

Mucosal Perfusion Preservation by a Novel Shapeable Tissue Expander for Oral Reconstruction

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Background: There are few methods for expanding oral mucosa, and these often cause complications such as tissue necrosis and expander eruption. This study examines mucosal blood perfusion following insertion of a novel shapeable hydrogel tissue expander (HTE). The canine model used subgingival insertion of HTE following tooth extraction and alveolar bone reduction. The primary goal of this study was to gain understanding of epithelial perfusion and reparative responses of gingival mucosa during HTE expansion.

Methods: Nine Beagle dogs underwent bilateral premolar maxillary and mandibular tooth extraction. Three to four months later, HTE-contoured inserts were implanted submucosally under the buccal surface of the alveolar ridge. After removal and following a 6- to 7-month period of healing, new HTE implants were inserted at the same sites. The area was assessed weekly for tissue perfusion and volume of expansion. Biopsies for histological analysis were performed at the time of expander removal.

Results: Within 2 weeks following the second insertion, blood flow returned to baseline (defined as the values of perfusion measurements at the presurgery assessment) and remained normal until hydrogel full expansion and removal. Volume expansion analysis revealed that the hydrogel doubled in volume. Histological assessment showed no macrophage or inflammatory infiltration of the mucosa. No superficial fibrosis, decreased vascularity, or mucosal change was seen.

Conclusion: Maintenance of adequate tissue perfusion is a clinically important aspect of tissue expander performance to reduce risk of device loss or injury to the patient, particularly for areas with a history of previous surgeries. (*Plast Reconstr Surg Glob Open* 2017;5:e1449; doi: 10.1097/GOX.0000000000001449; Published online 28 August 2017.)

INTRODUCTION

Reconstructive procedures utilized by plastic surgeons often benefit from extra epithelial and supportive tissue

that can be obtained through tissue expansion techniques. Although widely used for skin applications, tissue expansion has less of a role in oral and maxillofacial surgery. Expansion of oral mucosa for the surgical grafting of lost alveolar bone (vertical ridge augmentation) has been particularly challenging because of atrophy following loss of teeth.

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Similarly, challenges have been reported in expanding oral mucosa for cleft palate reconstruction, where alveolar bone grafting is a key part of the procedure.¹ Current practice to cover a bone graft relies on a tension-free mucosa coverage that involves superficial split-thickness flap and a pedicle flap. Many variations of these techniques have been developed^{2,3} though reduction of perfusion in the flaps has been shown.^{4,5} Oral mucosa stretched over a bone graft can necrose because of poor vascularization, exposing the vulnerable bone graft and putting it in contact with microbes of the oral cavity.^{6,7} Exposure of the bone grafts for vertical ridge augmentation, as shown in Figure 1, is a major cause of prerestorative complications that range from significant volume reduction in the final healing stages to complete loss of the bone graft.^{6,8-15} Osmotically controlled hydrogel tissue expanders (HTEs) have been shown to offer a promising new method,¹⁶ applicable both for skin¹⁷⁻²⁰ and oral mucosa (for the purpose of bone grafting).^{7,21} However, rapid expansion of an HTE can lead to ischemia and subsequent necrosis of the expanding skin or oral mucosa, leading to loss of the device and exposure of alveolar bone.^{21,22}

The optimal HTE would be easily inserted, readily altered to the shape and size needed, and designed to expand at a rate that allows constant blood perfusion to the mucosal flap.²² Hydrogel devices are inserted beneath closed mucosa and expand gradually by reaction with moisture from the surrounding stroma. Controlling the mechanical properties of speed and expansion pressure is essential for preserving tissue perfusion while promoting adequate expansion. In this study, a novel osmotic HTE with optimized expansion properties was evaluated for movement, volume change, and mechanical strength of the device, as well as for perfusion in the expanding mucosa. Perfusion effects and device failure rates were

studied in the canine model assessing risks for clinical applications.

METHODS

Canine Model of Tissue Expansion

The study was approved by the Indiana University Institutional Animal Care and Use Committee. Nine female Beagle dogs (Marshall BioResources, North Rose, N.Y.) aged 13 months were used. The animal model of mucosa undergoing surgical manipulation following loss of teeth was created via bilateral extraction of premolar maxillary and mandibular teeth from each dog (a total of 14 teeth). After 3 months, an initial set of HTEs was placed in each of the 4 extraction sites for each dog, via full-thickness flap, using a single curved vertical incision. The 4 sites were in the right and left maxilla, and right and left mandible in the buccal mucosa adjacent to the dental extraction sites. The HTEs were removed on day 42 postinsertion. The dogs were allowed to recover for 3 months, and at this point, the model was considered to be established. A set of HTEs was placed again within the previously surgically manipulated oral mucosa sites, for a period of 42 days. Blood perfusion in the oral mucosa and expander volume were evaluated at each of the extraction sites in each dog immediately before and after the HTE insertion. The insertions were evaluated once a week for 6 weeks. Full-thickness mucosa and soft-tissue biopsies were collected from 7 predetermined locations for each insertion site (Fig. 2).

