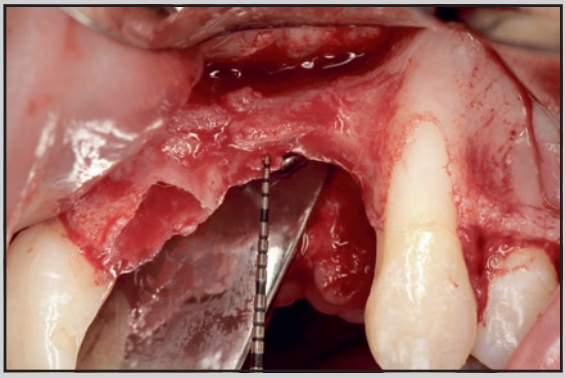
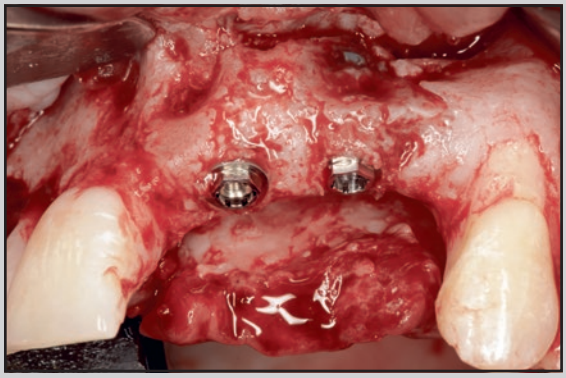
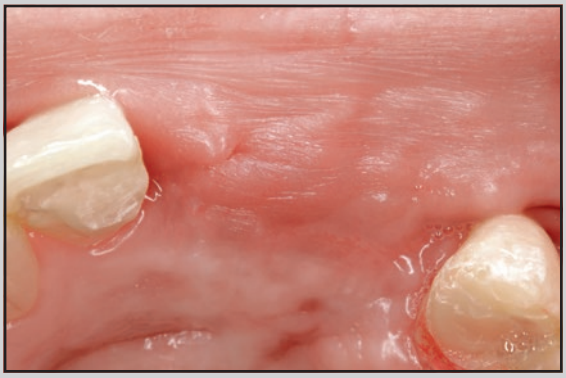
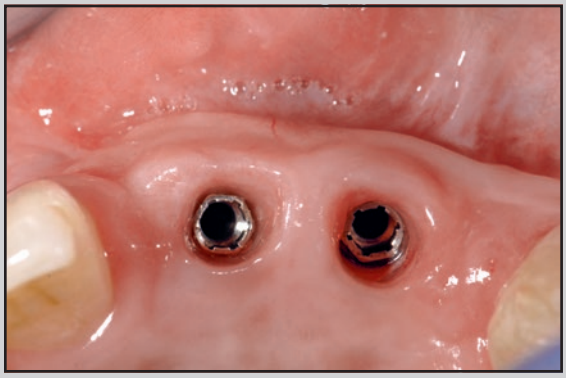
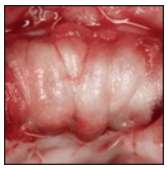





Evaluation of the Combination of Strip Gingival Grafts and a Xenogeneic Collagen Matrix for the Treatment of Severe Mucogingival Defects: A Human Histologic Study



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Predictable and effective surgical techniques that aim to increase the width of keratinized gingiva, relocate the mucogingival junction, and deepen the vestibule often involve soft tissue autografts; however, soft tissue autograft supply is limited and its harvesting is associated with patient morbidity. With a strip autograft and xenogeneic collagen matrix (XCM) technique combination, autograft harvest requirements and patient morbidity are reduced. In this histologic evaluation, 12 strip autograft/XCM biopsy samples were compared with 3 reference samples of palatal strip autografts. Tissue morphology, keratin, and collagen expression appear identical, indicating that the combined grafting technique provides desired and physiologically normal keratinized gingiva.
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To alleviate the need for an extensive autograft harvest, the strip gingival autograft technique was introduced by Han et al.[1] This technique utilizes thin strips of free gingival autografts placed parallel to one another and fixed to the most apical extension of the prepared periosteal bed, leaving the exposed periosteum between the graft strips to heal by secondary intention. However, this surgical approach is technically demanding, time consuming, and may still cause discomfort for patients, as multiple strips of autografts have to be harvested and secured in parallel to help cover large portions of the periosteal bed, which would otherwise have to heal through secondary intention.

