PERI-IMPLANT SOFT TISSUE AUGMENTATION: CONTRIBUTIONS TO A CONTEMPORARY ISSUE IN IMPLANT DENTISTRY

PhD Thesis

by

DR. LÁSZLÓ PÁRKÁNYI, DMD

UNIVERSITY OF SZEGED, DOCTORAL SCHOOL OF CLINICAL MEDICINE

SUPERVISOR: DR. EMIL SEGATTO, DDS, PhD
UNIVERSITY OF SZEGED, FACULTY OF DENTISTRY, DEPARTMENT OF PERIODONTOLOGY

University of Szeged

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LIST OF PUBLICATIONS PROVIDING THE BASIS OF THE THESIS

1.Stähli A*, Párkányi L*, Aroca S, Stavropoulos A, Schwarz F, Sculean A, et al. The effect of connective tissue graft or a collagen matrix on epithelial differentiation around teeth and implants: a preclinical study in minipigs. *Clin Oral Investig* 2023 Jun 10 [epub ahead of print].

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2.Párkányi L, Fráter M. Thickening the attached gingiva around implants - the "H technique" - case presentation [Feszes íny szélesítés implantátumok körül – „H" technika esetismertetés]. *Fogorv Szle* 2018;111(1):12-5.

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RELATED PUBLICATIONS

Chackartchi T, Romanos GE, Parkanyi L, Schwarz F, Sculean A. Reducing errors in guided implant surgery to optimize treatment outcomes. Periodontol 2000 2022;88(1):64-72.

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ABBREVIATIONS

aJE	the most apical extent of the junctional epithelium
aN	the most apical part of the surgically exposed root surface
ASCTG	autogenous subepithelial connective tissue graft
BW/cBI-PIMM	biologic width around teeth/biologic width around implants
CAF	coronally advanced flap
cB	the most coronal level of the bone
cBI	the most coronal level of bone in contact with the implant
cC	the most coronal extent of new cementum
CDH	clinical defect height
CEJ	cementoenamel junction
CM/XCM	xenogeneic collagen matrix
DCTG	deep connective tissue graft (autogenous)
GM	gingival margin
HJE	height of junctional epithelium
IS	implant shoulder
JE	junctional epithelium
LKE	length of the keratinized epithelium
LNKE	length of the non-keratinized epithelium
PIMM	peri-implant mucosal margin
SB	the bottom of the sulcus
SCTG	superficial connective tissue graft (autogenous)
SD	sulcus depth
VB	the bottom of the vestibulum

I. INTRODUCTION

I.1. Background

The current treatment approaches for the rehabilitation of partially and fully edentulous patients with implant-supported restorations have shown remarkable success and survival rates, even in high-risk populations [1-7]. In 2018, half a decade after the groundbreaking studies of Brånemark and his colleagues [8, 9], it was estimated that 12 to 18 million dental implants were sold worldwide per annum [10], and a steady rise was forecast, indicating the vast popularity of this therapeutic approach - obviously not unrelated to its safety and predictability as corroborated by countless studies. Not less important is the fact that implant-supported restorations offer a significantly higher oral health-related quality of life than their conventional counterparts, especially removable dentures [11, 12]. Thus, it comes as no surprise that dental implant therapy has become the leading approach in oral rehabilitation, if not the mainstream.

Implant failure, however, is still a relevant and prevalent problem [13]. And while, for instance, a 15-year survival rate of 94% in a large sample of more than 10,000 implants is excellent by any measure [7], it also means that 6% of the placed implants were lost. Most long-term follow-up studies, often reporting on thousands of implants, document similar rates, which raises the question if this is the inherent limit of the approach or there are further, potentially manageable factors.

Implant failure is commonly attributed to a combination of factors. Some of these are manageable, some are more difficult to address. Parameters such as age, sex, smoking, systemic diseases, implant location in the maxilla, bone quantity and quality, and implant surface treatments and features have been found to be associated with implant failure [14-16]. In terms of temporal occurrence, dental implant failure is classified as early and late. Early implant failure refers to cases where clinical mobility of the implant is observed prior to the placement of the final prosthesis. This is also referred to as implant rejection and is associated with poor bone quality and quantity, systemic diseases, osteoporosis, medications (especially corticosteroids and bisphosphonates), smoking, infection, lack of primary stability, and surgical trauma. By a careful clinical approach involving thorough history taking, diagnosis by the appropriate and necessary means, meticulous surgical planning, minimally invasive surgery, appropriate follow-up and good patient education, the chance of early failure can be minimized. Late implant failure occurs within 1-3 years after implant placement. The most commonly cited

risk factors include excessive loading, peri-implantitis, bruxism, retained subgingival dental cement, inadequate prosthetic construction/fit, and traumatic occlusion [17].

Among these factors, peri-implantitis is the least easily manageable and potentially most destructive. Peri-implantitis is a degenerative and irreversible condition affecting the hard and soft tissues surrounding dental implants. It is characterized by bone loss, reduced osseointegration, the formation of deep pockets, and the presence of purulent exudate [18]. With reported prevalence rates up to 56% [19], peri-implantitis is a major contributor to late implant failure and loss, whose prevention and management is in the focus of intensive research [20]. Conservative treatment methods can effectively address mucositis and moderate forms of peri-implantitis. These approaches encompass various manual ablation techniques, laser-assisted systems, and photodynamic therapy, which can be complemented by the administration of local or systemic antibiotics. Through these interventions, the restoration of osseointegration is achievable. More advanced cases necessitate resection and regenerative treatment. Although the available treatment modalities yield promising results [21-23], the management of peri-implantitis is still a challenge, and the affected implant can be lost despite our best efforts. Therefore, it is best to take a preventive approach from the very beginning of the implant therapy.

A growing body of evidence suggests that peri-implant soft tissue augmentation, where applicable, can play a key role in keeping the peri-implant tissues healthy and thus it can lead to higher rates of implant survival [24]. In other words, this is not merely an aesthetic question. The role of the amount of keratinized tissue and the choice between autogenous subepithelial connective tissue grafts (ASCTGs) and xenogeneic collagen matrices (XCMs) are recurring issues in the recent literature of this topic [25-28]. The studies presented in this thesis aimed to contribute to these ongoing debates by providing histologic data from an animal model and presenting a surgical technique for peri-implant soft tissue augmentation using a xenogeneic collagen matrix.

I.2. On the importance of the peri-implant soft tissues in peri-implant health

As said, it can be regarded as an established fact that the soft tissues surrounding dental implants play a crucial role in the success and survival of implant restorations [24, 29, 30]. While an ideal soft tissue environment greatly contributes to aesthetic outcomes [31, 32], it has a significantly more substantial impact on the success of the implant. Therefore, this aspect holds great importance not only within the aesthetic zone but also beyond it. The thickness of

the peri-implant mucosa directly affects the stability of the marginal soft tissues and the preservation of the underlying bone [33-35]. Whether keratinized mucosa, per se, plays a role, has been discussed for some time [36, 37], but it remains controversial. This controversy is best exemplified by two recent systematic reviews published in high-prestige journals only one year apart, in 2021 and 2022. Both reviews utilized correct methodology; however, they arrived at directly opposing conclusions regarding the role of keratinized mucosa. The first review, conducted by Ramanauskaite and colleagues and published in May 2021 in Clinical Oral Implants Research, clearly concluded that reduced keratinized tissue width (< 2 mm) is associated with an increased prevalence of peri-implantitis, plaque accumulation, soft-tissue inflammation, mucosal recession, marginal bone loss, and greater patient discomfort [26]. In contrast, the second review, conducted by Ravidá and co-workers and published in June 2022 in Clinical Implant Dentistry and Related Research, arrived at the contrary conclusion that the impact of keratinized mucosa width (either <2 mm or ≥ 2 mm) as a risk factor for developing peri-implant disease remains low [38]. While the authors of the latter study acknowledge “the need for future controlled studies with proper sample sizes and longer follow-up” to validate their findings, it is evident that the controversy surrounding the role of keratinized tissue remains unresolved. Research has not yet yielded a decisive answer to this question. Nevertheless, our clinical experience supports the significance of keratinized tissue width in peri-implant health, and the studies presented in this thesis are based on this assumption.

After tooth loss, during the healing process, the alveolar ridge undergoes varying degrees of resorption, primarily affecting the horizontal dimension [39]. Alveolar involution is not limited to hard tissues alone; it consistently involves a decrease in volume and keratinization in the soft tissues [40]. Tooth loss, whether due to caries or periodontal reasons, most commonly occurs first in the molar region [41]. The decrease in keratinized tissue is particularly pronounced in the molar region, resulting in a significant absence of it around restorations fixed on molar implants [42]. The molar region is more challenging for patients to access, making it inherently more difficult to clean. In the case of implants, the peri-implant mucosa is more prone to inflammation when exposed to a certain amount of plaque compared to the gingival area adjacent to retained teeth [43, 44]. Thus, soft tissue augmentation in the molar region can be especially important. Acknowledging this and seeking a less invasive solution for a patient requiring molar tooth replacement, we devised the "H-technique," a method for augmenting soft tissue. Subsequently, we discovered its broader applicability in various regions. The latter

part of this thesis introduces the said technique and outlines our experiences in implementing it.

In summary, preserving the volume of soft tissues surrounding implants is crucial to prevent implant failure and loss. This typically involves grafting, traditionally referring to autogenous connective tissue grafting in this context. Nevertheless, xenogeneic collagen matrices are also being increasingly utilized for this purpose, yielding favorable outcomes.

I.3. Grafting: connective tissue graft or collagen matrix?

The main clinical indications for soft tissue grafting are recession coverage, keratinized tissue gain, and augmentation of soft tissue volume [30]. Different surgical techniques utilizing different materials have been proposed to achieve soft tissue augmentation in terms of thickness and width, but harvesting of ASCTGs from the palate is still considered as the gold standard [45-50].

However, despite the well-known benefits of ASCTGs, there are significant drawbacks and limitations associated with this method. These include the morbidity and pain associated with the donor site, which burdens the patient, and the limited availability of donor tissue from the palate, which restricts the number of treatable sites at any given time [51-53].

To overcome the limitations of ASCTGs, alternative biomaterials have gained importance [54, 55]. These biomaterials offer advantages such as reduced surgical time, decreased morbidity, and improved patient acceptance [56, 57]. However, such a biomaterial must exhibit favorable biological behavior to support modeling and remodeling processes, and it should demonstrate long-term volume stability [49]. Three-dimensional structures that can serve as scaffolds, promoting cell attachment, migration, proliferation, and differentiation, are necessary to create an appropriate environment for the formation of tissue-like structures [58]. Xenogeneic collagen matrices (XCMs) have been proposed as a promising alternative to ASCTGs and have been utilized for soft tissue augmentation around dental implants and root coverage therapy, yielding favorable outcomes [12,19].

In their 2021 systematic review and meta-analysis, Vallecillo and colleagues concluded that XCMs were an effective alternative to ASCTGs in terms of both keratinized mucosa width and gingival thickness, even if SCTGs were still somewhat superior [27]. DeAngelis and co-workers compared the clinical outcomes of XCM used at the time of implant placement as an alternative to ASCTG, for soft tissue augmentation. They found that at 12 months after surgery,

XCM yielded clinical results comparable to ASCTG in terms of soft tissue augmentation on the buccal and occlusal sides [45]. The authors note that the patients in the collagen matrix group indicated significantly less pain than those in the ASCTG group as measured on a visual analog scale. Hadzik and colleagues reported on a 5-year follow-up of soft tissue augmentation around dental implants with ASCTG and XCM. The authors used the same XCM as we did in the studies presented in this thesis (Mucograft, Geistlich-Pharma AG, Switzerland) and came to a similar conclusion as the Vallecillo group [27], stressing that soft-tissue augmentation with XCM causes significantly less pain during speaking and chewing compared to ASCTG harvested from the palate [28]. The latest clinical trial by the DeAngelis group examined the question of XCM versus ASCTG for soft tissue augmentation at immediately placed single implants [59]. Forty-eight patients requiring a single implant-supported rehabilitation were enrolled and underwent either of two surgical procedures: immediate implant placement with ASCTG or immediate implant placement with XCM. Marginal changes in the peri-implant soft tissue and the facial soft tissue thickness were assessed after 12 months. Peri-implant health status, aesthetics, patient satisfaction, and perceived pain were also assessed. The patients in the ASCTG group exhibited a statistically significant reduction in mid-buccal marginal level recession and a significantly greater increase in facial soft tissue thickness compared to the patients in the XCM group. Using XCM, the aesthetic outcomes were favorable, and high levels of patient satisfaction were recorded. Yet, ASCTG yielded superior results in terms of both mid-buccal marginal levels and facial soft tissue thickness. Finally, it must be added that although XCMs have demonstrated good volume stability, their rapid biodegradation caused by enzymatic activity restricts their suitability as a complete replacement for ASCTGs [27, 54, 60-62].

Overall, it seems that for the purposes of peri-implant soft tissue augmentation, xenogeneic collagen matrices will not replace autogenous connective tissue grafting in the foreseeable future. Instead, the two options will coexist, probably with ASCTG still as the standard and XCM as its less invasive alternative, used at the clinician's discretion. Thus, research efforts should still be focused on both approaches, especially that even if ASCTG is the gold standard, several questions remain to be answered in connection with it.

I.4. Further questions regarding grafting

There is evidence suggesting that the development of gingival, palatal, and alveolar mucosa is predominantly determined by innate factors [63]. Additionally, it seems that the connective tissue derived from regions originally covered by keratinized epithelium and/or from the periodontal ligament has the potential to stimulate epithelial keratinization. These findings align with previous studies that reported similar outcomes, indicating that granulation tissue originating from the alveolar mucosa tends to induce the formation of a non-keratinized epithelium, while granulation tissue originating from the supra-alveolar connective tissue or the periodontal ligament tends to result in keratinized epithelium [64, 65].

Clinical observations suggest that in many instances, when palatal connective tissue grafts are covered by a non-keratinized mucosal flap, there is a failure of epithelial cell keratinization. These clinical observations align with earlier observations indicating that connective tissue grafts obtained from deeper layers of the palatal connective tissue may not possess the same capacity to induce keratinization as grafts harvested from more superficial layers. In a well-designed experiment, a thick palatal epithelial-connective tissue graft was excised and divided into two thinner grafts, one placed immediately subepithelially and the other closer to the bone [66]. These grafts were transplanted into opposing areas lacking keratinized mucosa. Following a healing period of 3 months, biopsies were obtained and subjected to routine histology, immunofluorescence, and gel electrophoresis. The results demonstrated that while the epithelial-connective tissue grafts exhibited histologic and biochemical characteristics of keratinized tissue, resembling gingiva, the deep connective tissue grafts exhibited features associated with both keratinized and non-keratinized tissue. Similar findings have also been reported in human studies by other researchers, indicating that palatal connective tissue grafts or free gingival grafts transplanted into non-keratinized tissue areas may not consistently acquire the characteristics of keratinized tissue [67-69].

Hence, it is evident that connective tissue grafts obtained from the palate may not consistently promote keratinization at sites originally characterized by non-keratinized epithelium. This could be attributed to differences between palatal connective tissue grafts harvested from superficial or deeper layers, affecting their ability to induce keratinization. Interestingly, the potential for the regeneration of a keratinized tissue zone following a complete excision (e.g., gingivectomy) of keratinized tissues surrounding implants (i.e., removal of both free and attached mucosa) remains uncertain.

A bioresorbable porcine collagen matrix has been proposed as an alternative to connective tissue grafts, to augment the width of keratinized tissue around implants and address single and multiple recessions associated with natural teeth and implants. Available evidence suggests that the use of this XCM may contribute to an increase in keratinized mucosa around implants [51, 70-74] and, to some extent, aid in widening the keratinized tissue when employed for recession coverage around teeth [75-77]. However, this remains to be confirmed, as well as the hypothesized difference between superficial and deep connective tissue grafts in terms of their ability to induce keratinized tissue. A major part of this thesis deals with these issues.

II. OBJECTIVES AND HYPOTHESES

This thesis summarizes two studies, both of which investigated issues related to peri-implant soft tissue augmentation.

The first study [78] examined the differences between superficially and deeply harvested palatal connective tissue grafts and xenogeneic collagen matrices in terms of their impact on epithelial keratinization around teeth and implants that lacked gingiva or keratinized mucosa, respectively. The study was conducted in Göttingen miniature pigs using histologic methods. We hypothesized that both superficial and deep connective tissue grafts would induce similar levels of keratinization at teeth and implants. Additionally, we expected that the outcomes achieved with collagen matrices would be comparable to those achieved with connective tissue grafts, without any significant inferiority.

