Submucosal Injection of Micronized Acellular Dermal Matrix: Analysis of Biocompatibility and Durability

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Background: Posterior pharyngeal augmentation is a recognized treatment for velopharyngeal insufficiency in selected candidates. To date, however, the procedure has failed to gain widespread acceptance because of the absence of an implant material with sufficient safety, durability, and biocompatibility. In this study, the use of micronized acellular dermal matrix injection for augmentation of the posterior pharynx was investigated. Using a porcine animal model, the safety and durability of posterior pharyngeal augmentation by micronized decellularized dermis was evaluated.

Methods: Twelve Yorkshire piglets were used in this study. Under general anesthesia, porcine-derived micronized acellular dermal matrix was injected into the submucosa of the right side of the pharynx. At 30 days, the animals were euthanized, and the implants and surrounding tissues were assessed grossly for degree of augmentation and histologically to determine the extent of host cell infiltration, vascularization, and matrix deposition and remodeling.

Results: No animal perioperative or postoperative morbidity resulted from the operations. When the animals were euthanized and the tissue was harvested at 30 days, there existed no evidence of gross augmentation on the experimental side of the pharynx in any of the specimens. Histologic analysis demonstrated trace amounts of residual implant, with extensive host lymphocytic infiltration of the material.

Conclusions: Although micronized acellular dermal matrix is a safe material when injected into the pharyngeal wall, this study demonstrated that it is not a durable implant at this site. The authors do not recommend its use for long-term soft-tissue augmentation of the posterior pharyngeal wall in patients with velopharyngeal insufficiency. (*Plast. Reconstr. Surg.* 120: 1156, 2007.)

Velopharyngeal insufficiency is diagnosed in 25 to 50 percent of patients following cleft palate repair and may also result from congenital or acquired neuromuscular disorders or from adenoidectomy.[1,2] Affected patients manifest hypernasal speech and may develop compensatory articulation errors that further compromise speech intelligibility. Nasal regurgitation of liquids is also a common occurrence from velopharyngeal insufficiency.

Many operations have been described to provide for complete closure of the velopharyngeal port, such as the posterior pharyngeal flap and sphincter pharyngoplasty. These procedures, however, result in significant alteration in nasopharyngeal air flow and may be associated with obstructive sleep apnea. In selected patients, posterior pharyngeal augmentation offers a simplified approach to closing the velopharyngeal gap.[3]

Historically, numerous substances have been either implanted or injected to augment the posterior pharyngeal wall, including petroleum jelly, paraffin, silicone, Teflon, bovine collagen, autologous fat, and autologous rib cartilage. Reported drawbacks of alloplastic materials include implant migration, infection, embolization, and extrusion. Moreover, the use of autologous grafts for pharyngeal wall augmentation is associated with unpredictable volume maintenance and donor-site morbidity.[4]

Micronized acellular dermal matrix (Cymetra; LifeCell Corp., Branchburg, N.J.) is a commer-

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cially available injectable graft material developed to provide soft-tissue augmentation. Its composition is similar to that of acellular dermal matrix sheeting (AlloDerm; LifeCell), which contains collagens, elastin, proteins, and proteoglycans. Micronization is achieved by homogenization in liquid nitrogen to produce microfractures rather than shredding of the ultrastructure. This process results in an injectate with a median particle size of 123 μm.[5] Previously, these components have been demonstrated to promote cell repopulation and revascularization. Reported uses of micronized acellular dermal matrix include vocal fold augmentation[6] and cosmetic/reconstructive soft-tissue augmentation.[7] To date, however, there have been no reports of its use in posterior pharyngeal wall augmentation.

We hypothesized that this material could add needed bulk to the posterior pharyngeal wall for the simple treatment of small to moderate gap velopharyngeal insufficiency. Because the injectable matrix provides for long-term soft-tissue augmentation by promoting fibrovascular ingrowth, we anticipated that its use would be associated with excellent biocompatibility and volume maintenance in the posterior pharynx. In short, the aim of this study was to assess the safety and durability of micronized acellular dermal matrix for submucosal augmentation in a porcine model.

