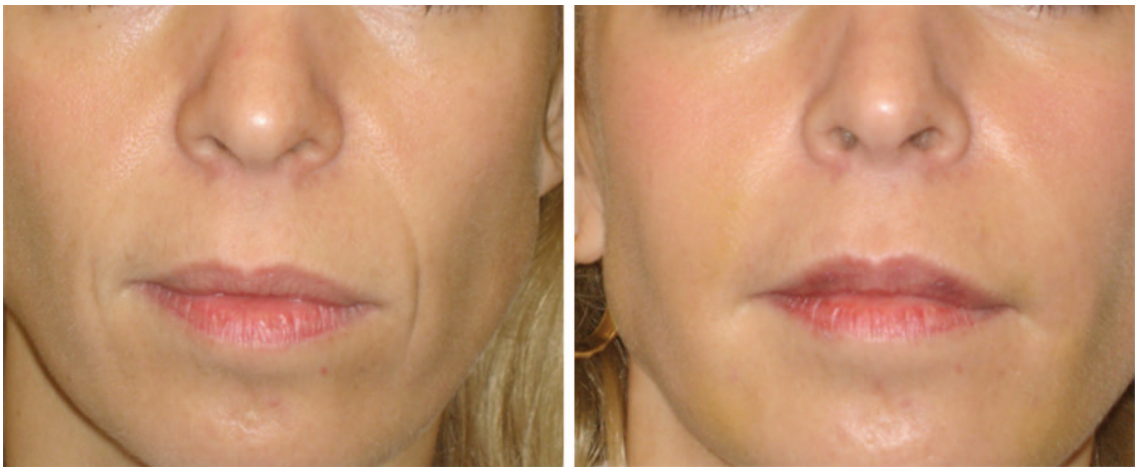

Fig. 3. Nasolabial fold correction. (*Left*) Preoperative view. (*Right*) Twelve months after 1 cc of injectable dermis to each nasolabial fold and the upper lip region.

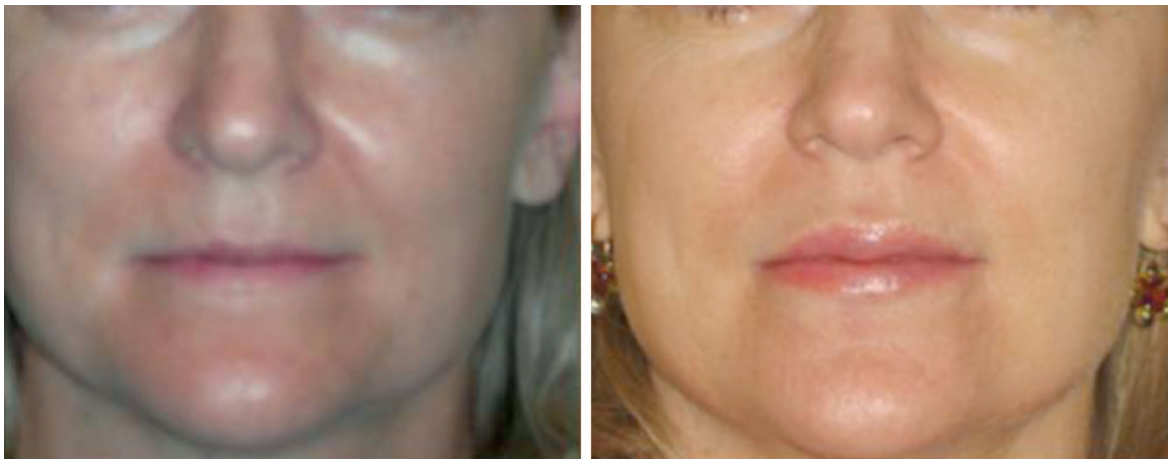

Fig. 4. Lip augmentation using autologous injectable dermis. This patient had 1.5 cc of injectable dermis applied to each lip. (*Left*) Preoperative view. (*Right*) Twelve months after grafting.