The second study [79] was a proof-of-concept case study exploring an XCM-based surgical technique derived from existing approaches used to manage peri-implant soft tissues, which we call "the H-technique" after the shape of the applied XCM. Conventionally, modifying the biotype and augmenting the thickness or width of the keratinized tissues around implants is performed as a distinct surgical procedure. This procedure can be carried out either during the uncovering of the implant and placement of the healing abutment or around previously positioned implants with healing abutments. Typically, a free gingival graft is employed for this specific procedure, which may result in considerable donor site morbidity and exhibit aesthetic disparities when compared to the adjacent soft tissues.

The objective of the technique we proposed was to achieve simultaneous modification of the biotype and augmentation of keratinized tissues using XCM during implant placement in an open healing approach. Such a technique is more comfortable for the patient, it eliminates the problem of donor site morbidity, and there is no need for multiple interventions, which shortens net healing time. Beyond these obvious advantages, we hypothesized that by using this technique, a sufficient amount of aesthetic keratinized tissue can be gained to ensure peri-implant soft tissue health even over a longer period.

The studies are presented in the above order.

III. PRESENTATION OF THE STUDIES

III.1. The effects of ASCTG and CTX on epithelial differentiation in minipigs

III.1.1. Study design and procedures

The study protocol was approved by the local Committee for Animal Research, University of Szeged, Hungary (No. 1-74-2/2015 MAB). Six Göttingen miniature pigs were used for the study. The husbandry and care of the animals before, during, and after surgery was handled at the Surgical Research Unit, University of Szeged, Hungary. The animals received standard food and water ad libitum. Animals were premedicated using ketamine (i.m. 20 mg/kg), xylazine (i.m. 2 mg/kg), atropine (i.v. 0.05 mg/kg) and midazolam (i.v. 0.5 mg/kg) to achieve the intubation status. Inhalation anaesthesia was performed with isoflurane (1.0 - 1.5 %). Fentanyl patches (5 - 10 mg/kg) were used for the intraoperative analgesia and the animals received antibiotic prophylaxis for three days (Duplocillin LA, 12,000 IU/kg).

The progression of the study procedures is outlined in Figure 1. On one side of the lower jaw, the second, third, and fourth premolars, as well as the first molar, were extracted. Following a healing period of 12 weeks, three 8 - 10 mm long Straumann Standard Plus RN tissue level implants (Straumann, Switzerland) were placed. After an additional 8 weeks of healing, a surgical procedure was performed to create a soft tissue dehiscence around the implants. On the contralateral side, isolated Miller Class II recession defects were surgically created around the second, third, and fourth premolars. This involved the complete removal of the buccal gingiva, bone, and root cementum using blades, bone chisels, and slowly rotating burs while rinsing with sterile saline, following previously established protocols [80, 81]. The resulting defects had a depth of approximately 5 mm and a width of 4 mm apically to the cemento-enamel junction. The exposed root and implant surfaces were left untreated for 4 weeks to facilitate soft tissue healing, plaque accumulation, and to better simulate a chronic recession-type defect.

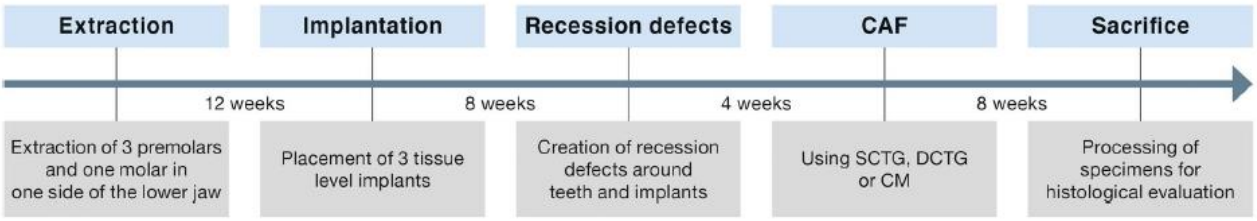

Figure 1. An outline of the progression of the study (CAF: coronally advanced flap, SCTG: superficial connective tissue graft, DCTG: deep connective tissue graft, CM: collagen matrix)

After a 4-week healing period, the defects underwent treatment. Initially, the exposed root surfaces of the teeth were thoroughly cleaned using Gracey curettes (Hu-Friedy, Chicago, IL, USA), while the implants received supramucosal cleaning with rubber cups and a polishing paste (Zircate, Prophy Paste; Dentsply, Konstanz, Germany). For teeth, a small bur (diameter 2 mm) was used to mark the most apical part of the previously surgically exposed root surface, creating a reference mark for the histometric analysis. Regarding the implants, clinical defect height (CDH) was measured at the mid-buccal aspect, from the implant shoulder (IS) to the bottom of the mucosal recession. The defects were then treated using a CAF (coronally advanced flap) technique as described by Allen & Miller [82], along with either a CM (collagen matrix) or a CTG (connective tissue graft). Two vertical releasing incisions, 6 mm longer than the recession defects, were made. In cases where a CTG was chosen, the required tissue was harvested from the palate, following the technique described by Hürzeler & Weng [83], with dimensions measuring 0.5 mm less than the size of the vascular bed in mesio-distal length and 5 mm in corono-apical direction. The defects in each quadrant were then randomly assigned to either of the following three treatment groups: CAF + superficial CTG (SCTG) around teeth and implants, or CAF + deep CTG (DCTG) around teeth and implants, or CAF + CM (Mucograft®, Geistlich, Wolhusen, Switzerland) around teeth and implants. The flaps were closed with 6-0 monofil (Polypropylene, Stoma, Emmingen-Liptingen, Germany) suture material. Sutures were removed at 2 weeks. The animals were euthanized after 8 weeks of healing.

III.1.2. Histology

The lower jaws were removed and chemically fixed by immersion in 10% buffered formalin supplemented with $CaCl_2$ for 3 weeks. The specimens were rinsed in running tap water, dehydrated in ascending concentrations of alcohol, and embedded in methyl methacrylate, as previously described [84, 85]. Each tooth and implant was sectioned parallel to its longitudinal axis in a bucco-lingual direction, resulting in two to three undecalcified ground sections of ~ 500 µm thickness. The sections were ground to a final thickness of 80 µm, superficially stained with toluidine blue and basic fuchsin and the two central-most sections were used for descriptive and histomorphometric analyses. Figure 2 shows a section of a tooth and an implant.

The descriptive analysis was conducted using direct microscopic observation. The sections stained with toluidine blue/fuchsin were examined to assess keratinization/non-keratinization, as well as the presence/absence and extent of inflammation. To provide a basis for comparison, one untreated first molar per animal was used as control.

For the histomorphometric analyses, the ground sections were digitized using a Zeiss Axio Imager.M2 microscope (Zeiss, Germany) equipped with an automatic scanning stage and a digital camera. The measurements were carried out on the buccal side of the sections, by the same experienced and calibrated investigator, using the ZEN (Zeiss Efficient Navigation, Zeiss, Germany) software.

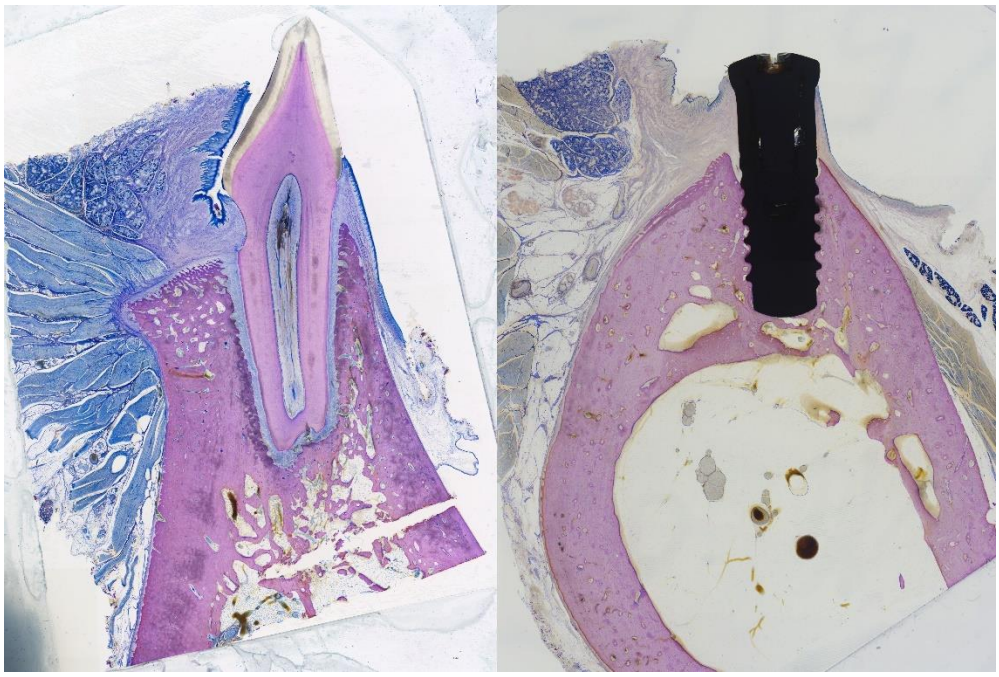

Figure 2. Histologic sections illustrating the tissue samples used for descriptive and histomorphometric analyses.

III.1.3. Outcomes and statistical analysis

The descriptive histologic analysis primarily focused on evaluating keratinization and signs of inflammation. Additionally, it considered the general appearance of the epithelium, the impact of the graft on tissue spatial configuration, the level of the bone crest, and the extent of graft integration into the surrounding tissue. Miscellaneous observations were also recorded.

As for the histomorphometric analysis, specific landmarks were determined around teeth and implants and distance measurements were performed using these landmarks. The landmarks and the measured distances are defined in Figures 3 and 4.

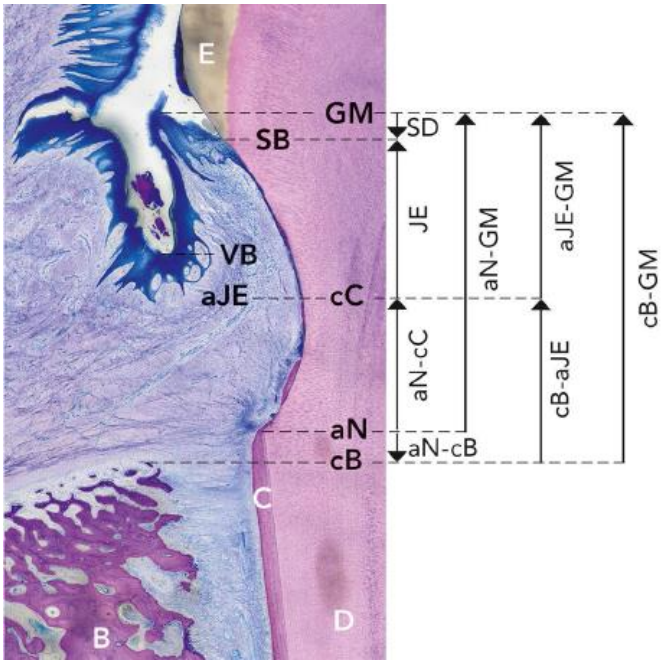

Figure 3. Landmarks around teeth for the histomorphometric measurements and the measured parameters (distances). **GM:** gingival margin; **SB:** sulcus bottom; **VB:** the bottom of the vestiblum; **aJE:** the most apical extent of the junctional epithelium; **cC:** the most coronal extent of new cementum; **aN:** the most apical part of the surgically exposed root surface; **cB:** the most coronal level of bone; **SD:** sulcus depth; **JE:** junctional epithelium; **aN-cC:** vertical gain of new cementum; **aN-cB:** from the most apical part of the surgically created root surface to the bone crest; **aN-GM:** from the most apical part of the surgically created root surface to the gingival margin; **aJE-GM:** length of junctional epithelium plus sulcus depth; **cB-aJE**: from the most coronal level of bone to the apical extent of the junctional epithelium; **cB-GM:** biologic width; **C:** cementum; **D:**dentin; **B:** bone; **E:** enamel

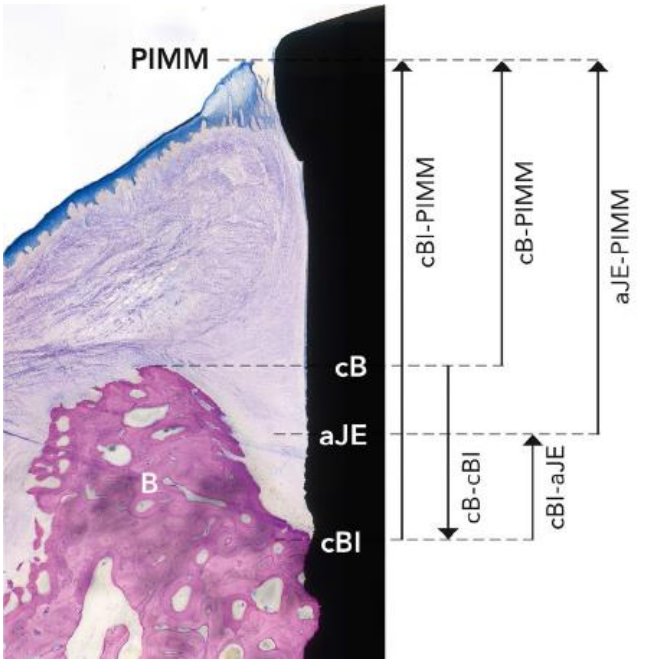

Figure 4. Landmarks around implants for the histomorphometric measurements and the measured parameters. **PIMM:** peri-implant mucosal margin; **aJE:** the most apical extent of the junctional epithelium; **cBI:** the most coronal level of bone in contact with the implant; **cBI-PIMM:** biologic width; **cB-cBI:** vertical distance from the bone crest to the most coronal bone level in contact with the implant; **cB-PIMM:** vertical distance from the bone crest to the peri-implant mucosal margin; **cBI-aJE:** the most coronal level of bone in contact with the implant to the most apical extent of the junctional epithelium; **aJE-PIMM:** length of junctional epithelium plus sulcus depth; **B:** bone.

The statistical analysis was conducted using GraphPad Prism 9, Version 9.3.1 (GraphPad Software, Inc. CA, USA). Mean values and standard deviations were calculated for each histomorphometric parameter, with the animal serving as the experimental unit for all comparisons (n = 6). Given the small sample size and the non-parametric distribution of the data, group differences were assessed using the Kruskal-Wallis test, followed by the Mann-Whitney test with Bonferroni correction for multiple comparisons. The significance level was set at $p < 0.05$.

III.1.4. Results - Descriptive histology

III.1.4.1. Control teeth

The oral gingival epithelium consisted of four strata and exhibited keratinization (Fig. 5a). Among all groups, the control teeth showed the most regular configuration of rete pegs in their keratinized epithelium (Fig. 5a). The junctional epithelium was short and terminated at or slightly apical to the cemento-enamel junction (Fig. 5b). All six teeth exhibited a healthy gingiva with minimal signs of inflammation that were within normal physiological range. Five teeth displayed very small gingival pockets (Fig. 5c), while one tooth exhibited substantial calculus and a slightly deeper gingival pocket. Although there was a relatively large distance between the cementoenamel junction and the bone crest in this tooth type, no signs of bone resorption or pathology were observed.

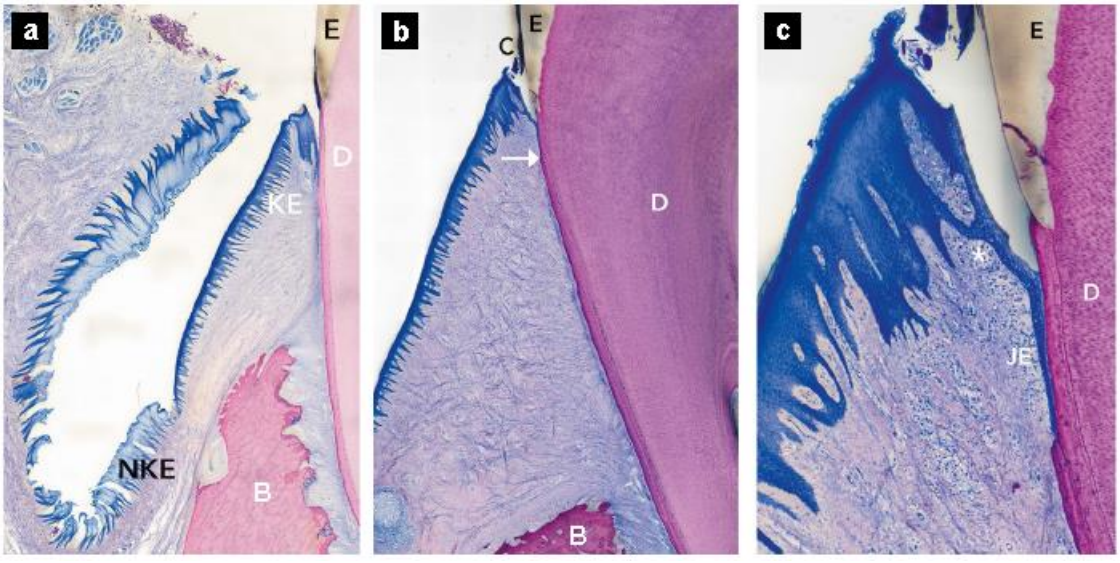

Figure 5. Micrographs illustrating the vestibulum (a) and the gingiva (b and c; c shows b at a higher magnification) around the control teeth. JE: junctional epithelium; KE: keratinized epithelium; NKE: non-keratinized epithelium; E: enamel; D: dentin; B: bone. The arrow indicates the apical end of the junctional epithelium.