Recently, a xenogeneic collagen matrix (XCM; Geistlich Mucograft) was introduced and evaluated.[2] A clinical evaluation combining XCM with strips of gingival autografts was also completed[3] (Fig 1). The primary outcome measure of the study was the increase of keratinized gingiva width from baseline to 12 months postprocedure. Twenty patients were enrolled, and all completed the evaluation. Despite a mean 43% shrinkage of the grafted area at 12 months, all treated sites exhibited a significant gain in keratinized gingiva with a mean width of 6.33 mm (standard deviation [SD]: ± 2.16). The combination graft was well accepted by patients, with minimal morbidity.

Clinically the regenerated tissue appeared to be keratinized; however, histologic proof was forthcoming.

Keratinized epithelium protects the body from oral cavity insults, mechanical damage, and water loss.[4] The proliferating cells that are necessary to continually renew this tissue reside in the basal layer, which is positive for keratin 5 and keratin 14. Upon movement of the cells coronally from the basal layer and concomitant differentiation, the expression changes to keratin 10 and keratin 1. In response to wounds or during various pathologic conditions, expression of keratin 6 occurs, which is associated with abnormal differentiation and, in most cases, hyperproliferation.[5] Assessing the expression patterns of these keratins reveals the state of the gingiva and serves to detect abnormal differentiation.[6–8]

The objective of the human biopsies and histologic analyses reported herein is to evaluate the soft tissue generated by the combined gingival strip + XCM technique, looking particularly for normal keratinized gingiva, when used to augment large areas of soft tissue in advanced regenerative procedures.

Materials and Methods

Patients who participated in the clinical study receiving the combination strip plus XCM technique were informed of the histologic investigation.[3] Ten patients agreed to participate by signing an informed consent, previously approved by the Ethics Committee of the University of Szeged, Hungary. The same surgeon (I.U.) treated all patients. After at least 6 months of healing, narrow (1.5-mm width) soft tissue biopsy samples were harvested through the grafted sites in an apicocoronal direction, coronal to the mucogingival margin, and including both gingival strip and XCM-augmented areas (Fig 1). Biopsy samples of autogenous free gingival graft strips representative of the strip graft therapy were also harvested from the palate to serve as reference histologic standards.

Biopsy samples were fixed overnight in 4% paraformaldehyde and subsequently dehydrated in progressive ethanol solutions with sodium chloride until further use for stainings. Samples were then further processed in a Microm STP 120 Spin Tissue Processor (ThermoScientific), embedded in paraffin, sectioned into 7-µm–thick longitudinal sections, and stained. Hematoxylin and eosin (H&E), Herovici, and immunofluorescence stains were employed according to standard protocols. H&E staining was used to identify tissue morphology, Herovici staining for maturation of collagen fibers, and immunofluorescence staining for different keratin proteins (10, 14, and 6), for an in-depth examination of the characteristics of the regenerated tissue.

For immunofluorescence staining, slides were first deparaffinized, washed, and then treated with primary and secondary antibodies for keratins, eg, antikeratin IgG (K6, PRB-169P, Biolegend; K10, M7002, Dako; K14, PRB-155P, Babco) and Cy3-conjugated anti-IgG (Covance). Image acquisition was performed with a Zeiss Imager.A1 with an Axiocam Mrm camera and EC Plan Neofluar objectives (10×/0.3 and 20×/0.5, ZEISS). Images were stitched together longitudinally using Adobe Photoshop CS3 (Adobe Systems).