Osmotic HTE Fabrication

The Restiex HTE (Akina, Inc., West Lafayette, Ind.) was used in the study. The manufactured hydrogels were sterilized by ethylene oxide (Andersen Sterilizer) and aseptically handled before use in surgery.

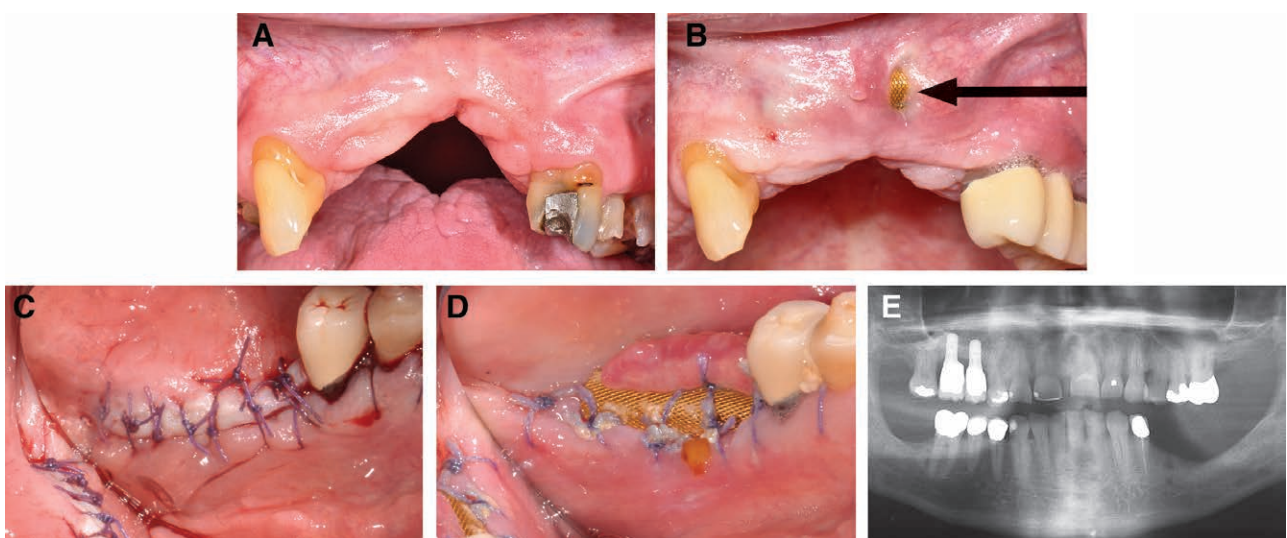


Fig. 1. Alveolar bone graft examples with fenestration (patient 1: A and B) and dehiscence (patient 2: C, D, and E). A, Significant alveolar bone defect from trauma. B, Fenestration first appeared 5 months after bone grafting (black arrow). C, Bone graft for a “vertical ridge augmentation,” left posterior mandible. A significant facial split-thickness flap was done to cover the bone graft. Note the blanching of the mucosa along the incision line. D, Dehiscence at the incision site 13 days following bone grafting. E, Panoramic radiograph of bone resorption on the left as compared with the right side; the radiograph was taken after the failed bone graft.

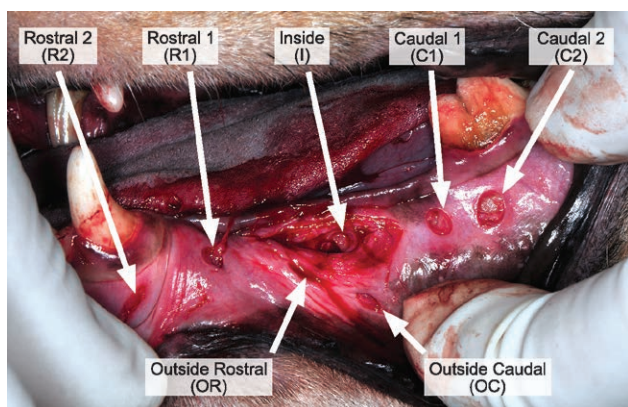


Fig. 2. Biopsy sites. Seven punch biopsies were obtained, through the anterior (rostral) and posterior (caudal) mucosal zones of the expansion site, and into the periosteum. A 2.0-mm circular punch biopsy knife was used.

Laser Speckle Contrast Imager Assessment of In Vivo Tissues

The blood flow at each insertion site was analyzed for a period of 1 minute using full-field, 780 nm Class 1 Laser Speckle Contrast Imager (LSI; FLPI, MOOR Inc., United Kingdom) at the low resolution/high-speed image acquisition rate of 25 Hz. The penetration depth of LSI in these measurements is approximately 1 mm. For each set of measurements, the 4 insertion sites were imaged using 2 regions of interest (ROIs) for each scan. At each insertion site, ROI-1 represents the area of mucosa directly overlying the hydrogel and ROI-2 encompasses a nearby surgically untouched mucosal area (control).