III.1.4.2. Teeth/SCTG

All teeth exhibited a normal, keratinized oral gingival epithelium consisting of four strata (Fig. 6a). The subepithelial connective tissue graft (SCTG) appeared to enhance the width of the gingiva and influence the spatial arrangement of the vestibulum, causing an elevation of the vestibulum base (Fig. 6a). Buccally, the bone crest level was consistently lower than lingually in all six teeth. The junctional epithelium was relatively long. Two teeth showed epithelial inclusions in the gingival connective tissue, one tooth exhibited food impaction, and another tooth displayed multinucleated giant cells surrounding a foreign body material. The connective tissue graft was clearly distinguishable, but the border region showed minimal evidence of graft tissue integration into the surrounding tissue (Figs. 6b and c). Gingival pocket formation, accompanied by supra- and subgingival calculus and biofilm, was observed in five out of six teeth (Fig. 6b). Peri-pocket inflammation was present in all teeth with gingival pockets.

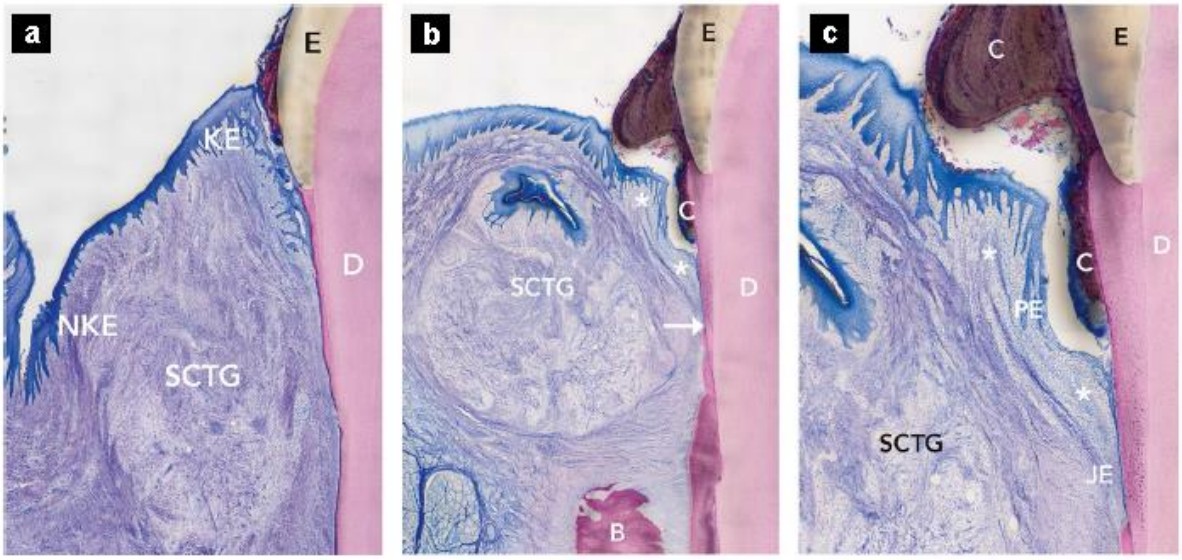

Figure 6. Micrographs illustrating the vestibulum (a) and the gingiva (b and c; c shows b at a higher magnification) around the teeth. JE: junctional epithelium; KE: keratinized epithelium; NKE: non-keratinized epithelium; PE: pocket epithelium; SCTG: superficial connective tissue graft; E: enamel; C: calculus; D: dentin; B: bone. The arrow indicates the apical end of the junctional epithelium.

III.1.4.3. Teeth/DCTG

All teeth exhibited a normal gingival epithelium with keratinization, composed of four strata (Fig. 7a). Due to its volume, DCTG appeared to influence the spatial arrangement of the gingiva and vestibulum, resulting in widened gingiva and elevation of the vestibular floor (Fig. 7a).

In all six teeth, the buccal side of the bone crest was positioned lower than the lingual side. The junctional epithelium varied in length, ranging from long to very long. Epithelial inclusions were observed in two teeth, food impaction in one tooth, and multinucleated giant cells surrounding foreign body material in another tooth. The connective tissue graft was clearly distinguishable from the surrounding tissue, exhibiting minimal signs of graft integration (Fig. 7b). Gingival pocket formation, accompanied by subepithelial calculus and biofilm, was observed in all six teeth. Peri-pocket inflammation was present in all teeth (Fig. 7c).

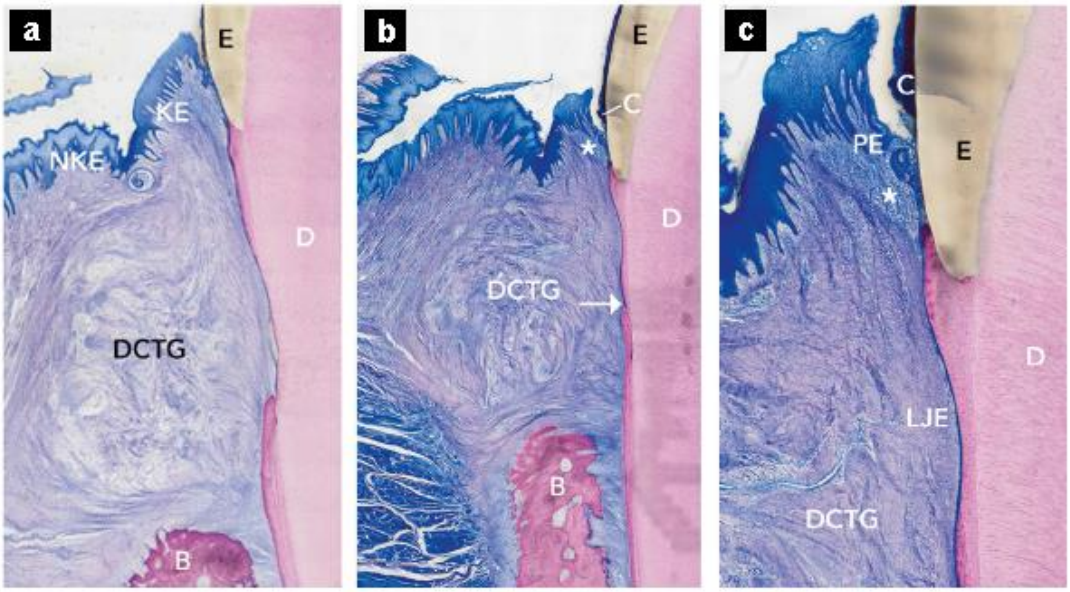

Figure 7. Micrographs illustrating the vestibulum (a) and the gingiva (b and c; c shows b at a higher magnification) around the teeth. KE: keratinized epithelium; LJE: long junctional epithelium; NKE: non-keratinized epithelium; PE: pocket epithelium; DCTG: deep connective tissue graft; E: enamel; C: calculus; D: dentin; B: bone. The arrow indicates the apical end of the junctional epithelium.

III.1.4.4. Teeth/CM

All teeth had a normal, keratinized oral gingival epithelium consisting of 4 strata (Fig. 8a). The spatial configuration of the keratinized and non-keratinized epithelium and the vestibulum were very similar to the situation around control teeth, i.e., the gingiva was thin and the bottom of the vestibulum was not elevated (Fig. 8a).

In all 5 teeth, the bone crest level was buccally lower than lingually. The junctional epithelium was either long or very long. Epithelial inclusions in the gingival connective tissue were not found. Food impaction was found in 1 tooth, a mini abscess in 1 tooth, and residual CM was found in the gingival connective tissue of all 5 teeth. The CM was partially integrated into the surrounding tissue and only remnants of the matrix could be detected (Fig. 8b and c). Gingival pocket

formation, subepithelial calculus, biofilm and peri-pocket inflammation were found in all 5 teeth (Fig. 8c).

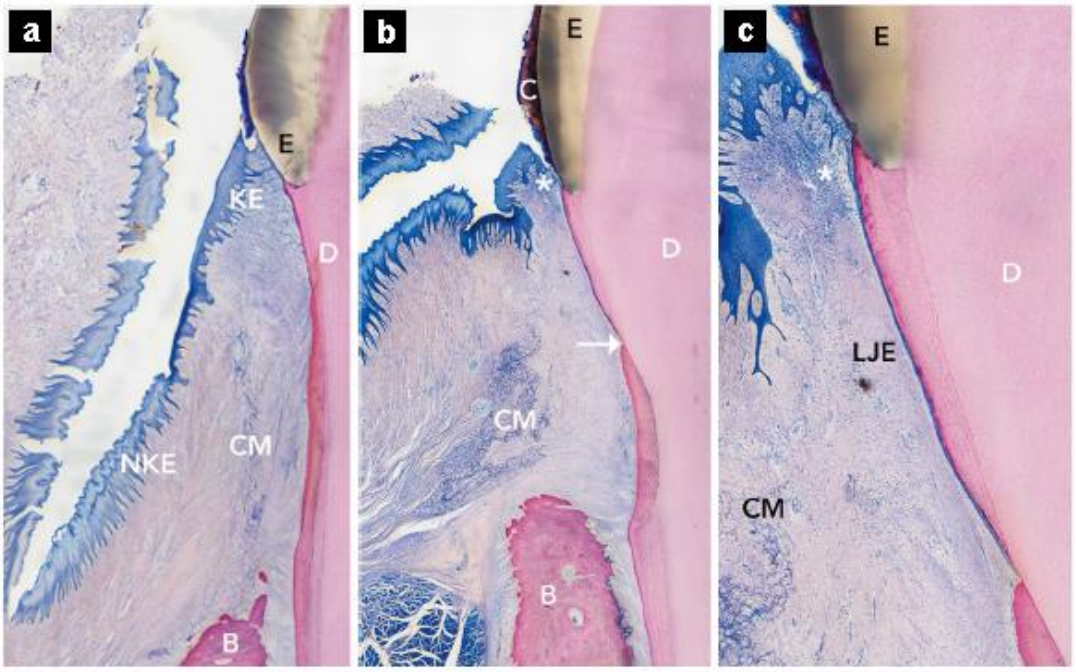

Figure 8. Micrographs illustrating the vestibulum (a) and the gingiva (b and c; c shows b at a higher magnification) around the teeth. KE: keratinized epithelium; LJE: long junctional epithelium; NKE: non-keratinized epithelium; PE: pocket epithelium; CM: collagen matrix; E: enamel; C: calculus; D: dentin; B: bone. The arrow indicates the apical end of the junctional epithelium.

III.1.4.5. Implants/SCTG

The peri-implant mucosa facing the graft exhibited characteristics of a keratinized epithelium, as shown in Fig. 9a (see also Fig. 12 top). A layer of soft connective tissue was observed between the epithelium and the SCTG. All 6 implants were non-submerged and displayed saucer-shaped bone defects on both the buccal and lingual aspects, as shown in Fig. 9b. One implant exhibited advanced bone loss, while remnants of dentin and cementum were found around another implant. In 4 implants, small pocket formation, calculus, biofilm, and mild inflammation were observed (Fig. 9c). Notably, there was a significant vertical distance between the peri-implant mucosal margin and the highest point of the bone. The junctional epithelium was long or very long, and its apical termination was consistently located below the bone crest (Fig. 9b). The SCTG was present around all implants, exhibiting a large and round shape. The precise positioning of the SCTG relative to the keratinized epithelium varied among the implants (Fig. 9a).

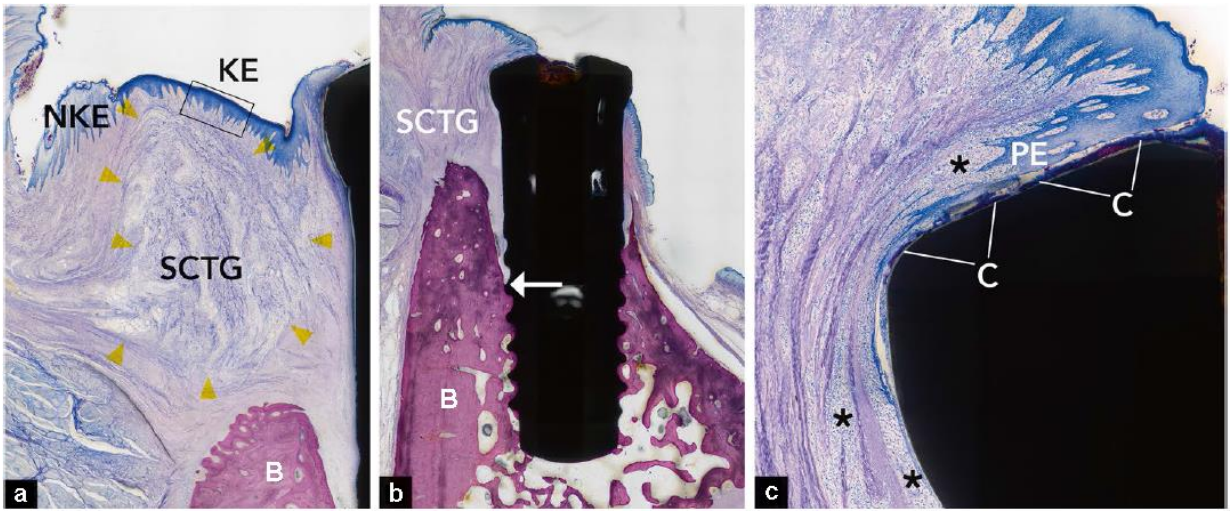

Figure 9. Micrographs illustrating the grafting area around the implant (a), the implant site (b) and a close-up of the implant at the coronal part (c). Note that (c) shows the same implant as (b), at a higher magnification. KE: keratinized epithelium; NKE: non-keratinized epithelium; PE: pocket epithelium; SCTG: superficial connective tissue graft; B: bone; C: calculus. Arrow: the apical end of the junctional epithelium. Yellow arrowheads: the border of the graft. The black rectangle in (a) indicates the area shown in Figure 12 (top).

III.1.4.6. Implants/DCTG

The epithelium of the peri-implant mucosa facing the graft exhibited characteristics similar to a keratinized epithelium (Fig. 10a, see also Fig. 12 middle). All six implants were non-submerged and displayed saucer-shaped bone defects both buccally and lingually (Fig. 10b). One implant showed advanced bone loss. Four implants exhibited small pocket formation, calculus, biofilm, and mild inflammation (Fig. 10c). The vertical distance between the peri-implant mucosal margin and the most coronal level of the bone was noticeably long, and the junctional epithelium was very long with its apical termination always below the bone crest (Fig. 10b). The DCTG surrounded all implants, appearing large and round-shaped, with its location relative to the keratinized epithelium varying among implants (Fig. 10a). A layer of soft connective tissue was present between the epithelium and the DCTG.

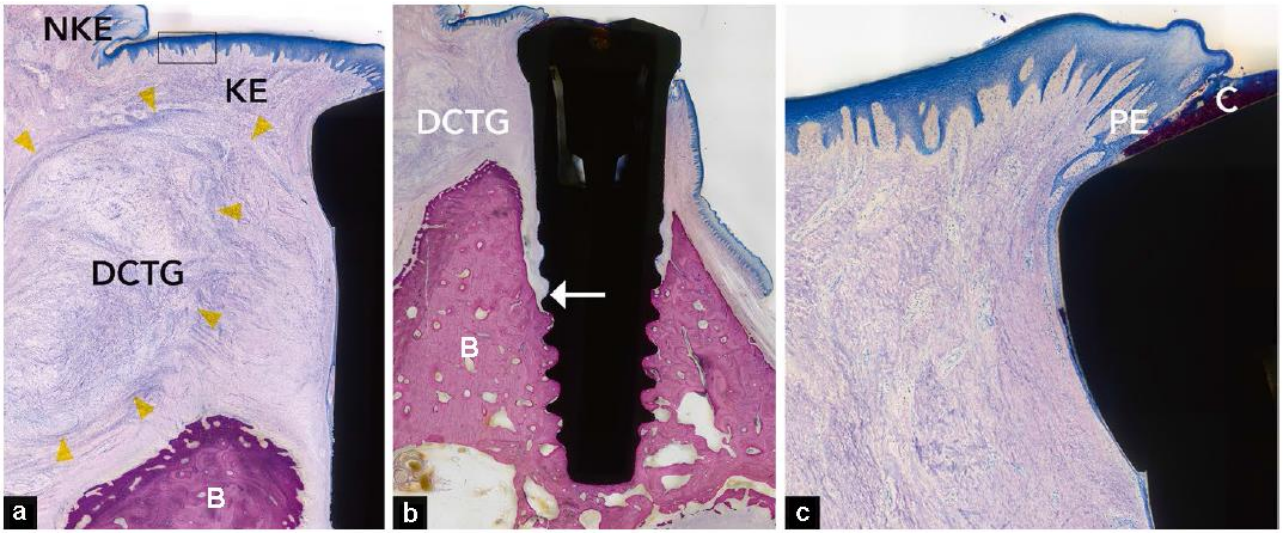

Figure 10. Micrographs illustrating the grafting area around the implant (a), the implant site (b) and a close-up of the implant at the coronal part (c). Note that (c) shows the same implant as (b), at a higher magnification. KE: keratinized epithelium; NKE: non-keratinized epithelium; PE: pocket epithelium; DCTG: deep connective tissue graft; B: bone; C: calculus. Arrow: the apical end of the junctional epithelium. Arrowheads: the border of the graft. The black rectangle in (a) indicates the area shown in Figure 12 (middle).