MATERIALS AND METHODS

Twelve 4-week-old Yorkshire piglets of unspecified gender were used in this study. All animals were housed and cared for at the large-animal facility at The Children's Hospital of Philadelphia. The experimental and procedural protocols were approved by the Institutional Animal Care and Use Committee of The Children's Hospital of Philadelphia.

The experimental injectate was prepared by mixing 1.0 cc of lyophilized porcine acellular dermal matrix powder with 1.4 cc of sterile, injectable saline to form a homogenous paste. Under general anesthesia, a total of 1.0 cc of this mixture, loaded in a 5-cc syringe, was injected transorally with a 19-gauge needle into the submucosal plane at the junction of the hard and soft palate (Fig. 1). A pediatric laryngoscope with a Miller blade was used for direct visualization. For standardization, the injection site was placed on the right side of midline in all animals so that the left side of the palate could serve as a control. The hard/soft palate junction was selected as the site of submucosal injection rather than the posterior pharyngeal wall, for ease of visualization.

At 30 days, the animals were euthanized by barbiturate overdose. The injection and control sites were assessed in each animal both in vivo and after tissue harvest. After fixing the samples in 10% formalin, the tissues were slowly dehydrated and embedded in paraffin. Subsequently, they were sectioned in the coronal plane at 5 μm and stained with

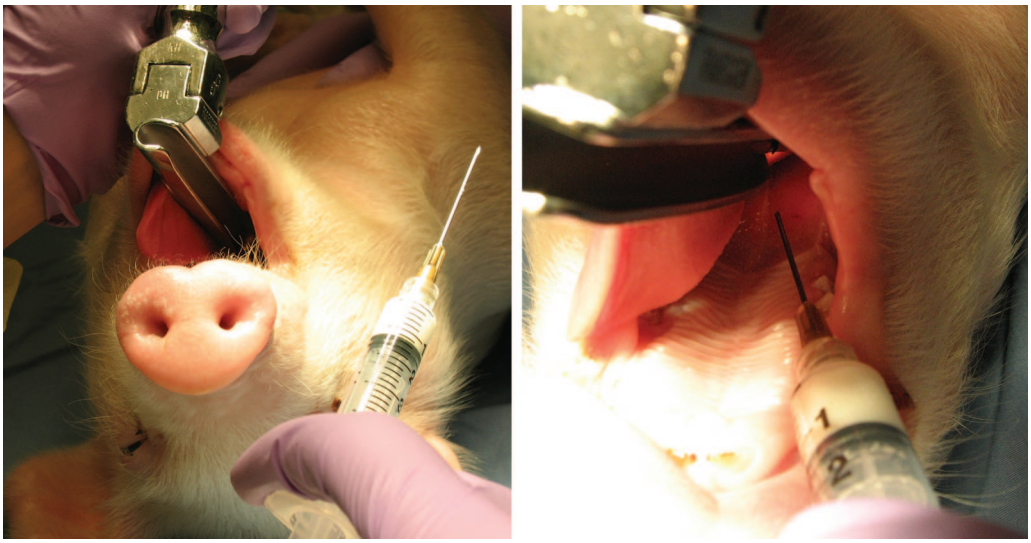

Fig. 1. Transoral injection of micronized acellular dermal matrix into the palate. (*Left*) Direct laryngoscopic visualization of the injection site. (*Right*) Using a 19-gauge needle loaded onto a 5-cc syringe, a total of 1.0 cc of the mixture of porcine-derived micronized acellular dermal matrix and saline is injected into the submucosal plane at the hard/soft palate junction.

hematoxylin and eosin. Under microscopic measurement, the samples were evaluated qualitatively for degree of pharyngeal augmentation and assessed histologically for evidence of vascular and fibroblast ingrowth.

RESULTS

Twelve animals underwent injections with micronized acellular dermal matrix. At the time of injection, all animals demonstrated marked augmentation at the site of injection (Fig. 2, *left*). All animals survived the initial procedures and postoperative period without complication. During the 30-day trial, there were no signs of infection, animal discomfort, or changes in feeding habits.

After the animals were euthanized at 30 days, the animals' mandibles were disarticulated to gain clear visualization of the hard and soft palate (Figs. 2 and 3). In all animals, there was observed to be no residual augmentation at the original injection site. The mucosa at the site of injection appeared to be similar to the surrounding palatal surface.