Assessment of Hydrogel Movement and Volume Expansion

FaroArm (FARO Technologies, Lake Mary, Fla.) laser surface scan (660 nm V2 laser probe, Class 2M) was used to obtain a 3-dimensional analysis and dynamics of hydrogel movement within the oral mucosa for 6 of the 9 dogs undergoing the procedure. Each scan was analyzed in chronological sequence to provide a graphic representation of the location of the expanding hydrogel. Any movement of the hydrogel during expansion was determined by the distance between the expander and a static point, usually the adjacent canine tooth.

Each HTE was photographed using a Nikon D90 camera (Nikon Corporation, Shinagawa, Tokyo, Japan) with a Nikon AF-S VR Micro-Nikkor 105 mm f/2.8G IF-ED lens, just before insertion and immediately after full expansion and removal. A ruler was placed in the frame of the photograph for calibration purposes. The camera was mounted on a stand positioned 50 cm away from the specimen. The images were analyzed using Olympus Microsuite 5 software and the dimensional change in width was determined.

Ultrasound images were obtained from the insertion sites of 3 dogs, using Vevo 2100 System (VisualSonics, Toronto, Canada). A MicroScan Transducer LK (40 MHz frequency) was used to scan the implant using B-mode to collect short and long axis views. The change in HTE volume was assessed by measuring their length, height, and width to estimate the volume at weekly time points during expansion.

Histologic Analysis

Immediately after hydrogel removal on day 42, 2.0-mm circular punch biopsies were taken through the anterior (rostral) and posterior (caudal) mucosal zones of the expansion site and into the periosteum at the deepest internal extent of the HTE (Fig. 2). The paraffin-embedded biopsies were oriented in each block so that 4-micron sections of the cylinders included both superficial and deep tissues. Histologic sections were stained with hematoxylin and eosin and with Masson's trichrome stain. Each section was graded for vascularity of the mucosal flap, extent of histiocytic infiltration near the gel, and thickness of the fibrous capsule. Each section was also examined for the presence of any retained hydrogel, chronic inflammatory cells, acute inflammatory cells, and proper orientation of the biopsy core to permit full-thickness evaluation. Hematoxylin and eosin stained slides were used to detect inflammatory cell infiltration and to identify the cell types involved.

Statistical Analysis

Mixed-model analysis of variance tests were performed to compare LSI relative perfusion in the HTE flaps at different insertion sites and at weekly intervals. Analysis of variance comparisons were also made over time and between different insertion sites within each dog. A significance level of 5% was used for all tests. Standard error of the mean values are represented in the graphs.

RESULTS

HTE Volume and Movement Assessment

FaroArm surface contour scanning (images not shown), revealed little to no movement of the expanding HTE, especially after the first 2 weeks postinsertion. The average distance of HTE shift was 2.7 mm. The analysis of the images showed a consistent linear expansion of 32%, which translated to 107% gain of oral mucosa linear dimension. The mandibular sites showed a slightly greater volume increase (21.5 mm³) than the maxillary sites, although the difference is not statistically significant (images not shown). The ultrasound data revealed a steady volume increase of 3.5% per day. The sonographic images of HTE implants showed a uniform hypoechoic zone with clearly defined borders that increased in size over the expansion period (Fig. 3). Similarly, Figure 3D shows the side-by-side comparison of HTE devices before insertion and after 6 weeks of expansion. None of the expanded HTE implants crumbled during removal. Only 5 implants were slightly fragmented (4 broke into 2 large pieces), and the rest was removed as a whole.

Tissue Perfusion Assessment in Oral Mucosa

The LSI blood flow assessment compared the perfusion of the area directly overlying the tissue expander with an area of nonoperated gingival mucosa nearby (control) (Fig. 4). Within the first 2 weeks, this perfusion ratio sometimes showed slight hypo- or hyperperfusion, although not in every case. The slight initial increase in perfusion may represent vasodilation from postoperative inflammation. Slightly decreased initial perfusion may represent