III.1.4.7. Implants/CM

The peri-implant mucosa facing the coronally located CM exhibited a keratinized epithelium, as shown in Figure 11a and b. One implant was lost in situ, while out of the 5 remaining implants, 2 were submerged and 3 were non-submerged. Saucer-shaped bone defects were observed around all implants, both buccally and lingually (Fig. 11b). Only one implant showed a very small pocket formation, whereas the rest of the implants had no pocket formation (Fig.11c). Healthy peri-implant soft tissue conditions with minimal inflammation were observed around all implants. The most coronal level of bone in contact with the implant (cBI) was located apically, resulting in a conspicuously long vertical distance between the bone crest and the most coronal bone in contact with the implant. The junctional epithelium was very long, with its apical termination consistently below the bone crest (Fig. 11b). Residual CM was detected in the soft connective tissue around all implants (Fig. 11a). The presence of CM varied in thickness and elongation, and its localization in relation to the keratinized epithelium differed between implants. A thick layer of connective tissue was observed between the epithelium and the CM.

Although all implants were surrounded by a collar of keratinized mucosa (Figs. 9a, 10a, 11a), the length of the mucosa could not be determined histomorphometrically due to variations in transmucosal healing and partial overgrowth of implant healing caps by peri-implant mucosa.

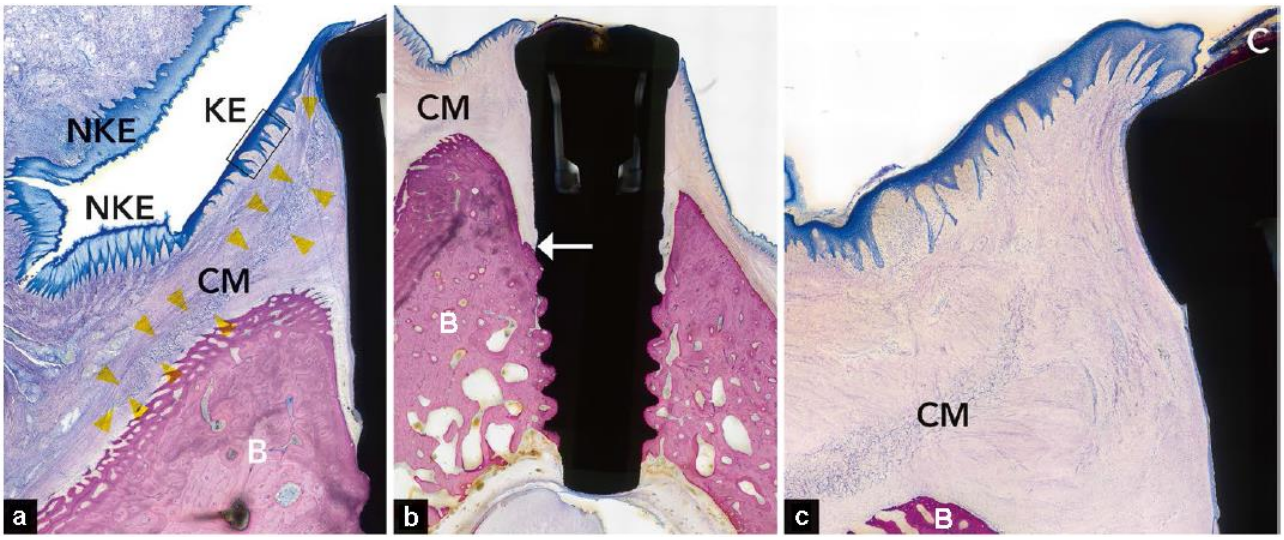

Figure 11. Micrographs illustrating the grafting area around the implant (a), the implant site (b) and a close-up of the implant at the coronal part (c). Note that (c) shows the same implant as (b), at a higher magnification. KE: keratinized epithelium; NKE: non-keratinized epithelium; PE: pocket epithelium; CM: collagen matrix; B: bone; C: calculus. Arrow: the apical end of the junctional epithelium. Arrowheads: the border of the graft. The black rectangle in (a) indicates the area shown in Figure 12 (bottom).

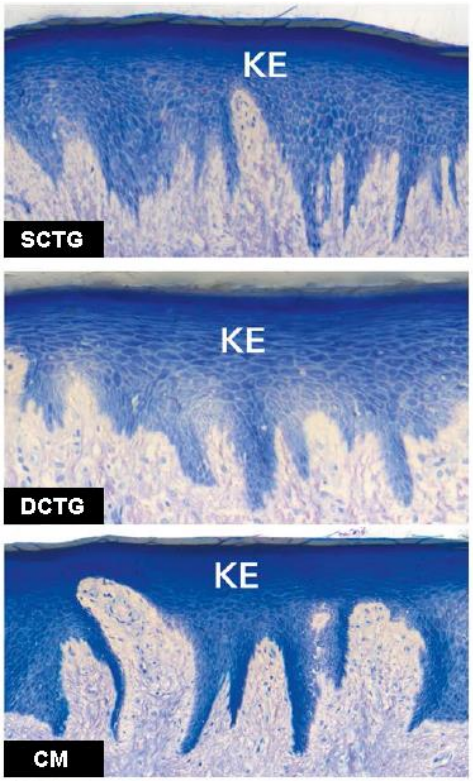

Figure 12. Keratinized epithelium around the grafts from the SCTG (top), DCTG (middle), and CM (bottom) groups. Magnified sections from Figs. 9a, 10a and 11a (indicated with a rectangle in the figures).

III.1.5. Results - Histomorphometry

III.1.5.1. Teeth

The findings of the histomorphometric analysis are presented in Table 1. The length of the keratinized epithelium was the smallest in the SCTG group. The KE:NKE ratio was consistent across all experimental groups, approximately 50:50. However, in the control teeth group, the ratio was 80:20 (SCTG: 49.92 ± 23.50% to 50.07 ± 23.05%; DCTG: 56.58 ± 13.60% to 43.41 ± 13.60%; CM: 53.38 ± 9.51% to 46.61 ± 9.51%; control: 83.49 ± 6.27% to 16.50 ± 6.27%). There was no statistically significant difference in the length of the keratinized epithelium between SCTG, DCTG, and CM. However, when comparing the CTG groups to the control group, both CTG groups were characterized by significantly shorter keratinized epithelium ($p=0.0025$ for SCTG and $p=0.0228$ for DCTG). There was no statistically significant difference between the control group and the CM group ($p=0.1814$). The length of the non-keratinized epithelium was similar across all experimental groups and the control group.

The increase in gingival height (aN-GM) was comparable across all three experimental groups, with values of 3.90 ± 0.81 mm for SCTG, 4.02 ± 1.40 mm for DCTG, and 4.21 ± 0.64 mm for CM. The biologic width (BW; cB-GM) was highest in the control group (5.14 ± 0.48 mm), where the crestal bone was situated further apically to the CEJ. Although not statistically significant, lower BW values were observed in the experimental groups (4.22 ± 0.65 mm for SCTG, 4.24 ± 0.88 mm for DCTG, 4.04 ± 0.41 mm for CM). There were significant differences between the control and experimental groups in two parameters: aJE-GM and HJE. However, no statistically significant differences were observed between the experimental groups for any of the assessed parameters. The distance aJE-GM was smallest in the control group, while the experimental groups exhibited longer junctional epithelium (SCTG: 3.29 ± 0.76 mm, DCTG: 3.01 ± 0.73 mm, CM: 2.91 ± 0.77 mm, control: 1.55 ± 0.48 mm). Significant differences were found between the control group and both CTG groups ($p=0.009$ for SCTG and $p=0.044$ for DCTG). A similar pattern was observed for HJE. The sulcus was shallowest in the control group, while the other groups showed deeper sulci with slight inflammation and pocket formation. The connective tissue adhesion (cC-aJE) was minimal in all test groups, indicating confluence or close proximity between the new cementum and the apical end of the JE. The mean vertical gain of new cementum (aN-cC) was highest in the CM group, followed by the SCTG and DCTG groups. Notably, the distance from the apical end of the notch (former level of the gingival margin) to the bone crest was positive in the CM group, indicating greater vertical bone growth compared to the two CTG groups, where this distance was negative.

III.1.5.2. Implants

The histomorphometric data of the implants are presented in Table 2. None of the parameters showed a statistically significant difference among the groups. The biologic width (cBI-PIMM), composed of cBI-aJE and aJE-PIMM, exhibited similar values in all three groups. Additionally, no significant differences were observed for the distance between the bone crest and the peri-implant mucosal margin (cB-PIMM). Notably, the distance between the apical end of the junctional epithelium and the peri-implant mucosa (aJE-PIMM), which represents the combined height of the junctional epithelium and sulcus depth, varied from 4.44 ± 1.24 mm to 5.35 ± 0.55 mm and was notably greater around implants compared to corresponding teeth. Conversely, the distance between bone on the implant and the apical end of the junctional epithelium (cBI-aJE), representing the connective tissue adhesion on the implant, was relatively short in all three groups. The height of the saucer-shaped bone deficiency (cB-cBI) was greatest in the CM group, followed by DCTG and SCTG, although without statistical significance.

Table 1. Descriptive statistics of the histomorphometric parameters measured around teeth. Conventions: **C:** control; **SCTG:** superficial connective tissue graft; **DCTG:** deep connective tissue graft; **CM:** collagen matrix; **LKE:** length of the keratinized tissue **LNKE:** length of the non-keratinized tissue; **aN:** the most apical point of the surgically exosed root surface; **GM:** gingival margin; **BW:** biological width; **aJE:** the most apical extent of the junctional epithelium; **HJE:** height of the junctional epithelium; **cC:** the most coronal extent of new cementum. The values are given in millimeters (mean±SD). The values in the table are rounded to two decimal places for enhanced readability and ease of interpretation.

	LKE	**LNKE**	**aN-GM**	**BW**	**aJE-GM**	**HJE**	**cC-aJE**	**aN-cC**
SCTG	0.87±0.92	0.79±0.51	3.90±0.81	4.22±0.65	3.29±0.76	2.52±0.72	0.02±0.06	0.58±0.60
DCTG	1.14±0.62	0.78±0.19	4.02±1.41	4.24±0.88	3.01±0.73	2.21±0.82	0.04±0.06	0.97±0.82
CM	1.40±0.76	1.16±0.25	4.21±0.64	4.04±0.41	2.91±0.77	1.90±0.68	0.01±0.05	1.29±0.86
C	5.01±0.97	0.94±0.28	N/A	5.14±0.48	1.55±0.48	1.02±0.35	N/A	N/A

Table 2. Descriptive statistics of the histomorphometric parameters measured around implants. Conventions: **SCTG:** superficial connective tissue graft; **DCTG:** deep connective tissue graft; **CM:** collagen matrix; **BW:** biologic width; **cBI:** most coronal level of bone in contact with the implant; **PIMM:** peri-implant mucosal margin; **cB:** the most coronal level of the bone (crest); **aJE:** most apical part of the junctional epithelium. The values are given in millimeters (mean±SD). The values in the table are rounded to two decimal places for enhanced readability and ease of interpretation.

	BW(cBI-PIMM)	**cB-PIMM**	**aJE-PIMM**	**cBI-aJE**	**cB-CBI**
SCTG	5.38±0.90	3.17±0.62	4.44±1.24	0.63±0.48	-2.16±0.80
DCTG	5.90±0.97	3.17±0.43	5.23±0.79	0.66±0.89	-2.68±1.04
CM	5.82±0.51	2.93±0.56	5.35±0.55	0.47±0.32	-2.89±0.27

III.2. The “H-technique” for the thickening of the attached gingiva around implants

III.2.1. Rationale

Tissue changes following tooth loss extend beyond the hard tissues and consistently involve a reduction in volume and keratinization of the soft tissues [40]. This decrease in keratinized tissue is particularly noticeable in the molar region, which means that the amount of keratinized tissue around implants placed in this region may be insufficient [42]. Assuming that keratinized tissue plays a role in maintaining peri-implant health [26], this is an issue that needs to be addressed. Furthermore, due to its location, the molar region poses challenges for patients in terms of accessibility and proper cleaning, and the peri-implant mucosa is more susceptible to inflammation when exposed to plaque compared to the gingival area around natural teeth [43, 44]. Consequently, soft tissue augmentation in the molar region seems to be an especially important issue.[1]

Typically, a separate surgical procedure is employed to modify the biotype and enhance the thickness or width of the keratinized tissues around implants. This can be carried out either during the uncovering of the implant and placement of the healing abutment or around previously positioned implants with healing abutments.

Usually, a free gingival graft is used for this purpose, which can lead to donor site morbidity and may not perfectly match the surrounding soft tissues in terms of aesthetics.

In 2017, we devised a CM-based technique to address these issues, called it “the H-technique” and published it as a proof-of-concept case study in the same year [79]. This technique aimed to reduce donor site morbidity, minimize the number of surgical interventions and separate steps (resulting in reduced healing time), and improve aesthetic outcomes. We noted already in that study that the favorable immediate outcome and the short-term success achieved with the proposed technique did not necessarily imply long-term success and that the case needed to be followed up. In preparation for this thesis, six years later, in 2023, we reevaluated the patient (who was otherwise asymptomatic) to assess the long-term performance of the technique. In this chapter, we present the case, the technique employed, and the follow-up.

[1] While our initial efforts concentrated indeed on partial edentulism in the molar region, we have since successfully applied the presented technique in a fully edentulous case as well. The 5-year follow-up of that case suggests that the technique is equally effective in full edentulism and other regions, whenever the soft tissues around adjacent implants need to be augmented. The reason the results are not reported here is because that particular case has not been published.

III.2.2. The case

Our middle-aged female patient visited our dental office to have her missing lower left molars (36, 37) replaced.

The patient is a non-smoker, systemically healthy, and maintains excellent oral hygiene. No signs of periodontal disease were detected. However, it was apparent even through a simple visual examination that the bone volume in the implant area and the quantity of soft tissues were not sufficient. Figure 13 illustrates the initial situation, where the remarkably thin mucosa on the edentulous alveolar ridge and the narrow, approximately 2 mm wide keratinized mucosa can be clearly observed. In this case, we encountered a dual challenge as we had to navigate suboptimal conditions for both the hard and soft tissues. Moreover, we aimed to adopt a minimally invasive approach whenever feasible.

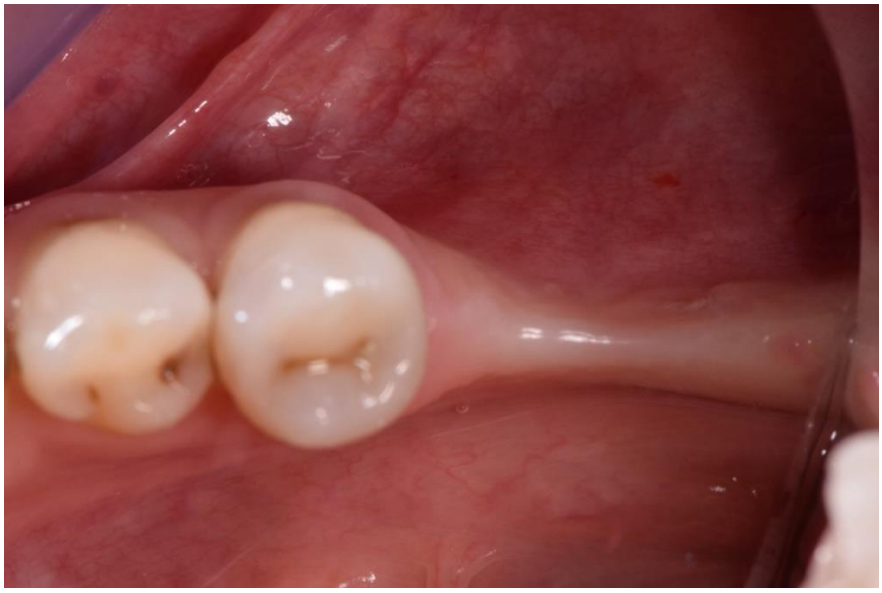

Figure 13. The initial situation: narrow, atrophied alveolar ridge with thin mucosa and a narrow strip of keratinized mucosa.