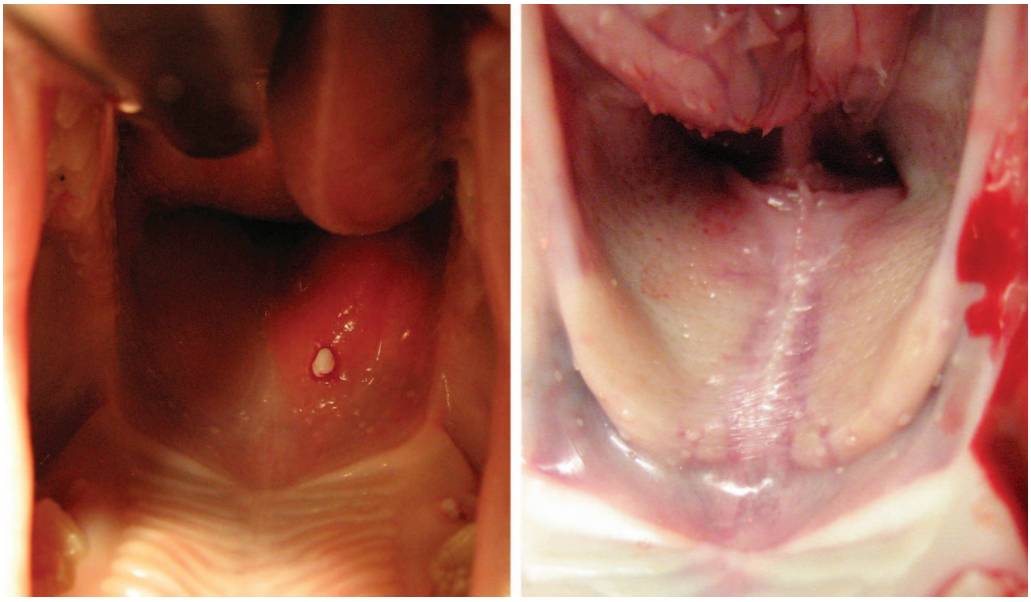

Fig. 2. Comparison of pig palates in situ immediately following micronized AlloDerm injection (*left*) and at 30 days after injection (*right*). The significant augmentation created at the time of injection on the right palate is not observed at 30 days.

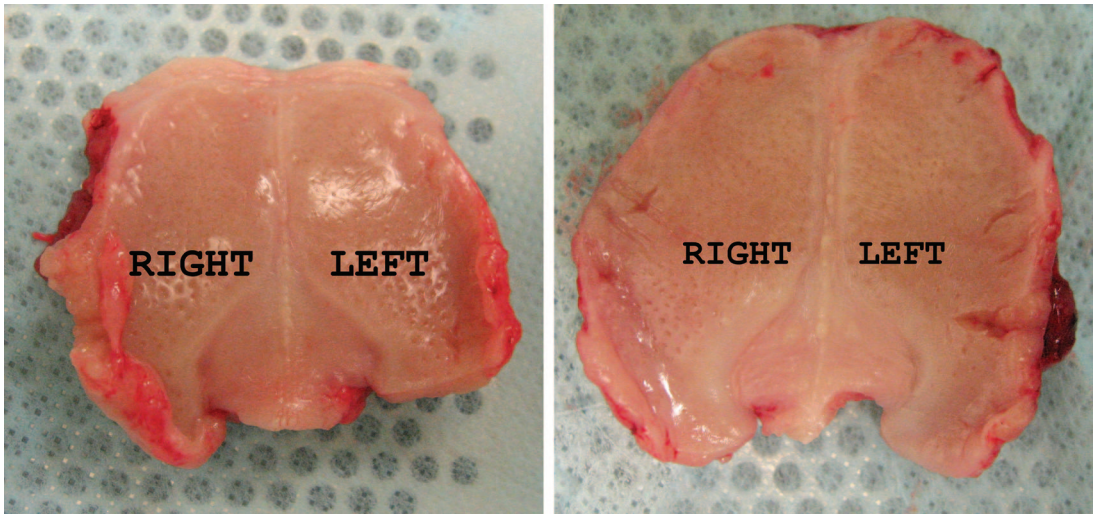

Fig. 3. Ex vivo examination of two specimens harvested at 30 days after injection demonstrates no gross evidence of tissue augmentation. There exists no perceptible difference in the experimental sides (labeled *right*) and the control sides (labeled *left*) within each specimen.