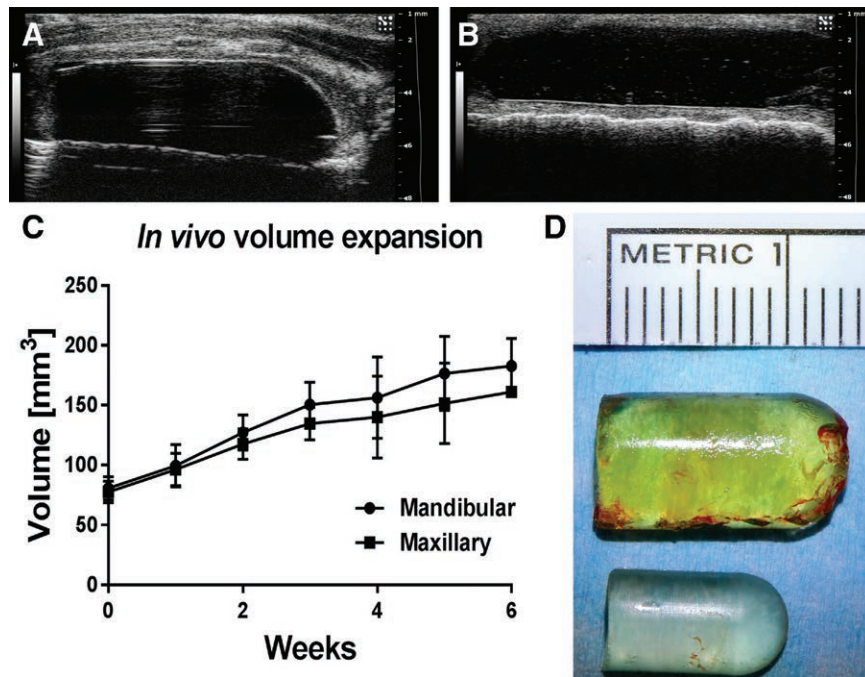


Fig. 3. Qualitative assessment of expanded tissue overlaying the hydrogel using ultrasound analysis of HTE volume change revealed volume doubling at a rate of 3.5% per day. A and B, Representative images of the same hydrogel pellet on days 8 and 45. The echogenic bright lines at the bottom of image represent the periosteum, and the echolucent dark zones are hydrogel. C, Change in volume expansion of the hydrogel for maxillary and mandibular sites in 3 dogs. D, Image showing the hydrogel before the insertion (bottom) and immediately after removal, after 6 weeks of tissue expansion (top). Physical condition of the expanded HTEs was judged as “whole,” “fragmented,” or “crumbled.”

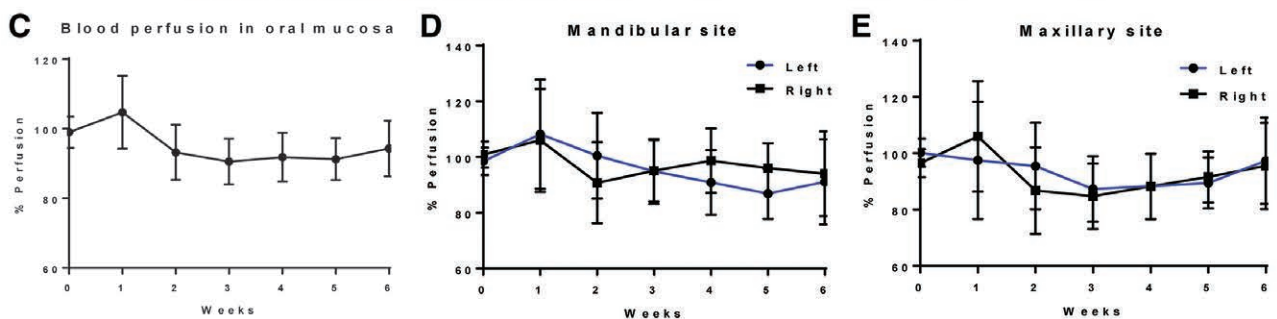
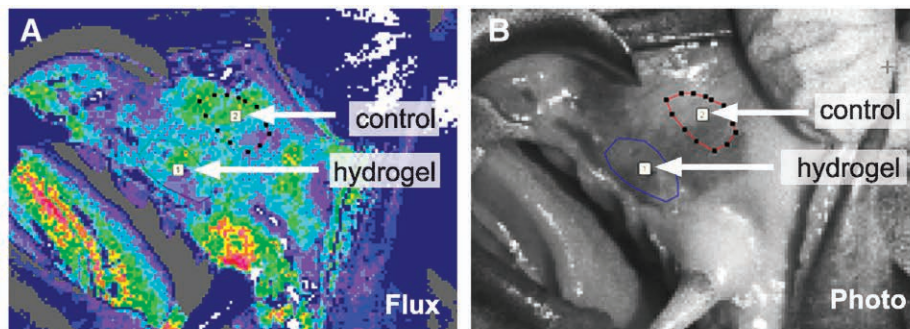


Fig. 4. Quantitative laser speckle contrast imaging assessment of blood perfusion in the superficial layers of oral mucosa overlaying the hydrogel compared with the control site indicates sufficient tissue perfusion maintained following the first 2 weeks after tissue expander insertion. The LSI technique uses the dynamic optical speckle of a low intensity laser shining on the mucosa to quantitatively measure microvascular blood flow. A and B, Flux (laser illuminated) and photographic (white light illuminated) images rendered by the laser speckle contrast imager in maxilla. Arrows mark outlined ROI: hydrogel and control site for comparison. C, Comparisons of weekly mean blood perfusion values expressed as the ratio of hydrogel and control ROI flux. D and E, Comparisons of weekly mean blood perfusion values for mandibular and maxillary sites.

stretching of the vessels before local vasomotor accommodation. After approximately 2 weeks, however, the blood flow ratio returned to a level congruent with the baseline ratio of that area before expander insertion. There were no clinical changes in the expanded area of mucosa such as erythema, blanching, edema, or necrosis.