Results

Fifteen biopsy samples, 3 of which were free gingival graft strips used only for reference, were retrieved from 10 patients between 6 months and 1.5 years postoperative. The biopsy samples were processed and analyzed. Representative sample sections are shown in Figs 2 and 3. The treatment-site samples were indistinguishable from the reference samples. Keratinized attached gingiva was distinguished histologically by the distribution and nature of inelastic (gingival) collagen fibers and long, slender (gingival) papillae, with defining surface layers of keratin. Keratins were appropriately expressed in the treated biopsy samples, indicating a normal differentiation pattern of oral keratinocytes. Accordingly, keratin 14 was expressed in the basal layer and detectable throughout the epithelium up to the most coronal layers of cells; keratin 10 was expressed in the first and more coronal suprabasal layers; and keratin 6 was expressed at a more apical level than keratin 10, in the hyperproliferative regions. There were also no obvious differences in the deposition of mature or immature collagen (collagen I and collagen III, respectively), as determined by Herovici staining.

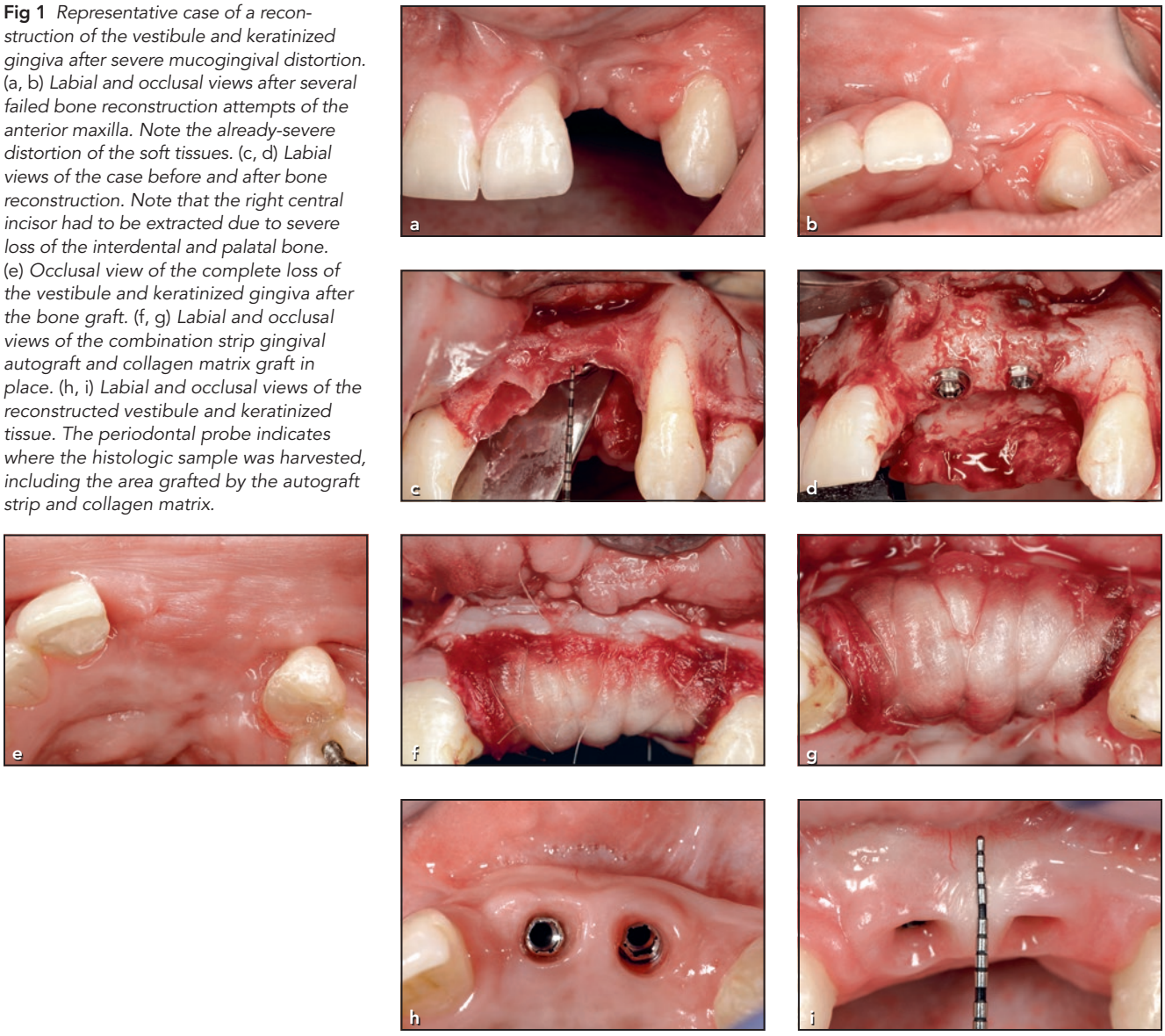

Fig 1 *Representative case of a reconstruction of the vestibule and keratinized gingiva after severe mucogingival distortion.* (a, b) *Labial and occlusal views after several failed bone reconstruction attempts of the anterior maxilla. Note the already-severe distortion of the soft tissues.