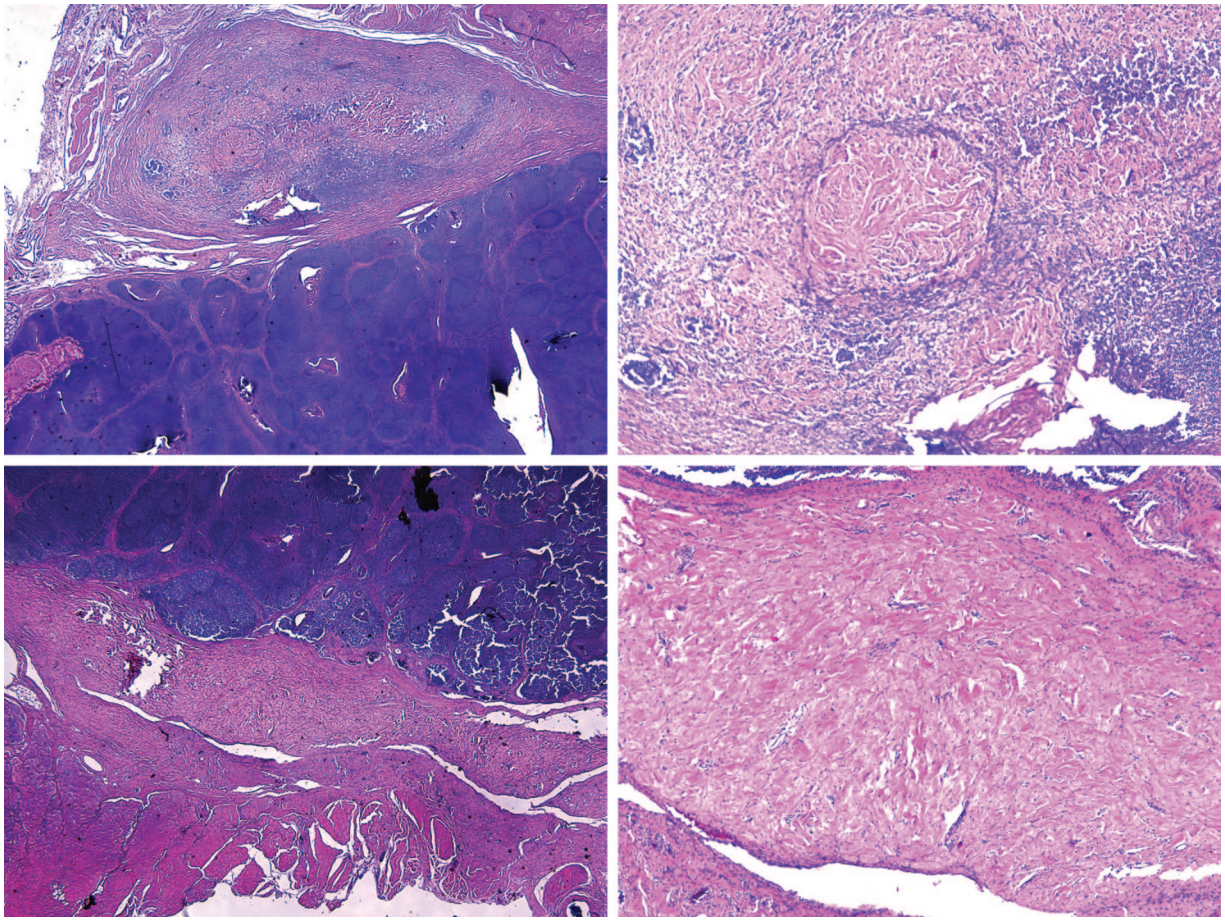

Fig. 4. Histologic analysis of palates at 1 month after injection with micronized AlloDerm at low (*left*) and high power (*right*). There is some evidence of fibroblast invasion of implant. At high power, there is a large host lymphocyte infiltration (hematoxylin and eosin, original magnification at low power, ×4; original magnification at high power, ×10).