Histological Assessment of the Oral Mucosa Biopsies

For each HTE site, a total of 7 biopsies were obtained (Fig. 2). Each soft-tissue biopsy was stained with hematoxylin and eosin and with Masson's trichrome stain. The tissue samples around the HTE implant showed no infiltration by chronic or acute inflammatory cells, minimal dense fibrosis, and minimal retained microscopic hydrogel particles after the expander removal (a thin, uniform fibrous capsule surrounding the expanded HTE was grossly observed at the time of removal). Evaluation of these parameters was confirmed by comparison of HTE-related biopsies with control biopsies from nearby unoperated mucosa. Importantly, a slight increase in the vascular density of the tissue flaps was seen in biopsies adjacent to HTE on day 42, suggesting that neovascularization had taken place. This assessment compared the mucosal vascular density in 2 expanded flap sites (rostral and caudal) with the vascular density at 2 control sites (rostral and caudal). The average vascular density for control sites varied from sparse to focally dense, whereas in the areas associated with the expander vascular density, it ranged from focally dense to diffuse dense neovascularity. No difference in vascularity was observed between maxillary and mandibular sites (Fig. 5A). No other changes were detected by histologic examination (Fig. 5B, C).

DISCUSSION

Bone grafting in oral mucosa is a challenging clinical problem because increased bone volume must be accompanied by increased mucosa to allow engraftment and healing of the new graft. Vascular deficiency within soft tissue or mucosal flap tension pose risk of bone graft exposure.⁴⁻⁶ Even slight exposure markedly decreases engraft-

ment of the new bone and creates a need for secondary procedures to manage the complications.⁶

Oral mucosa expansion can be a useful technique for palate reconstructions, extirpation of benign oral lesions, and bone grafting to replace resorbed alveolar ridge. Rapid tissue expansion, however, may cause epithelial necrosis as well as discomfort, expander migration, hematoma, infection, and wound dehiscence.²² Current surgical techniques for covering bone graft with mucosa result in decreased perfusion in the surgically elevated flaps.^{4,5} Figure 1A, B demonstrated a soft-tissue fenestration that first appeared 5 months after a split-thickness flap was placed over a bone graft. Based on the findings presented by Kaner et al.,²³ we believe that reduced blood perfusion in the oral mucosa covering bone grafts is associated with decreased bone oxygenation, reduction of the volume and quality of the bone graft, and overall decline of the healing process.²⁴

More tools are needed that address the concerns of safe and effective tissue expansion.

Self-expanding HTE devices are an attractive alternative to the use of silicone "balloons," which are commonly utilized in plastic surgery. Osmotic devices, carefully formulated to achieve the proper rate and final volume of expansion, are designed for full implantation without the presence of exposed tubes or ports. HTE for use in oral mucosa before a bone graft has shown significantly improved outcomes in dogs²⁴ and humans,²¹ when compared with the traditional split-thickness advancement of the oral mucosa over bone grafts.

Currently HTEs being developed for human use by Oxtex Ltd and Osmed^{GmbH} are enveloped in a silicone capsule that prevents the surgeon from reshaping and resizing the expander. HTEs developed by Oxtex Ltd have been shown to successfully expand palate oral mucosa in pigs.²⁵ HTEs developed by Osmed^{GmbH}, which use a screw to secure it in the desired position, were reported to erupt during oral mucosa expansion in 17%²¹ and 27%⁷ of cases in human subjects. Nevertheless, it is encouraging that Kaner and Friedmann²¹ reported mean vertical bone gain