III.2.3. The treatment

Based on CBCT imaging, we planned to place standard-diameter (4.1 mm), short-length (36: 8 mm; 37: 6 mm) Straumann Roxolid implants with screw-retained crown restorations in the area of the missing lower molars (36, 37). This approach allowed us to avoid bone grafting and spare the patient from an additional invasive procedure with a relatively long healing period.

For the augmentation of soft tissues, we also aimed to adopt a minimally invasive approach. Therefore, we decided to use a xenogeneic collagen matrix and opted for a minimally invasive surgical technique.

The essence of the soft tissue augmentation treatment was to restore the thickness and keratinization of the soft tissues around the implants in the same surgical step as implantation [86]. In order to avoid the need for autogenous connective tissue (thus reducing invasiveness and eliminating donor site morbidity), mucosal thickening and keratinization were achieved using a three-dimensional collagen matrix [87]. The applied technique also employed an open wound healing approach, where the healing abutments play a crucial role.

The crestal incision was made in the middle of the thin keratinized strip to ensure that both flap edges contained, even in small amounts, the cells necessary for keratinization. Following the placement of implants, the high primary stability (35 Ncm) allowed us to provide the implants with healing abutments during the surgery (Figure 14).

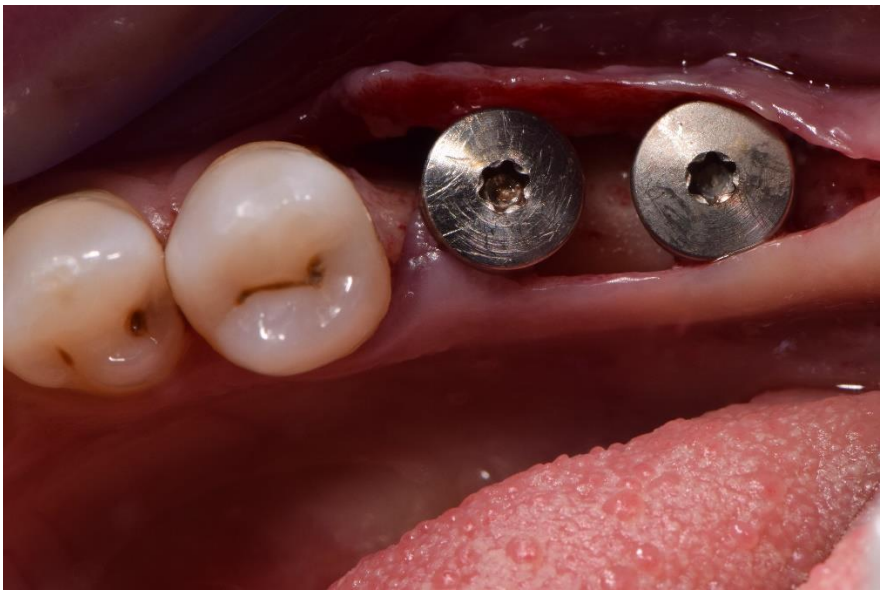

Figure 14. Crestal incision and the implants with the healing abutments after placement (before soft tissue augmentation).

To thicken the mucosa of the buccal and lingual flaps and promote keratinization between the flap edges, we used a collagen matrix (Mucograft®, Geistlich Pharma AG, Wolhusen, Switzerland), which was shaped in an "H" configuration (Figure 15).

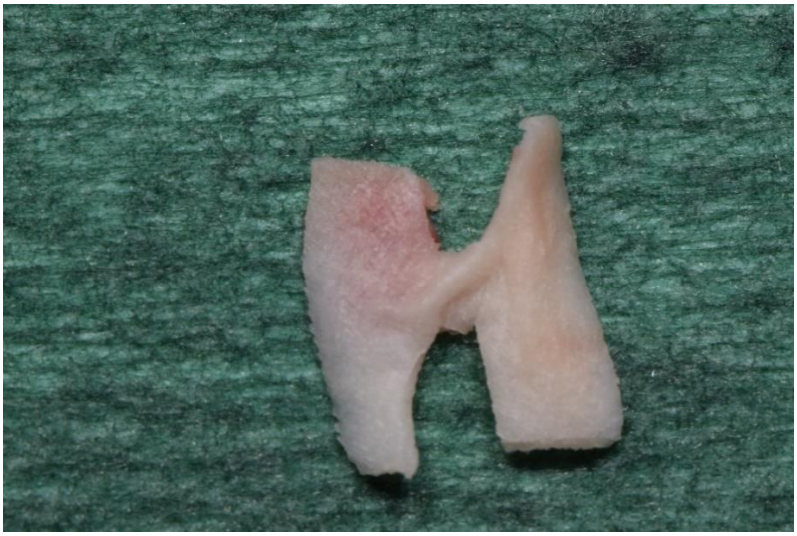

Figure 15. The collagen matrix cut out in an H-shape to thicken the mucosa of the buccal and lingual flaps and promote keratinization between the flap edges.

The technique later received its name from this. The middle connecting part of the H shape (the horizontal line in the letter H) covered the exposed denuded ridge surface between the two implants, while the outer parts (the vertical lines in the letter H) were placed beneath the flap edges using the tunnel technique. This way, the collagen matrix simultaneously provided thickening of the mucosa (both buccally and lingually) and widening of the keratinized tissue. The immobility of the collagen was largely provided by the healing screws themselves, along with a cross-linked horizontal mattress suture between the implants. The schematic representation of the matrix placement is shown in Figure 16, while Figure 17 illustrates the actual postoperative situation.

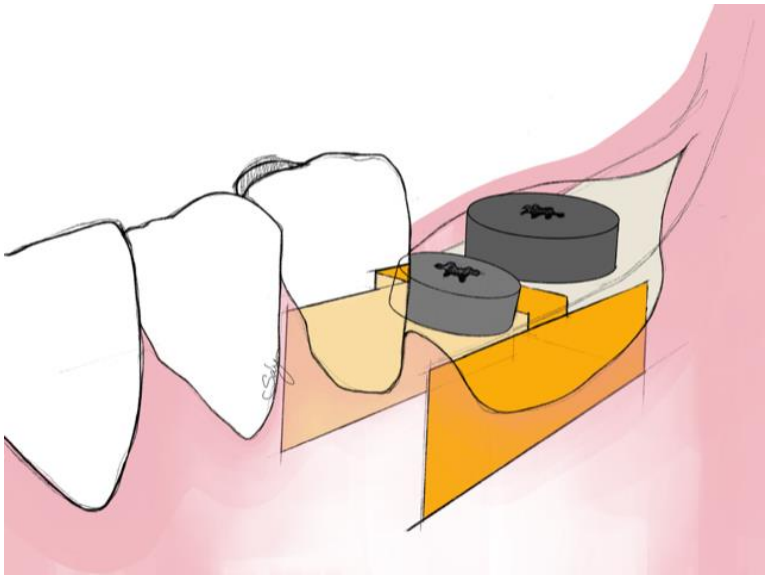

Figure 16. Schematic representation of the application of the H-shaped matrix (orange). Artwork courtesy of Dr. Tekla Sáry.

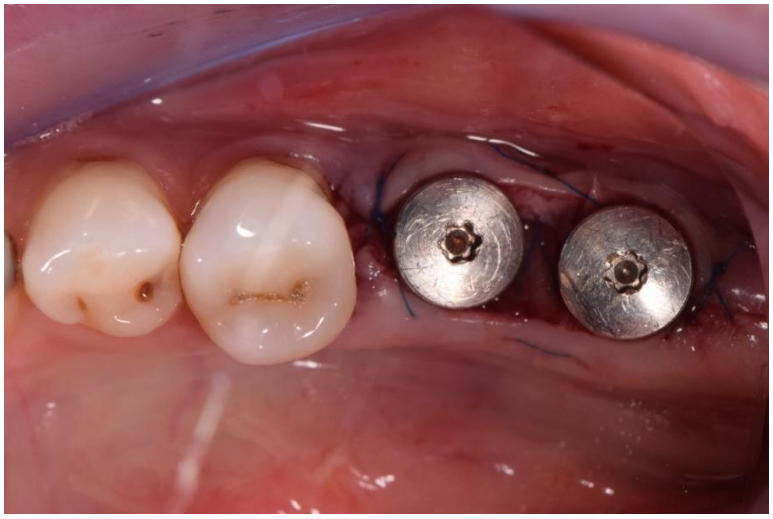

Figure 17. The postoperative situation. The collagen matrix is readily visible inside the wound. Mattress sutures had been applied to immobilize the matrix.

III.2.4. The immediate results and the one-year follow-up

The sutures were removed two weeks after the surgery. Following this short-term healing, the thickened biotype and initial keratinization were already visible on the exposed collagen matrix surfaces (Figure 18).

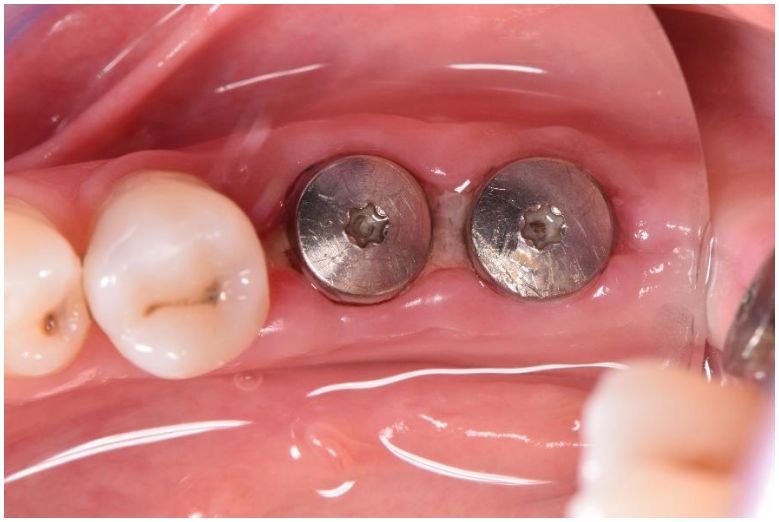

Figure 18. Early healing: the clinical situation after the removal of the sutures (2 weeks after surgery)

Upon complete healing (at two months), the thickening of the soft tissues and widening of keratinization in the area were clearly visible, without any discernible differences in color or texture

compared to the surrounding tissues, unlike with traditional free gingival graft techniques (Figure 19).

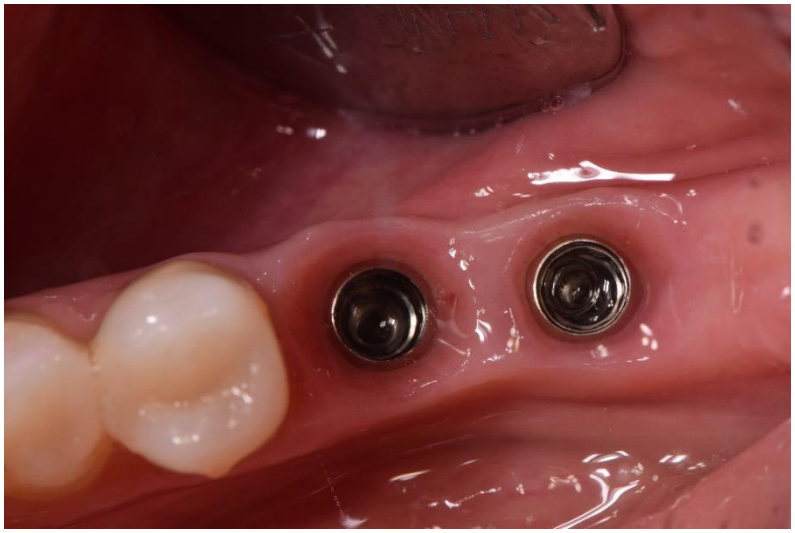

Figure 19. The clinical situation at 2 months right before the delivery of the implant crowns.

Screw-retained fixed dental restorations were placed on the implants, and even after a one-year follow-up, a healthy, stable, and aesthetically pleasing soft tissue profile was observed. This was, of course, greatly influenced by the patient's optimal individual oral hygiene, despite the difficult accessibility of the area (Figure 20).

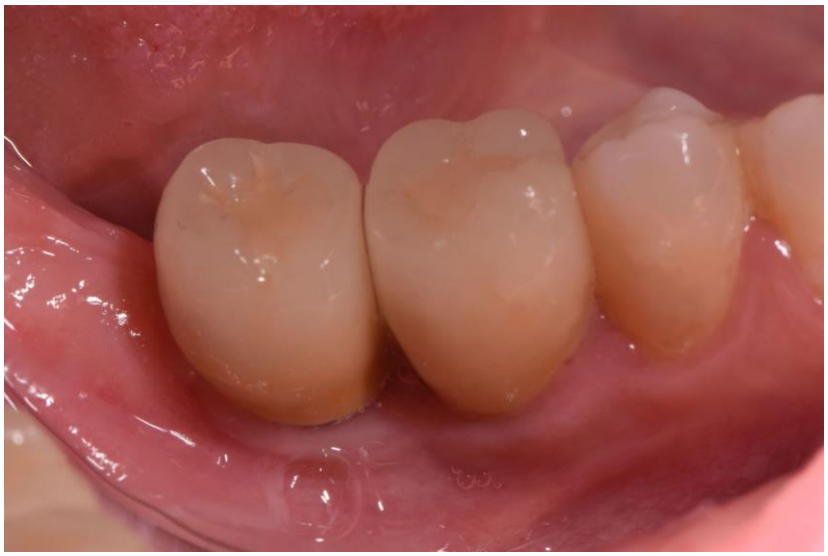

Figure 20. The clinical situation 1 year after the surgery. The soft tissues are healthy and esthetic.

III.2.5. The situation six years later

Six years later, in July 2023, the patient returned for a follow-up appointment. During the examination, the patient reported no issues with the implants, prosthetic crowns, or the soft tissues surrounding the implants. The assessment revealed that the soft tissues were in a healthy and aesthetically pleasing condition, closely resembling the state observed six years before (Figures 21 and 22).

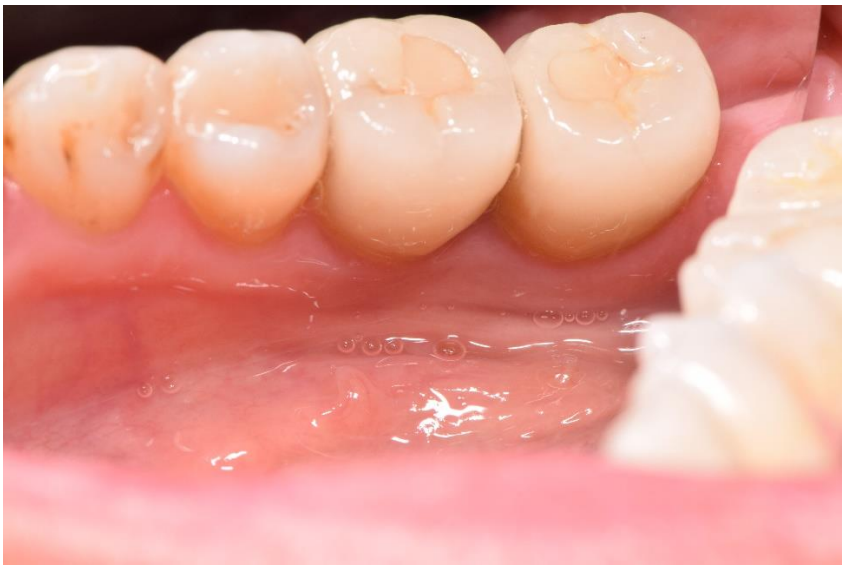

Figure 21. Clinical situation at the 6-year follow-up (lingual aspect)

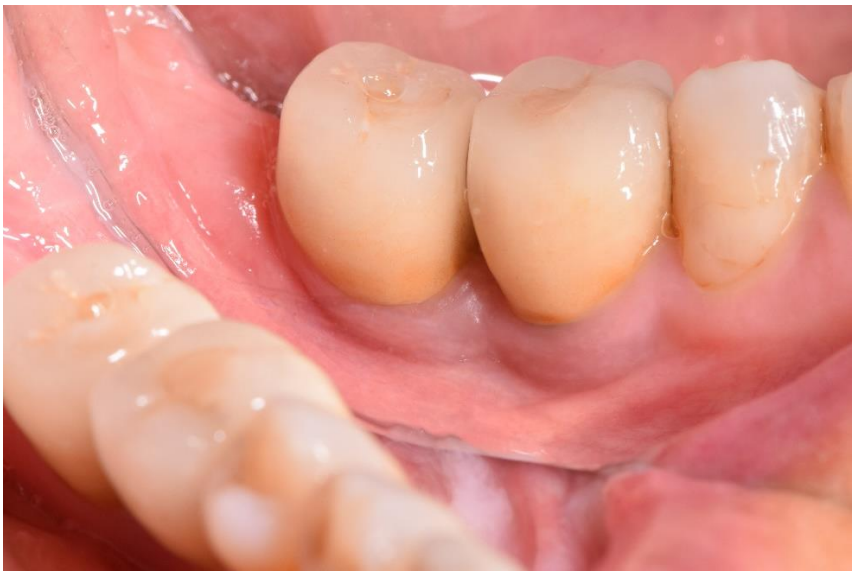

Figure 21. Clinical situation at the 6-year follow-up (buccal aspect)

IV. DISCUSSION

This thesis explores the topic of peri-implant soft tissue augmentation, specifically focusing on the use of xenogeneic collagen membranes (XCM) as an alternative to autogenous grafts. We put special emphasis on the aspect of keratinization, which has been a subject of debate within the existing literature. To address these questions, we used two vastly different perspectives: an animal study with histology and a clinical case study.