On histologic examination, fragments of micronized dermis were present in the submucosal plane in all of the specimens. However, the injected acellular dermis was present in only trace amounts in specimens examined 30 days after the injection. Minimal fibrovascular ingrowth was observed in the vicinity of the injected dermis. Rather, there was a significant infiltration of lymphocytes at all of the injection sites. No histologic changes were noted in the overlying mucosa (Fig. 4).

DISCUSSION

Using a porcine animal model, we have investigated the use of micronized acellular dermis for posterior pharyngeal wall augmentation in the treatment of small to moderate gap velopharyngeal insufficiency. Our specific goals were to evaluate the safety and durability of this material when injected into the pharynx through gross and histologic analyses.

Previous studies have demonstrated the safety of decellularized dermis in its intact and micronized forms when used as an implant.[8] However, to date, there have been no studies that have evaluated the safety and durability of micronized acellular dermis in the oropharyngeal submucosa. All of the animals in our study tolerated the injection of micronized dermis in the pharynx well. There were no postoperative infections or changes in feeding habits.

With respect to implant durability, others have demonstrated an early loss of soft-tissue augmentation when micronized acellular dermal sheeting is used at other sites, such as the lips and skin.[9,10] Sclafani et al.[7] evaluated the clinical and histologic properties of both intact and micronized acellular dermal grafts after subdermal/intradermal implantation in 25 human subjects. The investigators found that at 1 month, acellular dermal sheets and micronized dermal matrix implants exhibited a mean volume persistence of 82.8 and 24.6 per-

cent, respectively. The authors demonstrated a rapid loss in clinical augmentation when micronized acellular dermal matrix implants were injected in the subdermal plane rather than intradermally. They speculated that dispersion of the injectate within the subdermal plane may have accounted for its rapid attenuation.

Our experience suggests that micronized acellular dermis does not persist to any clinically significant degree within the oropharynx following submucosal injection. In all of the animals studied, there was no visible soft-tissue augmentation 1 month after injection. Histologic examination at this time demonstrated only microscopic traces of implant on all samples. Moreover, there appeared to be a cellular host immune response to the implant, evidenced by the presence of lymphocytes at the site of the remaining injectate.

It is uncertain why resorption of micronized acellular dermis occurs so rapidly at this site. Perhaps the pharyngeal submucosal plane allows for rapid dispersion of the substance, as observed in previous studies involving subdermal injection of micronized acellular dermal matrix. Furthermore, in the preparation of commercially prepared human micronized acellular dermal graft (Cymetra), micronization of the dermal sheeting may make the injectate more susceptible to host immunity. Specifically, the resulting median particle size of micronized acellular dermal graft is 123 μm (range, 59 to 593 μm). More than one-fourth of these particles are 52 μm or less, increasing the likelihood for host phagocytosis (S. Griffey, Ph.D., LifeCell Corp., unpublished data, 1999).[7]

Absolute indications for posterior pharyngeal wall augmentation have yet to be fully delineated. Furlow et al.[11] described a cohort of velopharyngeal insufficiency patients in whom posterior pharyngeal augmentation by Teflon injection was attempted. In this study, successful correction of patient velopharyngeal insufficiency was unrelated to either size of velopharyngeal gap (range, 0 to 10 mm) or cause of velopharyngeal insufficiency (cleft palate versus other). Rather, velar mobility appeared most predictive of successful outcome. Until a successful implant material is found and tested clinically, the effective limits of posterior pharyngeal wall augmentation will not be fully known. In those patients who ultimately are found to be candidates for posterior pharyngeal augmentation, there remain clear advantages that favor the use of an alloplastic injectate, such as minimal patient postoperative recovery time and the absence of donor-site morbidity. The use of substances such as calcium hydroxylapatite for soft-tissue enhancement appears promising as a safe and durable agent, although site-specific studies will be required.[12,13]

CONCLUSIONS

From our experience with the animal trial presented in this article, we believe that although micronized acellular dermis is well tolerated when injected into the oropharyngeal submucosa, it fails to demonstrate any degree of durability at this site. We do not advocate its use in the treatment of velopharyngeal insufficiency where long-term augmentation of the posterior pharyngeal wall is desired.

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DISCLOSURE

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