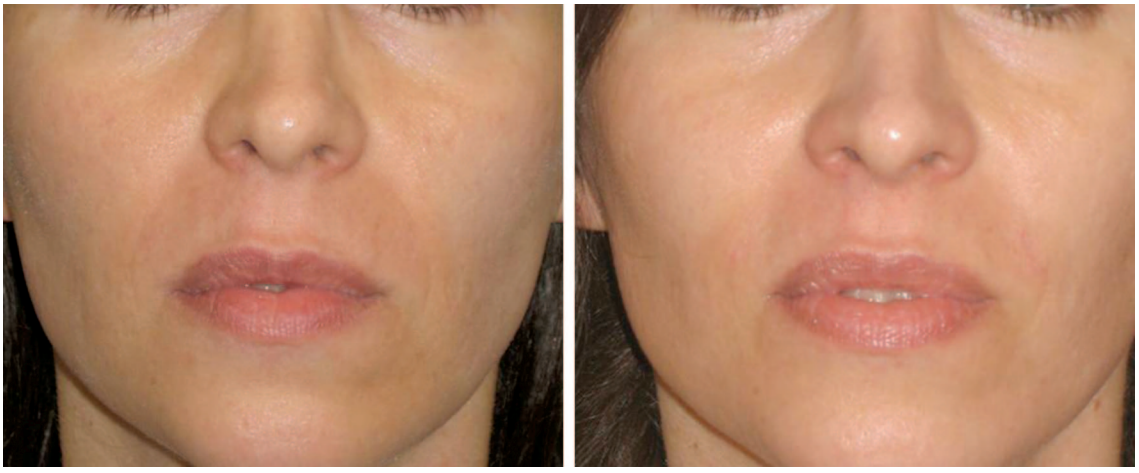

Fig. 5. Nasolabial fold correction. (*Left*) This patient complained of a "woody" feeling in her nasolabial folds 6 months after grafting. This resolved at 12 months. (*Right*) Her result at 12 months.

One obvious limitation to this technique is donor-site morbidity. Given the 116,000 abdominoplasty patients reported by the American Society of Plastic Surgeons in 2011,[6] the application may be limited. However, the rising rate of cesarean deliveries, 32.8 percent in 2010,[7] suggests that every year approximately 1.3 million are performed. Women presenting for nasolabial fold correction or lip augmentation might also have a cesarean scar requiring revision. Applying basic volumetric analysis to our process, a sheet of dermis that is 0.6 mm thick and 4 × 4 cm in size theoretically yields approximately 1 cc of processed injectable dermis (0.06 cm × 4 cm × 4 cm = 0.96 cm^3, 1 cc). Of the dermal elements available from an abdominoplasty ellipse, 5- to 10-cc volumes of autologous injectable dermis can read-

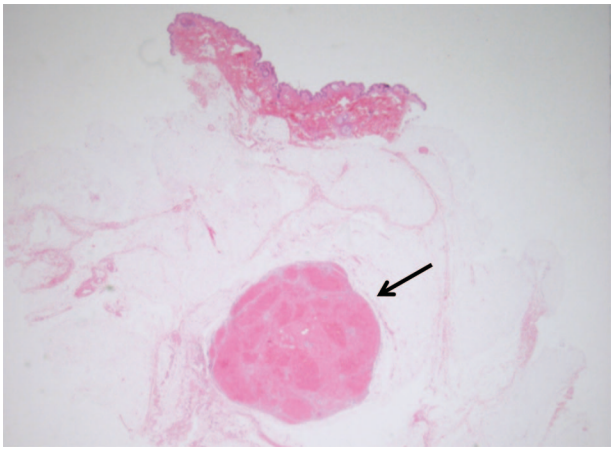

Fig. 6. Injectable dermis graft was identifiable in the subcutaneous fat (*arrow*) at 7 months and the recipient-site native dermis (*above*) in an en-bloc biopsy from a thighplasty patient.