* (c, d) *Labial views of the case before and after bone reconstruction. Note that the right central incisor had to be extracted due to severe loss of the interdental and palatal bone.* (e) *Occlusal view of the complete loss of the vestibule and keratinized gingiva after the bone graft.* (f, g) *Labial and occlusal views of the combination strip gingival autograft and collagen matrix graft in place.* (h, i) *Labial and occlusal views of the reconstructed vestibule and keratinized tissue. The periodontal probe indicates where the histologic sample was harvested, including the area grafted by the autograft strip and collagen matrix.*

Discussion

The need for a minimum amount of keratinized gingiva around teeth and implants to preserve the health and stability of the periodontium and peri-implant tissues is controversial but generally accepted. There are certain clinical situations in which soft tissue augmentation by mucogingival surgical techniques can be justified and indicated[9–14]; despite the controversy, clinicians prefer seeing keratinized gingiva. Major bone augmentations can result in severe translocations of the mucogingival junction and loss of the vestibule, which can limit lip mobility.[15–18] Surgical techniques aimed at increasing the width of keratinized gingiva, relocating the mucogingival junction, and deepening the vestibule include apically repositioned flaps and periosteal fenestrations.[19–21] Although the short-term outcome of these procedures is favorable in many cases, rebound typically occurs within a few months, and achieved tissue gains may be lost.[22,23] To achieve more stable results, soft tissue