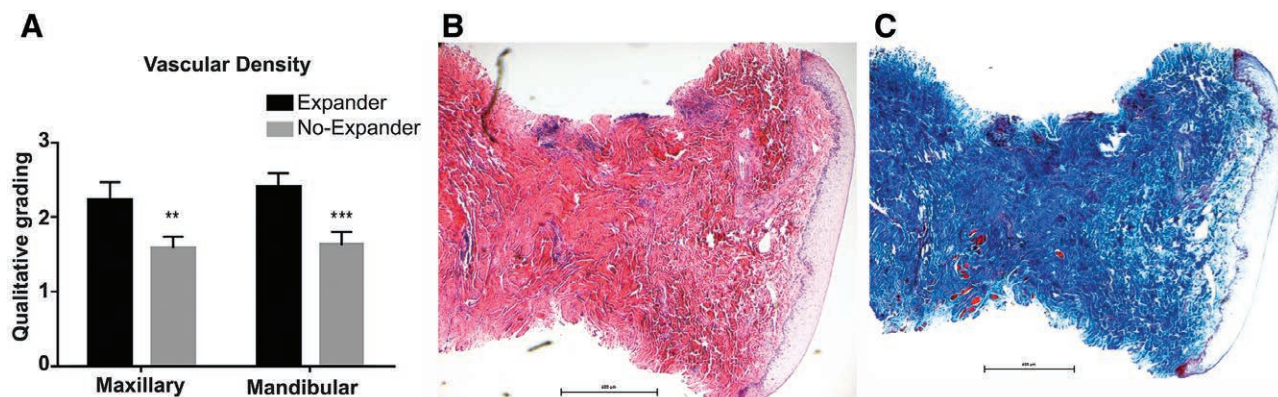


Fig. 5. Histological assessment of the oral mucosa at the time of tissue expander removal indicates no concerning pathological changes, fibrosis, or inflammation, and satisfactory vascularity for both first and second insertions. A, Biopsies from the expander sites (OC, OR) and nonexpander sites (C2, R2) were assessed for vascular density. Score 0 indicated absence of vessels; score 1, scattered small vessels seen mainly at high power (sparse); score 2, frequent small vessels, mainly capillaries (dense); and score 3, many variable sized vessels including large thin-wall neovascularity usually associated with granulation tissue (very dense; ** $P = 0.029$, *** $P = 0.007$). B and C, Histological assessment of tissue in the same dog, mandible, (B, hematoxylin and eosin; C, trichrome).

of 7.5 ± 2.4 mm in 12 patients using HTEs before bone grafting.

The Restiex HTE in the current study causes slow, steady expansion of oral mucosa with only a 3% incidence of device extrusion. Additionally, the lack of a silicone shell allows the expander to be cut to a desired size at the time of insertion (Fig. 6A).

The availability of HTEs that allows a more personalized approach to tissue expansion and being aware that adequate blood perfusion is critical for preventing tissue necrosis²⁶ has resulted in a hypothesis that gradual tissue expansion by a fully implanted device would preserve mucosal perfusion and reduce extrusion of the expanding HTE. Both the rate and final volume of Restiex® HTEs can be adjusted by altering the components in the polymerization formulation. To mimic frequent clinical settings where a bone is grafted in a location with a history of trauma and past surgical procedures, this study used a canine model in which a pre-existing scar tissue from a previous expansion was developed. The evaluation of HTE performance in tissues previously exposed to surgery, which reflects a common occurrence in medical practice, will allow for collection of data that represent the more clinically relevant and challenging scenarios. The main focus of this study is to assess tissue perfusion of expanding oral tissue, particularly since the importance of slight perfusion changes in the mucosa may be underestimated because of the normal rich oversupply of blood vessels in these tissues. To assess the perfusion of oral mucosa, we employed an LSI to record the average perfusion of the entire area of expanding tissue to a depth of 1 mm. The LSI overcomes the limitations of other mucosal perfusion mea-

surement techniques to combine both high spatial accuracy in ROI and high temporal resolution of changes over a wide range of time intervals. The LSI assessment of blood flow in the superficial mucosa over the HTE revealed no circulatory compromise relative to adjacent unexpanded mucosa. The histological assessment revealed no pathological findings and no inflammatory responses. The biopsies showed an increase in the vessel density as other investigators have noted in expanded skin.²⁷ Moreover, the Restiex HTE doubled in volume size and retained its shape and uniform structure throughout the expansion. At the same time, we observed little to no migration of the device. Most likely a thin fibrous capsule that formed within 1 week had secured the hydrogel in position.²⁸ To establish the animal model of previous tissue manipulation, we have subjected dogs to initial tissue expansion, with associated fibrous capsule formation. Although the capsule presence was not observed after the 3 months of healing time during the insertion of our tested HTEs and had likely undergone full resorption, we do acknowledge that the effect of a previously present capsule on the vasculature is not known. In addition, our histology data showed little to no vascularity within the fibrous capsule region.

Although histological changes occur in all tissues following surgical procedures, changes in oral mucosa are less pronounced, though it continues to exhibit altered morphology, following surgery or trauma.^{29,30}

HTEs offer the significant advantage of self-expansion, reducing the frequency of appointments, discomfort, and risk of infection associated with silicone balloon inflation. Because self-inflating tissue expanders cannot be controlled during expansion, HTEs must have precise physical