Regardless of the perspective, the primary aim of our research was to determine the viability of XCM as a practical alternative to autogenous grafting, which is a more invasive approach with longer healing time and potential donor site morbidity. Our objective was to contribute to the ongoing discussion surrounding these issues by presenting the findings from our publications. Overall, our results provide positive indications and support the use of XCM as a suitable option for peri-implant soft tissue augmentation, offering clinicians an alternative approach to consider.

Our animal study focused on investigating the healing characteristics around teeth and implants following recession coverage. We used either a superficial or deep connective tissue graft from the palate or a collagen matrix for the procedure. The evaluation involved descriptive histologic and histomorphometric analyses to assess any differences among the groups regarding the healing pattern, epithelial keratinization, and dimensions of soft and hard tissues around teeth and implants.

Regarding keratinization, all groups showed the formation of keratinized epithelium around both teeth and implants. In the case of teeth, the three experimental groups achieved similar lengths of keratinized epithelium, although significantly shorter compared to the control group with unaffected teeth. The length of non-keratinized epithelium remained similar for both the control and experimental groups. These findings suggest that the difference in keratinized epithelium between control and experimental teeth might be strongly influenced by the recession defect surgically created during the procedure. As for implants, determining the length of keratinized tissue was not possible due to incomplete transmucosal healing observed in some implants, leading to varying healing conditions.

In the minipig model, previous studies have assessed the extent of keratinized tissue in response to gingival recession defect treatment. When comparing CAF alone to CAF+CM, CAF alone resulted in approximately 1 mm more keratinized tissue width [81]. The average amount of keratinized tissue measured 2.66 ± 0.42 mm before CAF+CTG treatment and increased to 3.83 ± 0.47 mm 12 weeks after treatment [14]. In our study, the keratinized epithelium at the experimental teeth measured 0.86 ± 0.92 mm (SCTG), 1.13 ± 0.62 mm (DCTG), and 1.44 ± 0.76 mm (CM). These differences in measurements might be partially attributed to variations in the histometric evaluation method and the

absence of baseline measurements (i.e., before CAF preparation). Instead, we compared the values after 8 weeks with those of a control tooth. Additionally, our study exclusively used mandibular teeth and implant sites, while the other studies included both maxillary and mandibular sites [80, 81].

The findings in this study, showing that CAF+CTG and CAF+CM resulted in a similar increase in keratinized tissue, are consistent with previous clinical studies [19, 20], where the average gain in keratinized tissue was 1.26 mm for CAF+CTG and 1.34 mm for CAF+CM [75, 76].

This study did not find significant differences in the inherent characteristics of superficial and deep connective tissues in inducing keratinization at the recipient site, contrary to what was suggested by Ouhayoun and colleagues [66]. Nonetheless, when interpreting these results, it should be considered that the connective tissue grafts were covered with a relatively thick layer of flap, which might have limited the direct influence of cells within the grafts on the epithelium. Indeed, a recent review with meta-analysis further supported the superiority of superficial grafts, reporting a mean recession coverage of 89.3% for deeper connective tissue grafts and 94.0% for de-epithelialized superficial connective tissue grafts [88].

The influence of inflammatory processes on tissue keratinization remains a subject of ongoing debate. Animal studies involving experimentally induced chronic or acute inflammation have shown no significant effect on tissue keratinization [89, 90]. Conversely, reducing gingival inflammation has been associated with the occurrence of sulcular keratinization [91]. In our current study, we observed pocket formation with subgingival calculus and inflammatory processes in nearly all experimental teeth and around implants that received a connective tissue graft (CTG). In contrast, implants treated with a collagen matrix (CM) exhibited no pocket formation and displayed healthy peri-implant soft tissue conditions with minimal, physiologically normal inflammation.

Surprisingly, no significant difference in epithelial keratinization was observed between the inflamed and non-inflamed conditions. One possible explanation for the contrast in pocket formation between the CM and CTG groups at the implants is related to the characteristics of the grafts. The voluminous, spherical CTGs might have substantially lifted the bottom of the vestibulum, potentially hindering a tight seal between the flap and teeth/implants and thereby favoring plaque-induced inflammation. On the other hand, the less voluminous and flatter CMs did not cause such an elevation of the vestibulum's bottom, allowing for undisturbed healing around the implants.

One intriguing discovery was that both superficial and deep connective tissue grafts showed minimal signs of degeneration or integration into the surrounding tissues after an 8-week healing period. This phenomenon was observed in both teeth and implants. Presently, there is limited knowledge regarding the temporal sequence of tissue degradation or integration of transplanted

connective tissue grafts from the palate. The seminal studies conducted by Karring and colleagues in monkeys not only addressed the question of epithelium specificity but also provided insights into the healing process ranging from a few days to 12 months [92]. At the 3-month mark, the transposed tissues exhibited partial degeneration [93, 94]. However, it is crucial to consider that the surgical techniques and animal species differed between the latter and the current study.

In this study, the experimental groups demonstrated similar results in terms of biologic width (BW). It is worth noting that the BW at the control teeth averaged 5.1 mm, which is notably higher than what is typically observed in other species or in humans [95]. However, in the SCTG and DCTG groups around teeth, the junctional epithelium (JE) measured 2.51 ± 0.72 mm and 2.21 ± 0.81 mm, respectively, which is significantly longer than at the control teeth. These results strongly suggest that the surgical manipulation of the soft tissue triggered a repair process with the JE migrating apically.

These findings are consistent with previous studies in dogs [96] and minipigs, where treatment with CAF alone resulted in 2.79 ± 0.77 mm and CAF+CM in 2.26 ± 0.23 mm of JE [15]. Interestingly, at the implants, the JE was even longer, with the distance cB-PIMM averaging 3.17 ± 0.62 mm and 3.17 ± 0.43 mm for SCTG and DCTG, respectively, while slightly less for CM. It is possible that the connective tissue grafts may induce some form of bone resorption, akin to root resorptions that have been rarely described [97-99].

There are several limitations to consider regarding the applicability of the miniature pig model in this research. Firstly, the anatomical differences in the vestibulum between miniature pigs and humans pose a challenge for this type of surgical procedure. Other studies have utilized the miniature pig model for coronally advanced flap surgeries after connective tissue or biomaterial transplantations in various mandibular and maxillary positions. As a result, the three experimental groups displayed different vestibulum characteristics, with the CM group showing deep, the CTG groups showing very shallow, missing, or directly rising vestibulum conditions. These anatomical differences, along with the thickness of the transplanted materials, likely contributed to the variations observed between the CM and the two CTG groups.

Additionally, standardizing the harvesting of superficial and deep connective tissue from the palate proved to be challenging. Adaptations in implant placement and positioning were necessary due to anatomical variations, which deviated from the human situation and may have led to the occurrence of saucer-shaped defects. Furthermore, postoperative care and plaque control during the

healing phase were difficult to implement in the animal model, resulting in signs of inflammation and calculus formation around most of the teeth and implants.

Another limitation was the difficulty in performing horizontal measurements along all levels for both teeth and implants in all samples. The inclusion of control teeth, despite being molars, provided a comparison with normal histomorphometric parameters around teeth (i.e., junctional epithelium, soft connective tissue height, bone level, etc.). However, it's important to note that the experimental teeth were premolars and not fully comparable to the control teeth.

Finally, the study involved a relatively small number of teeth and implants, and the treatments were performed by two different surgeons. This may have introduced some inter-operator variation into the results.

Overall, while the miniature pig model provided valuable insights, these limitations must be considered when interpreting the study's findings.

Regarding our case study, it admittedly represents a smaller practical contribution to the topic of XCM in peri-implant soft tissue augmentation. At the time of the publication of our study, all major elements of the technique (such as open healing and XCM) were well-known. XCM was known and generally accepted, but the debate around whether it can induce keratinized tissue growth was also known [100]. At the same time, it was also not clear whether keratinized tissue per se played a role in peri-implant health at all [101]. Since then, as we showed in the introductory chapter of this thesis, much has been published about these issues, predominantly indicating that keratinized tissue does have a role and that XCM is capable of inducing keratinized tissue growth. Regardless of these unresolved issues, we required a minimally invasive solution for a situation where two adjacent implants needed to be placed in the severely atrophied posterior mandible. By applying short implants to avoid bone augmentation and opting for XCM to augment peri-implant soft tissues without autografting, we successfully restored the thickness (and later the keratinization) of the soft tissues around the implants in the same surgical step as implantation. This approach minimized invasiveness, significantly shortened healing time, and reduced the patient's burden compared to conventional multistep approaches. The procedure's novelty lay in integrating implantation and augmentation into the same step, as discussed before, and the unique application of XCM using an H-shape, which enabled simultaneous thickening of the mucosa (both buccally and lingually) and widening of the keratinized tissue. Within two months, we achieved a completely healed, healthy, and aesthetically pleasing surgical area with keratinized mucosa. The success of the approach was evident in both short- and long-term follow-ups at one year and six years, respectively. It is noteworthy that since

the publication of our case study, Han and colleagues reached success with XCM but with a different surgical technique in a similar situation [73]. They treated a 66-year-old healthy female patient with missing mandibular molars (36, 37). The teeth had been missing for 5 years. Soft tissue augmentation was carried out with XCM to augment the inadequate buccal keratinized mucosa. Following a two-month healing period, "the widths of mesial, medial, and distal buccal keratinized mucosa were 4, 3, and 3 mm, respectively, and the thickness of the augmented mucosa was 4 mm." These results and our own results provide clinical evidence that XCM can be a good and efficient alternative to autologous grafting in the posterior mandible (i.e., the molar region) and that XCM does induce keratinized tissue growth. As mentioned previously, another debated question is whether the presence of keratinized tissue contributes to peri-implant health. Indeed, the peri-implant tissues in this case were perfectly healthy even six years after the treatment, but given the excellent oral hygiene of our patient, it is not possible to determine the effect of keratinized tissue itself.

In essence, it can be said that our hypotheses regarding this technique have been confirmed. Applied in the posterior mandible, the single-step H-technique utilizing XCM resulted in favorable short- and long-term outcomes, both in terms of keratinized tissue restoration, peri-implant soft tissue health, and aesthetics. Based on the results presented in this thesis, as well as our additional experiences with the technique (not reported here), we propose that the approach can be safely and effectively employed not only in the molar region but also in other areas, including cases of full edentulism, where there is a need for soft tissue augmentation around adjacent implants.

Turning our attention to the study's limitations, case studies are often criticized for their limited generalizability, as results from a single case cannot be broadly applied. However, we believe that in dentistry, they play a crucial role in complementing evidence-based clinical practice. Dental scenarios frequently arise where addressing specific issues requires minor modifications or a combination of known approaches. These novel treatment concepts may not necessitate extensive preclinical and clinical trials but still require documentation and dissemination. The primary goal of a proof-of-concept study is not to provide definitive evidence or draw generalizable conclusions. Instead, it aims to gather preliminary data and evidence supporting the potential of a proposed intervention or idea. Such studies demonstrate that the proposed approach has a reasonable chance of success and could be further developed and studied in larger, more comprehensive trials or experiments. Our case study on the H-technique exemplifies this approach.

V. CONCLUSIONS

Through the studies covered in this thesis, we have demonstrated the following and we consider these to be the novel scientific findings related to the work that has been accomplished.

Regarding the histologic study:

- We have demonstrated that after peri-implant soft tissue augmentation with xenogeneic collagen matrix, keratinized tissue is formed. To our knowledge, we are the first to have demonstrated this with histological methods, corroborating widely reported clinical observations.
- All experimental teeth and implants receiving SCTG or DCTG showed pocket formation with subgingival calculus and inflammation, whereas implants treated with XCM displayed healthy peri-implant soft tissue conditions. This suggests that XCM outperformed autogenic SCTG/DCTG in this regard, representing an intriguing new finding that warrants further investigation.
- Contrary to previous suggestions, there was no discernible difference in the ability to induce keratinization between superficial and deep autogenic grafts.

Regarding the clinical case study:

- We have introduced the H-technique, a minimally invasive peri-implant soft tissue augmentation approach. This innovative technique involves simultaneous implantation and soft tissue augmentation during a single session, utilizing a specially shaped piece of XCM to thicken the mucosa (both buccally and lingually) and widen the keratinized tissue. We have demonstrated that the H-technique can serve as an excellent and less invasive alternative to conventional multistep techniques, offering remarkable short- and long-term results.

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APPENDIX

Clinical Oral Investigations
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RESEARCH

The effect of connective tissue graft or a collagen matrix on epithelial differentiation around teeth and implants: a preclinical study in minipigs

Alexandra Stähli[1] · László Párkányi[2] · Sofia Aroca[1] · Andreas Stavropoulos[3] · Frank Schwarz[4] · Anton Sculean[1] · Dieter D. Bosshardt[1,5]

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Abstract

Objectives This study aimed to histologically evaluate the healing at 8 weeks after coronally advanced flap (CAF) with either a superficial (SCTG) or deep palatal connective tissue graft (DCTG), or a collagen matrix (CM) to cover recession defects at teeth and implants.

Material and methods One mandibular side of 6 miniature pigs received each 3 titanium implants 12 weeks after extraction. Eight weeks later, recession defects were created around implants and contralateral premolars and 4 weeks later randomly subjected to CAF + SCTG, CAF + DCTG, or CAF + CM. After 8 weeks, block biopsies were histologically analyzed.

Results For the primary outcome, i.e., keratinization of the epithelium, all teeth and implants exhibited a keratinized epithelium with no histological differences among them also not in terms of statistically significant differences in length (SCTG 0.86 ± 0.92 mm, DCTG 1.13 ± 0.62 mm, and Cm, 1.44 ± 0.76 mm). Pocket formation was histologically seen at all teeth, around most implants with SCTG and DCTG, however not in the CM implant group. The connective tissue grafts showed hardly signs of degradation, whereas the CM was partly degraded and integrated in connective tissue. The mean gain in gingival height was similar in all experimental groups (SCTG 3.89 ± 0.80 mm, DCTG 4.01 ± 1.40 mm, CM 4.21 ± 0.64 mm). Statistically significant differences were found in the height of the junctional epithelium between the control teeth and the connective tissue groups ($p = 0.009$ and 0.044).

Conclusions In this animal model, the use of either a superficial or deep connective tissue graft or a collagen membrane did not seem to have any impact on the epithelial keratinization around both teeth and implants. All procedures (CAF + SCTG/DCTG/CM) resulted in a long JE that was even longer at implants.

Clinical relevance Deep/superficial palatal connective tissue graft yielded similar keratinization around teeth/implants. Given the absence of pocket formation and inflammatory processes at implants when using a CM, CAF + CM might bear potential clinical benefits.

Keywords Connective tissue graft · Keratinization · Minipig · Single tooth · Dental implants

Alexandra Stähli and László Párkányi contributed equally to the manuscript and share first author position.