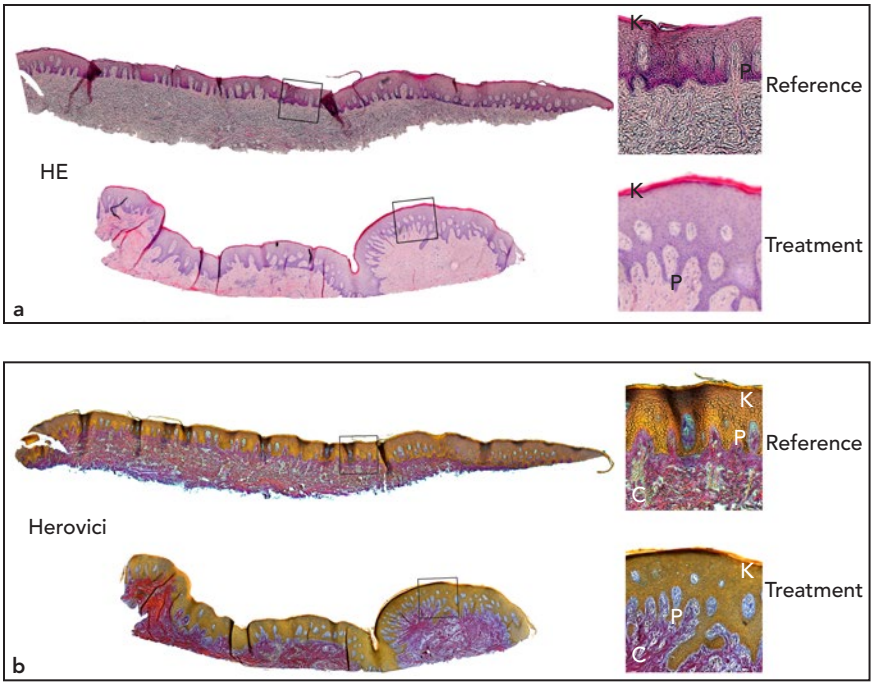

Fig 2 *Reference free gingival graft strip and treatment biopsy samples (×100) with hematoxylin and eosin (H&E) and Herovici stainings. Both the overall morphology of the epithelial, papillary, and reticular gingiva, as well as the specific staining for mature and immature collagen, indicated no difference between the reference and treatment samples.* (a) *Hematoxylin stains the epithelium predominantly blue, while the eosin stains connective tissue predominantly pink. The morphologies of the reference and treatment samples were indistinguishable.* (b) *Herovici stains immature collagen blue and more mature collagen red. The distribution of this staining did not differ between the reference and treatment samples, confirming that the connective tissue was indistinguishable between reference and treatment biopsies. K = keratinized epithelium; P = papillary gingiva; C = region stained for collagen.*