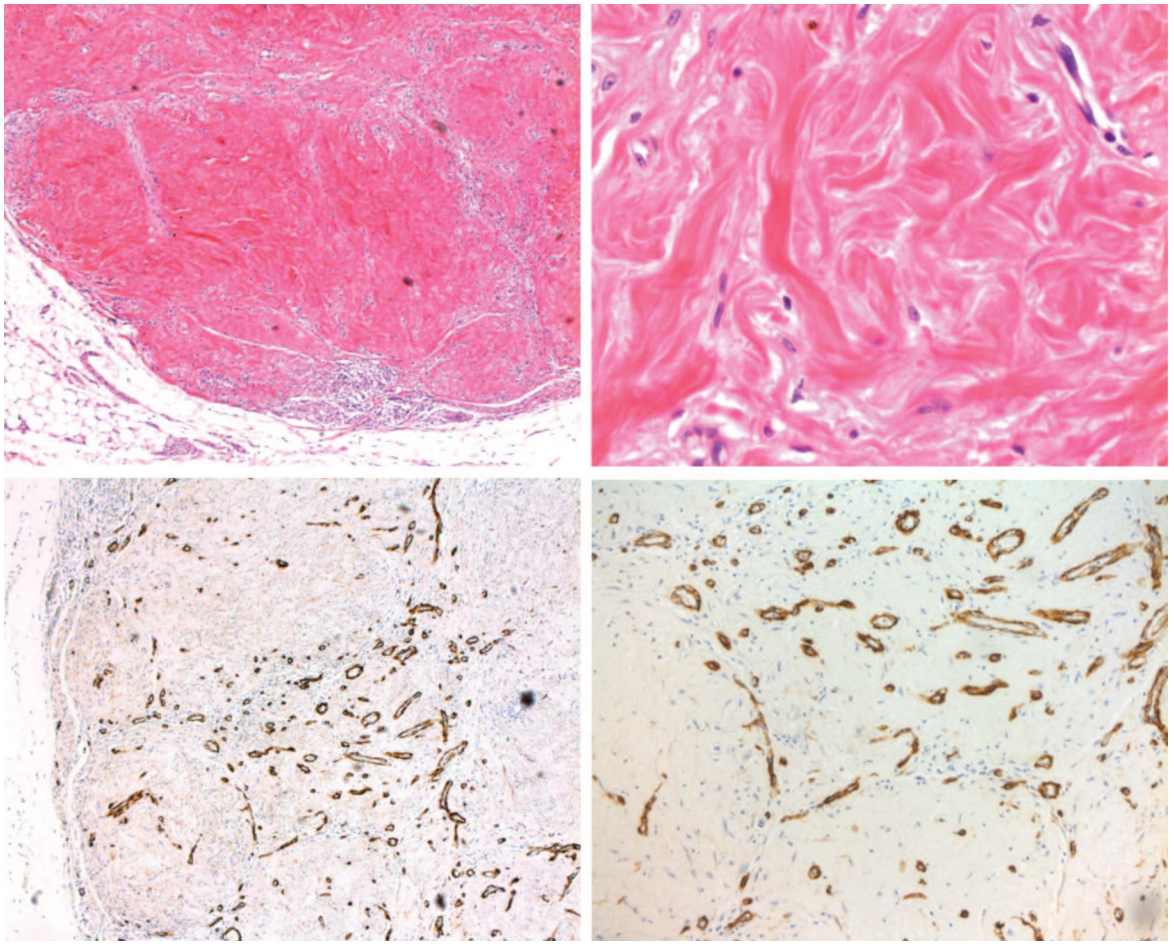

Fig. 7. (*Above, left*) Low-power view of 7-month harvested dermis graft stained with hematoxylin and eosin and (*above, right*) 40× high-power view showing collagen organization with nested fibroblasts, as typically seen in physiological reticular dermis. (*Below, left*) Low-power view and (*below, right*) 40× magnification of 7-month harvested dermis graft stained for CD31 (platelet endothelial cell adhesion molecule-1) endothelial cell marker, showing significant neovascularization of the dermal graft.

ily be processed. For smaller cesarean delivery scar revisions, 2 to 3 cc is feasibly obtained.

When fat transplantation is used as a volume-enhancing strategy, lips appear to be among the most difficult recipient areas in which to obtain consistent long-term results. It has been suggested that certain recipient sites may be "privileged" while others are less so.[8,9] Besides vascularity, suggested as a possible cause, other explanations may involve the relative mobility of the recipient site, due to the constant movement of the orbicularis sphincter, and mechanical positive pressure, which may negatively affect survival and the ability of grafted cells to take during the critical days after grafting.