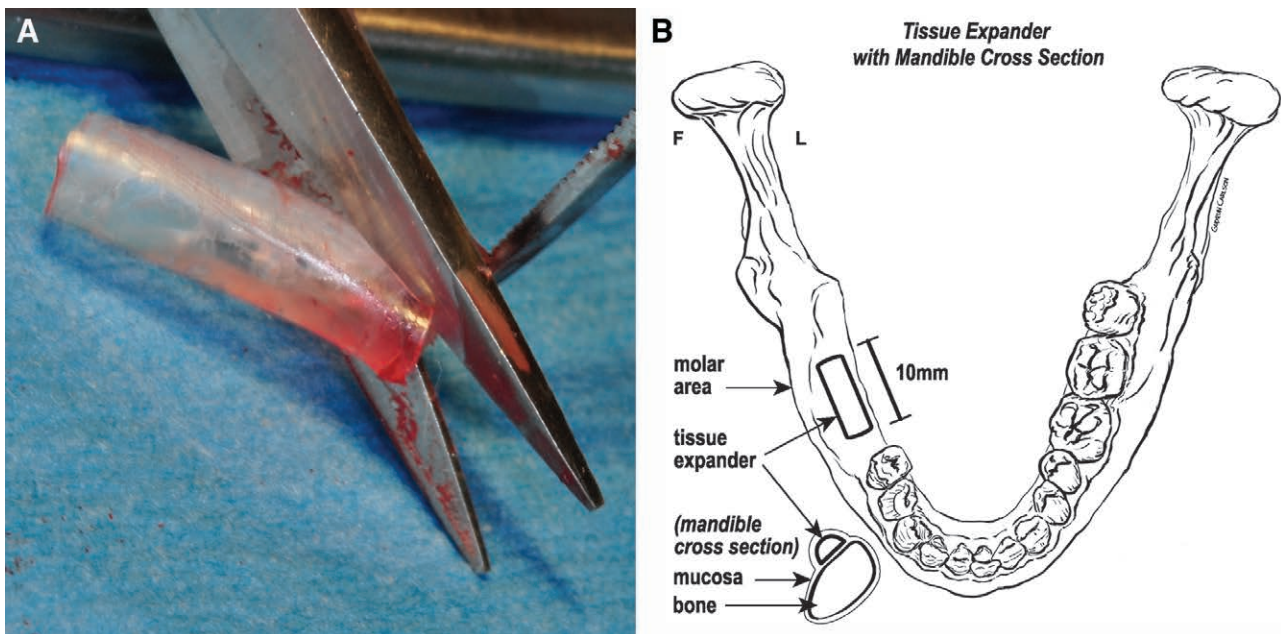


Fig. 6. Restiex HTE used in the study; illustration of tissue expander placement in the mandible. A, The dimensions of the HTE used in the study can be adjusted based on the desired size of the skin or mucosal flap to be generated. B, The HTE is comprised of a combination of hydrophilic polyethers and hydrophobic polyethers crosslinked by acrylate linkages. The hydrophobic polyesters are hydrolytically unstable and spontaneously degrade upon exposure to water in the warm, slightly alkaline environment of tissue interstices. As these portions are cleaved, the crosslinking density and overall hydrophobicity of the expander decrease, allowing the HTE to swell to a larger size over time. This mechanism causes the expander as a whole to swell at a slow, controlled rate over the course of 1–6 weeks.

characteristics (e.g., delay, force, rate, and volume of swelling) for the anatomic area of interest (e.g., mouth, nose, breast) to achieve optimal results and ensure patient safety.^{22,31} Although this study explored a novel Restiex HTE for use in dental reconstructions in our dog study and as illustrated for human use in Figure 6B, a similar HTE has shown remarkable results in closing 19 cleft palates in children.³² Investigation of Restiex HTE for other applications requiring epithelial expansion may be warranted.

In conclusion, we found that gradual tissue expansion with a volume increase of 3.5% per day allows maintenance of normal vascularization and perfusion in canine oral mucosa.

Adequate blood circulation, avoidance of postoperative trauma to the site, and elimination of the possibility of infection are necessary to ensure the expander remains in place and does not cause injury to the patient. The Restiex HTE preserved mucosal flap perfusion throughout the expansion phase and offered unique qualities such as minimal tissue reaction to the device and the ability to be shaped by the surgeon. This animal study indicates that a shapeable HTE may be an attractive device for various tissue expansion purposes in humans.