✉ Alexandra Stähli
alexandra.staehli@unibe.ch

[1] Department of Periodontology, School of Dental Medicine, University of Bern, Freiburgstrasse 7, 3010 Bern, Switzerland

[2] Department of Periodontology, Faculty of Dentistry, University of Szeged, 6720 Tisza Lajos Körút 64, Szeged, Hungary

[3] Department of Periodontology, Faculty of Odontology, Malmö University, Carl Gustafs Väg 34, 20506 Malmo, Sweden

[4] Department of Oral Surgery and Implantology, Goethe University, Theodor-Stern-Kai 7, 60596 Frankfurt, Germany

[5] Robert K. Schenk Laboratory of Oral Histology, School of Dental Medicine, University of Bern, Freiburgstrasse 3, 3010 Bern, Switzerland

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Introduction

Findings from a narrative review analyzing the biology and soft tissue wound healing around teeth and implants have indicated that tissue morphogenesis of the gingival, palatal, and alveolar mucosa appears to be primarily innately determined [1]. Furthermore, it also appears that the connective tissue originating from an area originally covered by keratinized epithelium and/or from the periodontal ligament possesses the potential to induce epithelial keratinization. These conclusions are in line with those made by others indicating that granulation tissue proliferating from the alveolar mucosa appears to induce the formation of a non-keratinized epithelium, whereas the one originating from the supra-alveolar connective tissue or from the periodontal ligament would lead to a keratinized epithelium [2, 3].

Based on the above-mentioned findings, connective tissue grafts (CTGs) harvested from the palate are nowadays routinely used for the treatment of soft tissue dehiscences/recessions around teeth and for increasing the width of keratinized tissue around teeth and implants. However, clinical observations indicate that in many cases when palatal CTGs are covered by a non-keratinized mucosal flap, keratinization of the epithelial cells fails to occur. These clinical observations are supported by findings from earlier studies suggesting that CTGs harvested from deep palatal connective tissue layers may not have the same potential to induce keratinization than grafts harvested from more superficial layers. In a nicely designed experiment, a thick palatal epithelial-connective tissue graft was excised and split into two thinner grafts. i.e., one immediately subepithelial and the other one closer to the bone [4]. The grafts were transplanted into contralateral areas lacking keratinized mucosa. Following a healing period of 3 months, biopsies were excised and examined by means of routine histology, immunofluorescence, and gel electrophoresis. The results showed that while the epithelial-connective tissue grafts displayed histological and biochemical characteristics of keratinized tissue (i.e., gingiva), the deep connective tissue grafts expressed features belonging to both keratinized and non-keratinized tissue. Comparable findings in humans were also reported by others indicating that palatal connective tissue grafts or free gingival grafts transplanted into areas of non-keratinized tissue may not always develop the characteristics of keratinized tissue [5–7].

Thus, it appears that CTGs harvested from the palate may not always induce keratinization at sites with originally non-keratinized epithelium, which may be explained by differences between the palatal connective tissue grafts harvested from superficial or deeper parts to induce keratinization. Interestingly, at present, it is still unknown, whether a zone of keratinized tissue may reform following complete excision (i.e., gingivectomy) of the keratinized tissues surrounding implants (i.e., excision of both free and attached mucosa). A porcine-derived bioresorbable collagen matrix (CM) has been suggested as a potential alternative to the CTG to increase the width of keratinized tissue around implants and to treat single and multiple recessions around natural teeth and implants. The available data indicate that the use of this CM may lead to an increase of keratinized mucosa around implants and [8–11], to a certain extent, to gain of keratinized tissue width when used for recession coverage at teeth [12, 13]. Whether superficial or deep CTGs induce a different degree of keratinized tissue is not known. Thus, the aim of this study is to explore to what extent differences exist between superficial (i.e., harvested from an immediately subepithelial area) and deep (i.e., harvested from an area close to the bone) parts of palatal CTGs in determining epithelial keratinization around teeth and implants completely deprived of gingiva or keratinized mucosa, respectively. We hypothesized that both superficial or deep CTGs induce similar keratinization at teeth and implants. Furthermore, it is unknown to what extent the application of a CM may replace the use of CTGs at teeth and implants.

Materials and methods

Surgical procedure

The study protocol was approved by the local Committee for Animal Research, University of Szeged, Hungary No 1-74-2/2015 MAB. Six Göttingen miniature pigs were used for the study. The husbandry and care of the animals before, during, and after surgery was handled at the Surgical Research Unit, University of Szeged, Hungary. The animals received standard food and water ad libitum. Animals were premedicated using ketamine (i.m. 20 mg/kg), xylazine (i.m. 2 mg/kg), atropine (i.v. 0.05 mg/kg), and midazolam (i.v. 0.5 mg/kg) to achieve the intubation status. Inhalation anesthesia was performed with isoflurane (1.0–1.5%). Fentanyl patches (5–10 mg/kg) were used for the intraoperative analgesia, and the animals received antibiotic prophylaxis for 3 days (Duplocillin LA, 12,000 U.I./kg).

The study design is summarized in Fig. 1a. In one side of the lower jaw, the second, third, and fourth premolars as well as the first molar were extracted. After 12 weeks of healing, three tissue level implants (8–10 mm long; Straumann®) were placed. After 8 weeks of healing, a soft tissue dehiscence was surgically created around the implants. Around the contralateral second, third, and fourth premolars, isolated Miller Class II recession defects were surgically created by completely removing

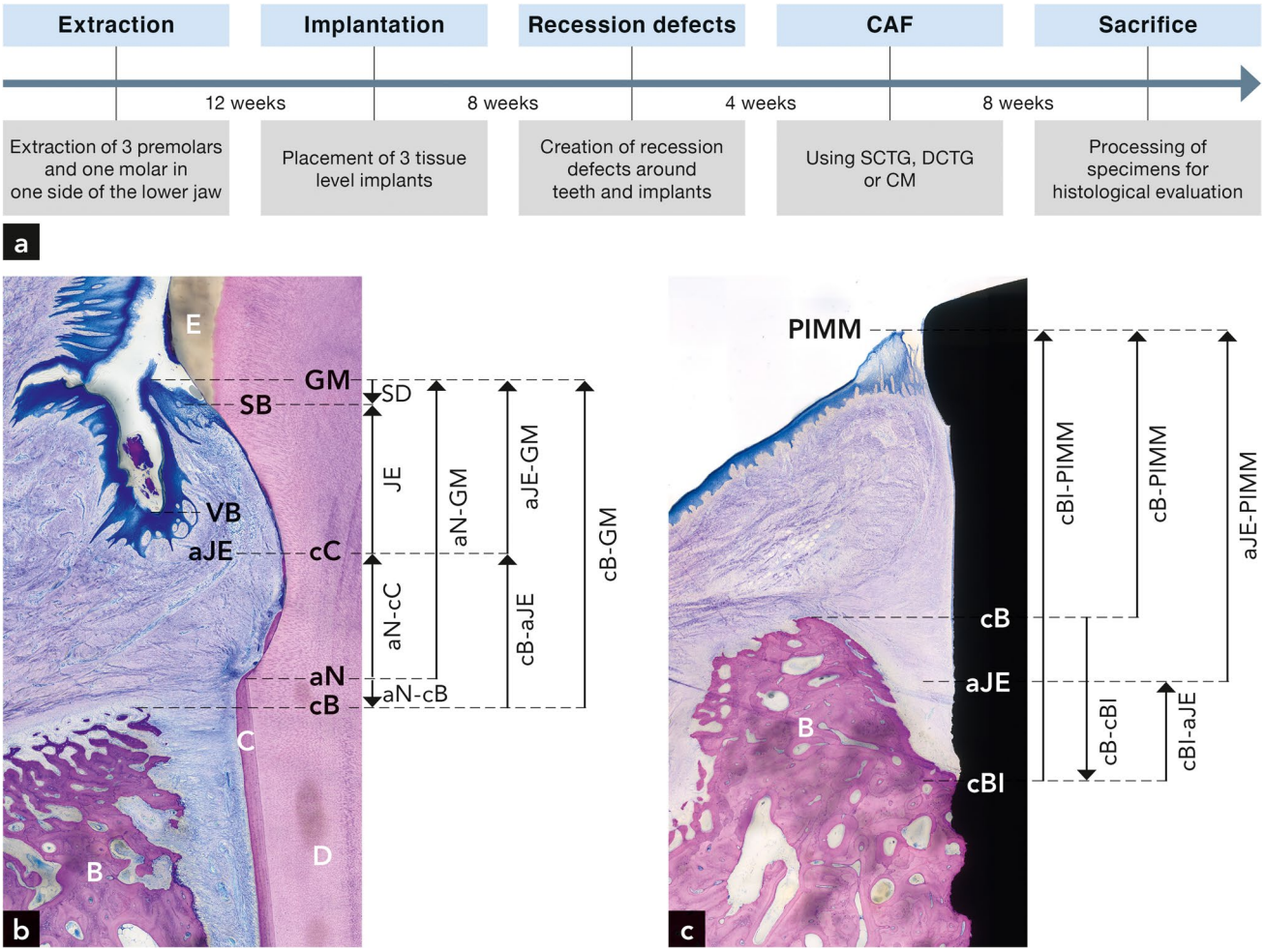


Fig. 1 **a** Flow diagram displaying the study design with the time-points of interventions and healing periods. **b** Landmarks around teeth for the histomorphometric measurements. GM, gingival margin; SB, sulcus bottom; VB, vestibulum bottom; aJE, the most apical extent of the junctional epithelium; cC, the most coronal extent of new cementum; aN, the most apical part of the surgically created root surface; cB, the most coronal level of bone; SD, sulcus depth; JE, junctional epithelium; aN-cC, vertical gain of new cementum; aN-cB, the most apical part of the surgically created root surface to the bone crest; aN-GM, the most apical part of the surgically created root surface to the gingival margin; aJE-GM, length of junctional epithelium plus sulcus depth; cB-aJE; the most coronal level of bone to the apical extent of the junctional epithelium; cB-GM, biologic width; c, cementum; d, dentin; b, bone. **c** Landmarks and distance measurements around implants. PIMM, peri-implant mucosal margin; aJE, the most apical extent of the junctional epithelium; cBI, the most coronal level of bone in contact with the implant; cBI-PIMM, biologic width; cB-cBI, vertical distance from the bone crest to the most coronal bone level in contact with the implant; cB-PIMM, vertical distance from the bone crest to the peri-implant mucosal margin; cBI-aJE, the most coronal level of bone in contact with the implant to the most apical extent of the junctional epithelium; aJE-PIMM, length of junctional epithelium plus sulcus depth; b, bone

the buccal gingiva, bone and root cementum using blades, bone chisels, and slowly rotating burs under copious rinsing with sterial saline according to previously described protocols [14, 15]. The so created defects measured about 5 mm in depth and 4 mm in width apically to the cemento-enamel junction. The exposed root and implant surfaces were left untreated for 4 weeks to allow soft tissue healing and plaque accumulation and to mimic closer a chronic recession-type defect.

After 4 weeks of healing, the defects were treated. First, the exposed parts of the roots of the teeth were meticulously cleaned with Gracey curettes (Hu-Friedy, Chicago, IL, USA); the implants received a supramucosal cleaning using rubber cups and a polishing paste (Zircate, Prophy Paste; Dentsply, Konstanz, Germany). Around teeth the most apical part of the before surgically exposed root surface was marked with a small bur (diameter 2 mm) to create a reference mark for the histometric analysis. At the implants, clinical defect height (CDH) was measured at the mid-buccal aspect from the implant shoulder (IS) to the bottom of the mucosal recession. The defects were treated using a CAF described by Allen and Miller (1989) and a CM or a

CTG. Two vertical releasing incisions were placed that were 6 mm longer than the recession defects. In case a CTG was selected, the needed amount of tissue was harvested from the palate according to the technique described by Hürzeler and Weng (1999) measuring 0.5 mm less than the size of the vascular bed in mesio-distal length and 5 mm in corono-apical direction.

Using a computer-generated randomization program, the defects in each quadrant were treated as follows:

(1) CAF + superficial CTG (SCTG) around teeth and implants
(2) CAF + deep CTG (DCTG) around teeth and implants
(3) CAF + CM (Mucograft®, Geistlich, Wolhusen, Switzerland) around teeth and implants

The flaps were closed with 6–0 monofil (Polypropylene, Stoma, Emmingen-Liptingen, Germany) suture material. Sutures were removed at 2 weeks. The animals were euthanized after 8 weeks of healing.

Histologic processing

The lower jaws were removed and chemically fixed by immersion in 10% buffered formalin supplemented with $CaCl_2$ for 3 weeks. The specimens were rinsed in running tap water, dehydrated in ascending concentrations of alcohol, and embedded in methylmethacrylate, as previously described [16, 17]. Each tooth and implant was sectioned parallel to its longitudinal axis in a bucco-lingual direction, resulting in two to three undecalcified ground sections of ~ 500 µm thickness. The sections were ground to a final thickness of 80 µm, superficially stained with toluidine blue and basic fuchsin and the two central-most sections were used for descriptive and histomorphometric analyses.

Descriptive histology

The descriptive analysis was performed directly under the microscope. Keratinization/non-keratinization as well as presence/absence and extent of inflammation were evaluated in the sections stained with toluidine blue/fuchsin. For comparative reasons, one untreated first molar per animal served as internal control for the descriptive analysis.

Histomorphometry

All ground sections were digitalized using a Zeiss Axio Imager.M2 microscope with an automatic scanning stage, a digital camera, and a stitching software called ZEN (Zeiss Efficient Navigation). All histometric measurements were performed at buccal sites blindly by one experienced and calibrated investigator using the ZEN software.

Primary outcome: keratinization of the epithelium

Measurements around teeth: The length of the keratinized tissue (from the gingival margin to the mucogingival junction), the length of the non-keratinized tissue (from the mucogingival junction to the bottom of the vestibulum), and the ratio between them were measured and calculated, respectively.

Measurements around implants: The length of keratinized tissue, length of non-keratinized tissue, and ratio of keratinized to non-keratinized tissue were planned to be measured. However, since many implants were submerged or partly submerged, these measurements were not possible.

Secondary outcomes

The following landmarks were identified around teeth (Fig. 1b):

– GM: gingival margin
– SB: sulcus bottom
– aJE: the most apical extent of the junctional epithelium
– cC: the most coronal extent of new cementum
– aN: the most apical part of the surgically exposed root surface, i.e., the gingival margin before flap advancement, marked with a notch
– cB: the most coronal level of bone = bone crest
– VB: the bottom of the vestibulum

The following vertical distance measurements were performed (Fig. 1b):

- aN-GM: gain in gingival height
- cB-GM: the biologic width, i.e., the vertical distance from cB to GM
- aJE-GM: length of junctional epithelium plus sulcus depth
- cC-aJE: length of connective tissue adhesion
- SB-GM: sulcus depth
- aN-cC: vertical gain of new cementum
- aN-cB: apical part of the notch to the bone crest

The following landmarks around implants were determined (Fig. 1c) according to Schwarz et al. [18]:

- PIMM: peri-implant mucosal margin
- aJE: the most apical extent of the junctional epithelium
- cBI: the most coronal level of bone in contact with the implant
- cB: the most coronal level of bone = bone crest

The following vertical distance measurements around implants were performed (Fig. 1c):

- cBI-PIMM: biologic width, i.e., vertical distance from cBI to the peri-implant mucosal margin
- cB-PIMM: vertical distance from the bone crest to the peri-implant mucosal margin
- cB-cBI: vertical distance from the bone crest to the most-coronal bone level on the implant
- aJE-PIMM: vertical length of junctional epithelium plus sulcus depth
- cBI-aJE: vertical length of soft connective tissue compartment

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9, Version 9.3.1 (GraphPad Software, Inc. CA, USA). Means and standard deviations for each histomorphometric parameter were calculated with the animal being the experimental unit for all the comparisons ($n = 6$). Due to the small sample size and the non-parametric distribution of the data, differences between groups were analyzed using the Kruskal-Wallis test followed by Mann-Whitney test with Bonferroni correction. The significance level was set at $p < 0.05$.

Results

The healing was uneventful in all animals without wound dehiscence or other major complications. Out of 18 teeth in the three groups, one tooth was lost in vivo (group CM). Furthermore, all 6 molars, used as internal control teeth, were available for the descriptive analysis.

Teeth

Descriptive histology

CAF + SCTG (Figs. 2a and 3a and b) All teeth had a normal keratinized oral gingival epithelium consisting of 4 strata (Fig. 2a). Owing to its volume, the SCTG appeared to widen the gingiva and to impact the spatial configuration of the vestibulum; i.e., it appeared to lift up the bottom of the vestibulum (Fig. 2a).