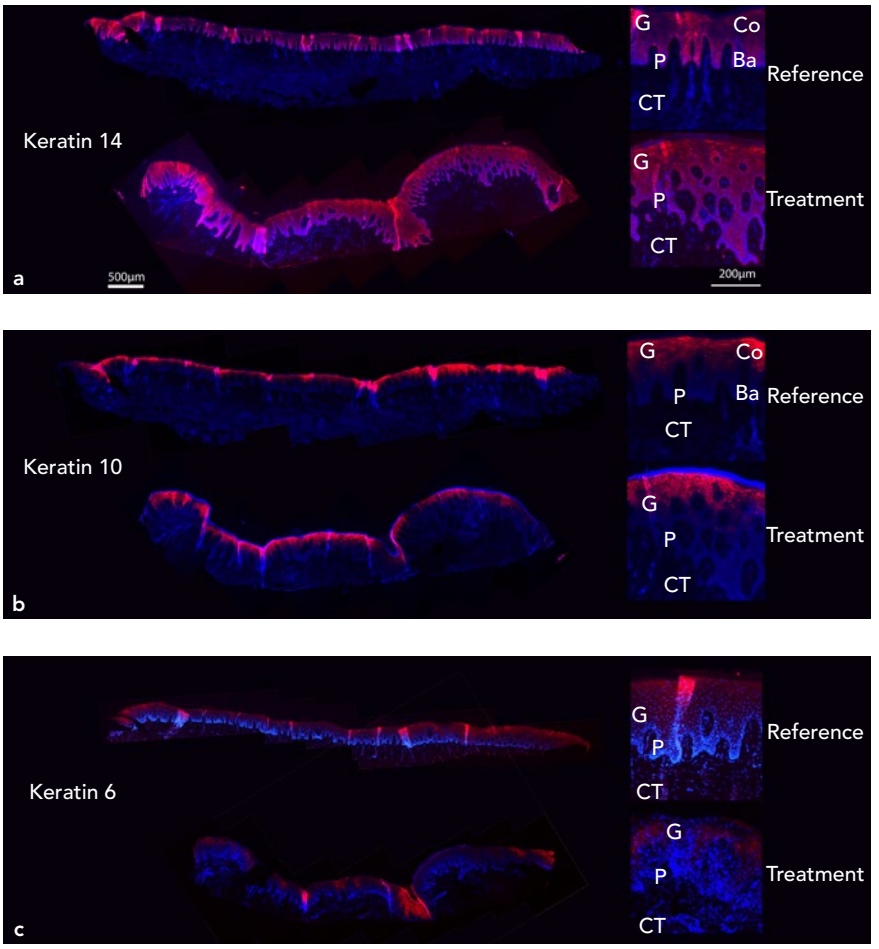

Fig 3 *Paraffin sections from reference and treatment biopsy samples were analyzed by immunofluorescence staining with the indicated antibodies.* (a) *Staining for keratin 14* (red) *and 4′,6-diamidino-2-phenylindole* (DAPI; cell nuclei, blue) *to confirm physiologic distribution of basal keratinocytes.* (b) *Keratin 10* (red) *and DAPI* (cell nuclei, blue) *staining begins in the suprabasal cell layers in both sample types, confirming that epithelial cells differentiate in a normal manner.* (c) *Keratin 6* (red), *DAPI* (cell nuclei, blue), *the marker for hyperproliferative epithelium, showed no differences between the two sample types and revealed a normal physiological distribution in both. G = gingiva; CT = connective tissue; P = papillary gingiva; Ba = basal epithelial cells; Co = corneal cornified epithelial cells; Pr = regions of hyperproliferative epithelium.*

autografts, either in the form of free gingival grafts[19,24,25] or free connective tissue grafts,[26] are currently the gold-standard treatments.[27]

Well-designed experimental studies have shown that transplanted autografts harvested from palatal gingiva are able to preserve tissue specificity, resulting in keratinized gingiva.[28] The cells responsible for determining this tissue specificity reside in the connective tissue underneath the epithelial basal lamina. When comparing epithelialized gingival autografts with free gingival connective tissue grafts, their ability to promote keratinized gingiva is similar, but free gingival grafts result in enhanced stability and less tissue shrinkage, though the esthetic outcomes are usually less favorable.[29] In both techniques, however, there is a need to harvest an autograft of sufficient size and dimension to achieve the desired outcome and compensate for shrinkage. Harvesting these soft tissue autografts from the palatal gingiva is usually associated with significant patient morbidity, mainly when there is a need to graft large mucosal areas, such as in advanced ridge bone–augmentation procedures.[30]

In order to overcome these challenges and disadvantages, one of the authors (I.U.) designed the strip autograft + XCM technique described herein. The technique is advantageous because the mucogingival junction is determined by the position of a single strip autograft, and the XCM covers what would otherwise be a periosteal bed subject to open healing. XCM appears to provide a "healing repository" for keratinized gingiva, which is "specified" by the strip autograft. Furthermore, autograft harvest and patient morbidity are minimized. According to patient pain measures reported in the original published case series,[3] on a 0 to 10 visual analog scale, mean pain experienced by the patients was 2.35, and pain medication was limited to a total of 25 mg of diclofenac potassium (Cataflam, Novartis Pharmaceuticals). Histologic staining and immunofluorescence examination revealed that the regenerated tissue was keratinized without obvious differences compared to "normal" keratinized tissue.

Ideally, the results obtained herein should be duplicated and verified by others. Also, in order to evaluate not only the soft tissue augmentation but also the patient outcomes reported, the strip autograft + XCM technique should be compared with other soft tissue augmentation techniques in controlled and randomized investigations with long-term follow-ups.

Conclusions

Using a combined strip autograft + xenogeneic collagen grafting technique for large areas of soft tissue augmentation, harvest requirements and patient morbidity are reduced, and the strip graft appears to serve as a mechanical barrier and cell source that maintains desired mucogingival junction position and generates desired keratinized gingiva. Comparing biopsy results of the strip autograft + xenogeneic collagen grafting technique to the harvested free gingival graft strips at 1-year post therapy, tissue morphology and keratin and collagen expression appear identical, indicating that the combined grafting technique provides not only desired but also physiologically normal keratinized gingiva outcomes.

Acknowledgments

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