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REFERENCES

- Weissler EH, Paine KM, Ahmed MK, et al. Alveolar bone grafting and cleft lip and palate: a review. *Plast Reconstr Surg*. 2016;138:1287–1295.
- Greenwell H, Vance G, Muninger B, et al. Superficial-layer split-thickness flap for maximal flap release and coronal positioning: a surgical technique. *Int J Periodontics Restorative Dent*. 2004;24:521–527.
- Greenstein G, Greenstein B, Cavallaro J, et al. Flap advancement: practical techniques to attain tension-free primary closure. *J Periodontol*. 2009;80:4–15.
- Mörmann W, Ciancio SG. Blood supply of human gingiva following periodontal surgery. A fluorescein angiographic study. *J Periodontol*. 1977;48:681–692.
- McLean TN, Smith BA, Morrison EC, et al. Vascular changes following mucoperiosteal flap surgery: a fluorescein angiography study in dogs. *J Periodontol*. 1995;66:205–210.
- Chiapasco M, Zaniboni M. Failures in jaw reconstructive surgery with autogenous onlay bone grafts for pre-implant purposes: incidence, prevention and management of complications. *Oral Maxillofac Surg Clin North Am*. 2011;23:1–15, v.
- Mertens C, Thiele O, Engel M, et al. The use of self-inflating soft tissue expanders prior to bone augmentation of atrophied alveolar ridges. *Clin Implant Dent Relat Res*. 2015;17:44–51.
- Pikos MA. Mandibular block autografts for alveolar ridge augmentation. *Atlas Oral Maxillofac Surg Clin North Am*. 2005;13:91–107.
- Esposito M, Grusovin MG, Worthington HV, et al. Interventions for replacing missing teeth: bone augmentation techniques for dental implant treatment. *Cochrane Database Syst Rev*. 2006;CD003607.
- Lundgren S, Sjöström M, Nyström E, et al. Strategies in reconstruction of the atrophic maxilla with autogenous bone grafts and endosseous implants. *Periodontol 2000*. 2008;47:143–161.
- Rocchietta I, Fontana F, Simion M. Clinical outcomes of vertical bone augmentation to enable dental implant placement: a systematic review. *J Clin Periodontol*. 2008;35:203–215.
- Tonetti MS, Hämmerle CH; European Workshop on Periodontology Group C. Advances in bone augmentation to enable dental implant placement: consensus report of the Sixth European Workshop on Periodontology. *J Clin Periodontol*. 2008;35:168–172.
- Waasdorp J, Reynolds MA. Allogeneic bone onlay grafts for alveolar ridge augmentation: a systematic review. *Int J Oral Maxillofac Implants*. 2010;25:525–531.
- Milinkovic I, Cordaro L. Are there specific indications for the different alveolar bone augmentation procedures for implant placement? A systematic review. *Int J Oral Maxillofac Surg*. 2014;43:606–625.
- Sanz-Sánchez I, Ortiz-Vigón A, Sanz-Martín I, et al. Effectiveness of lateral bone augmentation on the alveolar crest dimension: a systematic review and meta-analysis. *J Dent Res*. 2015;94:1288–1428.
- Asa'ad F, Rasperini G, Pagni G, et al. Pre-augmentation soft tissue expansion: an overview. *Clin Oral Implants Res*. 2016;27:505–522.
- Wiese KG. *Gewebedehnung mit osmotisch-aktiven Hydrogelsystemen – Entwicklung und tierexperimentelle Untersuchungen einer neuen Methode der Gewebeeexpansion [Tissue expansion with osmotic-active hydraulic gel systems – Development and animal experimental investigations of a new method of the tissue expansion]*. Berlin, Germany: Fachbereich Medizin der Georg-August Universität; 1998.
- Bergé SJ, Wiese KG, von Lindern JJ, et al. Tissue expansion using osmotically active hydrogel systems for direct closure of the donor defect of the radial forearm flap. *Plast Reconstr Surg*. 2001;108:1–5, discussion 6.
- Ronert MA, Hofheinz H, Manassa E, et al. The beginning of a new era in tissue expansion: self-filling osmotic tissue expander—four-year clinical experience. *Plast Reconstr Surg*. 2004;114:1025–1031.
- Ronert MA, Hofheinz H, Olbrisch RR. The beginning of a new era: self-filling tissue expander for defect coverage in a 3-year-old boy with a retroauricular nevus. *Plast Reconstr Surg*. 2003;112:189–191.
- Kaner D, Friedmann A. Soft tissue expansion with self-filling osmotic tissue expanders before vertical ridge augmentation: a proof of principle study. *J Clin Periodontol*. 2011;38:95–101.
- Rees L, Morris P, Hall P. Osmotic tissue expanders in cleft lip and palate surgery: a cautionary tale. *J Plast Reconstr Aesthet Surg*. 2008;61:119–120.
- Kaner D, Zhao H, Terheyden H, et al. Submucosal implantation of soft tissue expanders does not affect microcirculation. *Clin Oral Implants Res*. 2014;25:867–870.
- Kaner D, Zhao H, Arnold W, et al. Pre-augmentation soft tissue expansion improves scaffold-based vertical bone regeneration—a randomized study in dogs. *Clin Oral Implants Res*. 2017;28:640–647.

25. Swan MC, Bucknall DG, Czernuszka JT, et al. Development of a novel anisotropic self-inflating tissue expander: *in vivo* submucoperiosteal performance in the porcine hard palate. *Plast Reconstr Surg*. 2012;129:79–88.
26. Yeoh MS, Kim DD, Ghali GE. Fluorescence angiography in the assessment of flap perfusion and vitality. *Oral Maxillofac Surg Clin North Am*. 2013;25:61–6, vi.
27. Pang CY, Forrest CR, Neligan PC, et al. Augmentation of blood flow in delayed random skin flaps in the pig: effect of length of delay period and angiogenesis. *Plast Reconstr Surg*. 1986;78:68–74.
28. Tominaga K, Matsuo T, Kuga Y, et al. An animal model for subperiosteal tissue expansion. *J Oral Maxillofac Surg*. 1993;51:1244–1249.
29. Wong JW, Gallant-Behm C, Wiebe C, et al. Wound healing in oral mucosa results in reduced scar formation as compared with skin: evidence from the red Duroc pig model and humans. *Wound Repair Regen*. 2009;17:717–729.
30. Glim JE, van Egmond M, Niessen FB, et al. Detrimental dermal wound healing: what can we learn from the oral mucosa? *Wound Repair Regen*. 2013;21:648–660.
31. Swan MC, Goodacre TE, Czernuszka JT, et al. Cleft palate repair with the use of osmotic expanders: a response. *J Plast Reconstr Aesthet Surg*. 2008;61:220–221.
32. Kobus KF. Cleft palate repair with the use of osmotic expanders: a preliminary report. *J Plast Reconstr Aesthet Surg*. 2007;60:414–421.