When transplanted, dermis appears to be a more robust mesenchymal cell construct than fat. Its viability with storage has been described for more than half a century[10]; dermis can be stored for up to 5 weeks with proper refrigeration and storage medium and still be successfully grafted.[11] It is used in acellular, cadaveric form to position breast implants and tissue expanders[12] and to facilitate immediate tissue expansion placement and assist in final aesthetic outcomes in breast reconstruction.[13] Clinical success with partial- and full-thickness skin grafts compared with the best results of fat grafting supports the view that fibroblasts withstand the stresses of the recipient site far better than fat grafts.

If the physiologic durability of dermis is an advantage to survival, its fibrillar structure becomes its disadvantage when one attempts to render it into an injectable form. Enzymatic digestion has been used to render human and bovine dermal sources injectable,[14] but such processing im-

pairs cross-linking and is not easily compatible with a one-step surgical procedure. The mechanical morselization of injectable dermal micrografts that we suggest appears suitable as an intraoperative process, does not affect the quality of the dermis, and, once standardized, became readily reproducible.

The technique of applying injectable dermis in this series of patients did not involve overcorrection. Autologous injectable dermis, as with autologous fat and off-the-shelf long-lasting fillers such as hydroxylapatite gel pastes, should be considered as potentially nonabsorbable materials and must be placed in the appropriate planes to avoid unwanted consequences. Care should be taken to avoid overcorrection, to accurately morsel the tissue, and to place the graft smoothly without bolus injections. The dilution with 20% saline should be taken into account when intraoperatively evaluating volume augmentation endpoints.

Clinically, the two cases of graft firmness completely resolved over time. Remodeling of the dermis, similar to that seen in external skin grafts over time, may explain the transient firmness. Firmness could represent transient postgraft edema as well as be a reflection of the giant cell inflammatory response observed on the serial histological findings. The presence of a giant cell reaction may also suggest the possibility of epidermal inclusion cysts, possibly resulting from the graft being inadequately de-epithelialized at the time of dermal processing. However, the hematoxylin and eosin findings at 7 months demonstrated a diminution of giant cell response. By 12 months the response was barely present. If such a giant cell reaction was due to epithelial inclusions, cysts would be expected clinically and histologically, and one might expect to see increased giant cell activity over time. It is more likely that grafted dermis may elicit a long-term mild inflammatory response as part of a physiological wound healing. As for normal wound healing, in which the remodeling phase is known to last 1 to 2 years, the dermal grafts we studied had an inflammatory response progressively exhausting throughout 12 months. Importantly, the grafts were appropriately vascularized, fibroblasts were present and abundant, and the overall histological structure of the graft did not appear fibrotic. There was an absence of hyalinized thick collagen fibers characteristic of hypertrophic scarring; rather, both collagen fibers and fibroblasts appear to develop a more regular organizational pattern over time.

The histological demonstration of viable injectable dermal grafts over different periods of time has helped identify this technique as a potential option for autologous dermal fillers in various regions of the body, especially the face. Although this technique has previously been mentioned in the literature, to date, there has been no histological evidence of the nature of the volume maintenance, nor was there qualification of results in these grafts.

What seems apparent from the present study is that dermis, when rendered injectable and placed in the subcutaneous position, can (1) survive by diffusion, (2) receive a blood supply via angiogenesis, (3) maintain a fibroblast population of cells, and (4) undergo healing and remodeling without derangement into pathologic scarring.

CONCLUSIONS

Rendering dermis injectable can be performed as an "on-table" intraoperative process that requires 30 minutes on average. The long-term volume maintenance of the graft in the lip and nasolabial fold region appears to be stable at 12 months. The patients in this series, although probably undertreated, all had evidence of volume augmentation, suggesting that this type of graft is robust compared with fat grafting in these areas. Histological examination of injectable dermis transplanted into the subcutaneous position also suggests that grafted dermis survives as a viable unit, exhibiting viable fibroblasts, collagen persistence, organization, and neovascularization. The use of autologous injectable dermis should be considered in patients seeking abdominoplasty or with scars in need of revision in whom concomitant small-volume soft-tissue augmentation is desired.

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