In all 6 teeth, the bone crest level was buccally lower than lingually. The junctional epithelium was quite long. Epithelial inclusions in the gingival connective tissue were found in 2 teeth, food impaction at 1 tooth, and multinucleated giant cells around a foreign body material at 1 tooth. The connective tissue graft was clearly discernible. Circularly, the border region did hardly show any signs of graft tissue

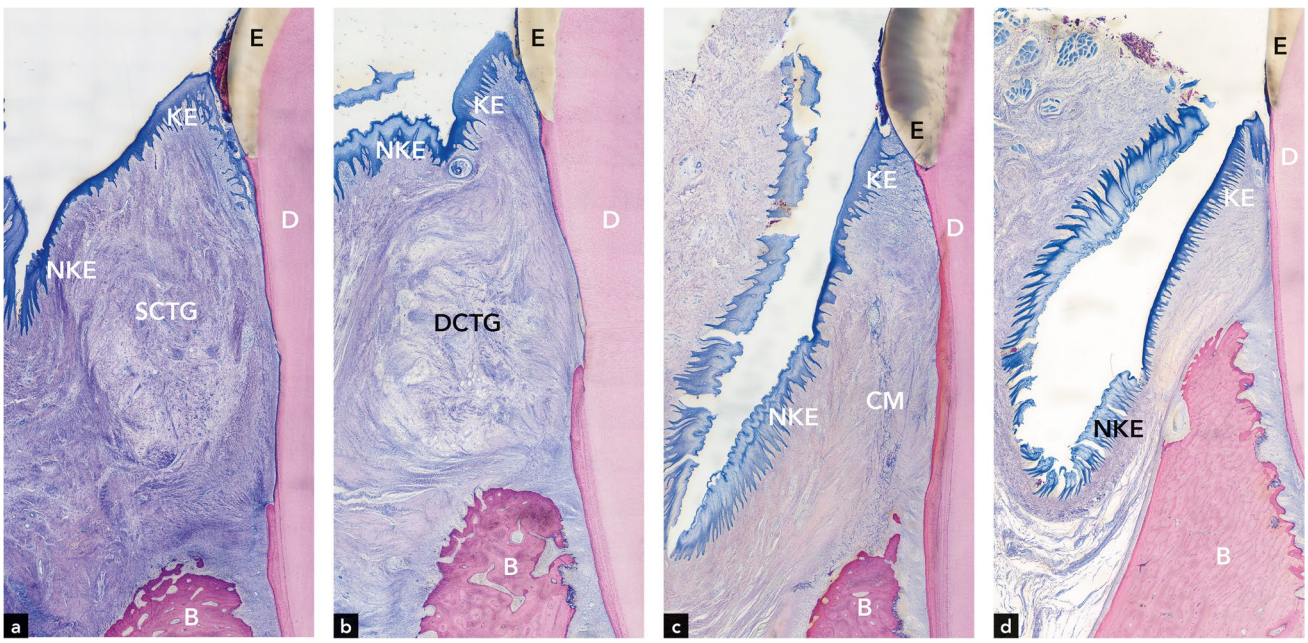


Fig. 2 Representative micrographs illustrating the vestibulum around teeth and the encapsulated configuration of the connective tissue grafts in **a** the SCTG group, **b** the DCTG group, **c** the CM group, and **d** the control group. KE, keratinized epithelium; NKE, non-keratinized epithelium; SCTG, superficial connective tissue graft; DCTG, deep connective tissue graft; CM, collagen matrix; E, enamel; D, dentin; B, bone

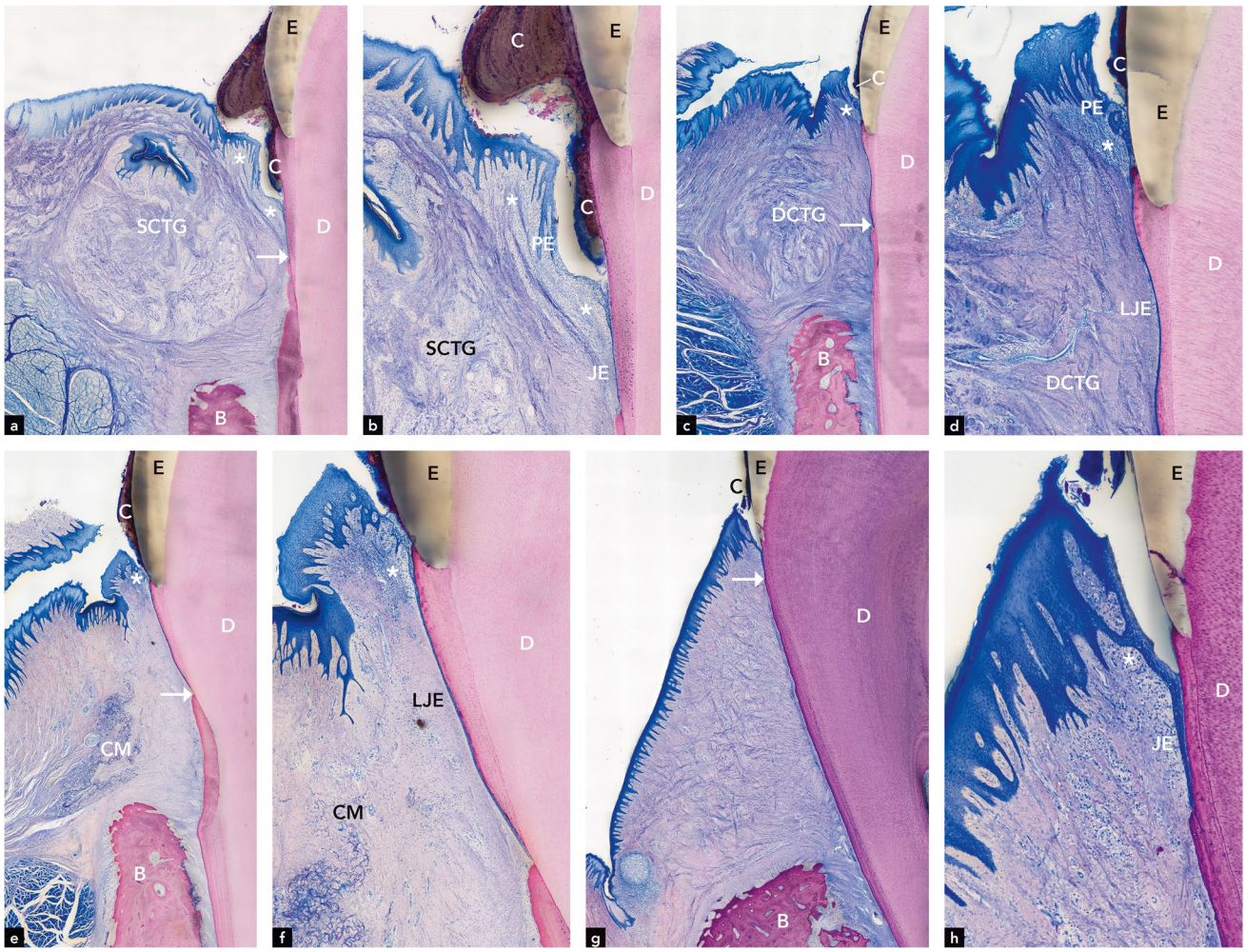


Fig. 3 Representative micrographs illustrating the gingiva at teeth in the SCTG group **a** and **b** (b= higher magnification of **a**) the SCTG group, (**c** and **d** higher magnification of **c**) the DCTG group, (**e** and **f** higher magnification of **e**) the CM group, and (**g** and **h** higher magnification of **g**) the control group. Arrows indicate the apical end of the junctional epithelium. E, enamel; C, calculus; D, dentin; SCTG, superficial connective tissue graft; DCTG, deep connective tissue graft; CM, collagen matrix; B, bone

integration into the surrounding tissue (Fig. 3a and b). Gingival pocket formation with supragingival and subgingival calculus and biofilm was found in 5 out of 6 teeth (Fig. 3b). Peri-pocket inflammation was found in all teeth with gingival pockets.

CAF + DCTG (Figs. 2b and 3c and d) All teeth had a normal, keratinized oral gingival epithelium consisting of 4 strata (Fig. 2b). Owing to its volume, DCTG appeared to impact the spatial configuration of the gingiva and the vestibulum; i.e., it appeared to widen the gingiva and lift up the bottom of the vestibulum (Fig. 2b).

In all 6 teeth, the bone crest level was buccally lower than lingually. The junctional epithelium was either long or very long. Epithelial inclusions in the gingival connective tissue were found in 2 teeth, food impaction in 1 tooth, and multinucleated giant cells around a foreign body material in 1 tooth. The connective tissue graft was clearly distinguishable from the surrounding tissue with hardly any signs of graft tissue integration into the surrounding tissue (Fig. 3c). Gingival pocket formation with subepithelial calculus and biofilm was found in all 6 teeth. Peri-pocket inflammation was also found in all teeth (Fig. 3d).

CAF + CM (Figs. 2c and 3e and f) All teeth had a normal, keratinized oral gingival epithelium consisting of 4 strata (Fig. 2c). The spatial configuration of the keratinized and non-keratinized epithelium and the vestibulum were very similar to the situation around control teeth; i.e., the gingiva was thin and the bottom of the vestibulum was not elevated (Fig. 2c).

In all 5 teeth, the bone crest level was buccally lower than lingually. The junctional epithelium was either long or very long. Epithelial inclusions in the gingival connective tissue

were not found. Food impaction was found in 1 tooth, a mini abscess in 1 tooth, and residual CM was found in the gingival connective tissue of all 5 teeth. The CM was partially integrated into the surrounding tissue and only remnants of the matrix could be detected (Fig. 3e and f). Gingival pocket formation, subepithelial calculus, biofilm, and peri-pocket inflammation were found in all 5 teeth (Fig. 3f).

Control teeth (untreated molars; Figs. 2d and 3g and h)) The oral gingival epithelium consisted of 4 strata and was keratinized (Fig. 2d). Of all groups, the keratinized epithelium of the control teeth demonstrated the most regular configuration of rete pegs (Fig. 2d).

The junctional epithelium was very short and terminated at or slightly apical to the cemento-enamel junction (Fig. 3g). All 6 teeth demonstrated a healthy gingiva with physiologically normal minimal signs of inflammation. Five teeth presented with very small gingival pockets (Fig. 3h), whereas in one tooth, massive calculus and a slightly deeper gingival pocket were found. The distance between the cemento-enamel junction and the bone crest was quite large in this tooth type, but no signs of bone resorption and pathology were observed.

Histomorphometry

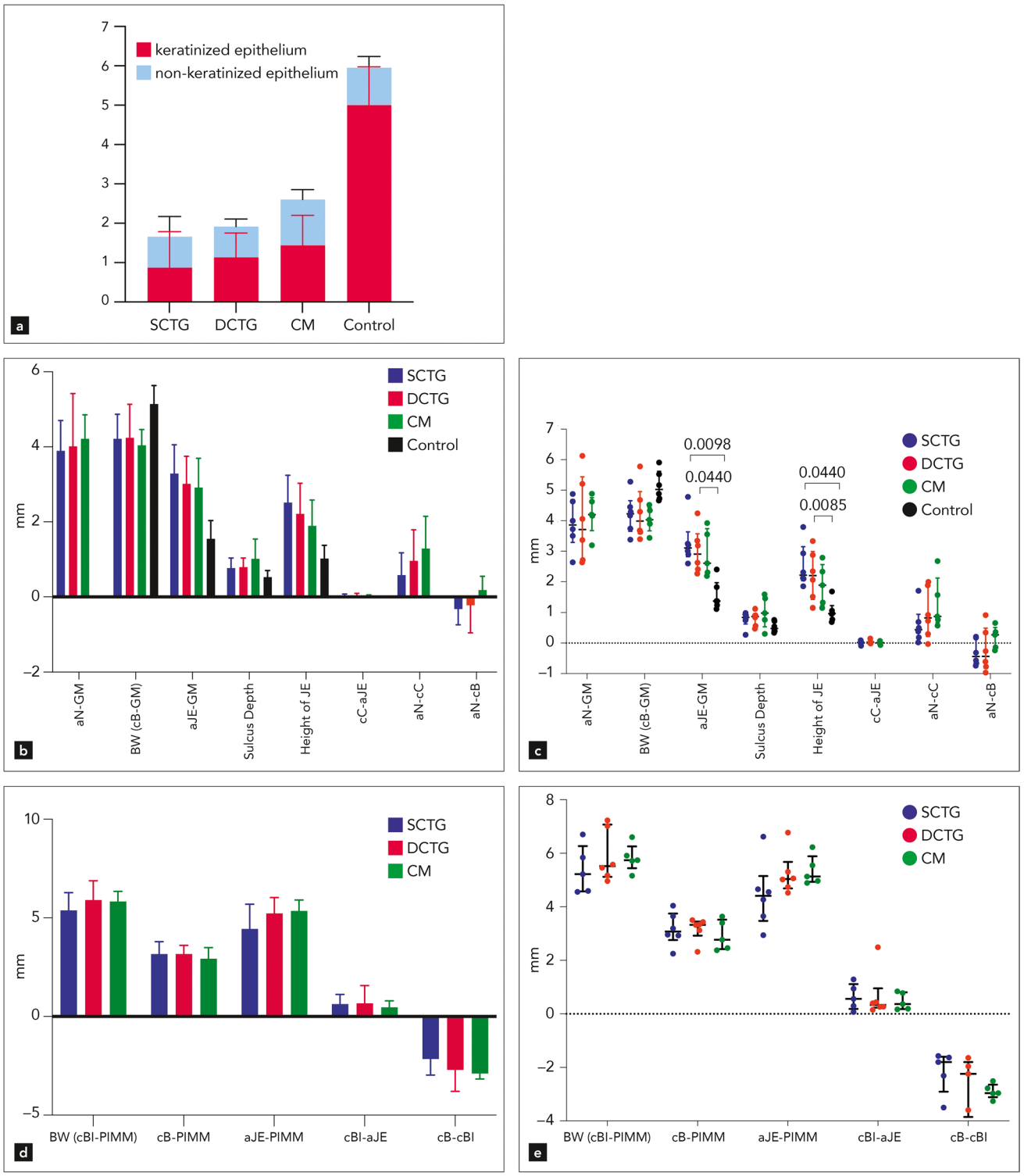
Epithelium

The results of the histomorphometric analysis are presented in Table 1 and Fig. 4a. The length of the keratinized epithelium was smallest in the SCTG group. The ratio keratinized epithelium to non-keratinized epithelium was similar among all experimental groups, i.e., about 50:50, however different in the control teeth where the ratio averaged 80:20 (SCTG: 49.92 ± 23.50% to 50.07 ± 23.05%; DCTG: 56.58 ± 13.60% to 43.41 ± 13.60%; CM: 53.38 ± 9.51% to 46.61 ± 9.51%; control: 83.49 ± 6.27% to 16.50 ± 6.27%). Comparing the 3 experimental groups with each other, no statistically significant difference could be discerned between the groups in terms of keratinized epithelium length for SCTG, DCTG, and CM (0.86 ± 0.92 mm, 1.13 ± 0.62 mm, 1.44 ± 0.76 mm). Compared to the untreated control tooth group, the keratinized epithelium in both CTG groups was statistically significantly shorter ($p = 0.0025$ and $p = 0.0228$). No statistically significant difference, however, did exist between the control tooth group and the CM group ($p = 0.1814$). The length of the non-keratinized epithelium was in all experimental groups and in the control group about the same.

Table 1 Assessed parameters at teeth

	Keratinized epithelium in mm	Non-keratinized epithelium in mm	Whole length of epithelium in mm	aN-GM in mm	BW (cB-GM) in mm	aJE-GM in mm	SB-GM	Height of JE (mm)	cC-aJE in mm	aN-cC in mm	aN - cB (mm)
SCTG	0.8697 ± 0.9203	0.7877 ± 0.5129	1.657 ± 1.115	3.897 ± 0.8092	4.220 ± 0.6507	3.290 ± 0.7675	0.7714 ± 0.2623	2.519 ± 0.7244	0.01742 ± 0.06075	0.5806 ± 0.5988	−0.3165 ± 0.4196
DCTG	1.136 ± 0.6201	0.7827 ± 0.1917	1.918 ± 0.6908	4.019 ± 1.407	4.245 ± 0.8891	3.014 ± 0.7376	0.7998 ± 0.2400	2.214 ± 0.8190	0.03753 ± 0.05828	0.9665 ± 0.8246	−0.2246 ± 0.7243
CM	1.440 ± 0.7649	1.163 ± 0.2503	2.603 ± 0.9012	4.214 ± 0.6438	4.046 ± 0.4188	2.916 ± 0.7778	1.018 ± 0.5233	1.898 ± 0.6849	0.005496 ± 0.04927	1.292 ± 0.8613	0.1827 ± 0.3700
control	5.008 ± 0.9706	0.9441 ± 0.2843	5.952 ± 0.7038		5.146 ± 0.4897	1.553 ± 0.4813	0.5285 ± 0.1778	1.024 ± 0.3509			

SCTG superficial connective tissue graft, *DCTG* deep connective tissue graft, *CM* collagen matrix, *aN* most apical part of the surgically exposed root surface, *GM* gingival margin, *BW* biologic width, *cB* most coronal level of bone, *aJE* most apical extent of the junctional epithelium, *SB* sulcus bottom, *cC* most coronal extent of new cementum



Vertical measurements

The results of the vertical measurements are presented in Table 1 and Fig. 4b and c. The gain in gingival height (aN-GM) was similar for all 3 experimental groups (3.89 ± 0.80 mm for SCTG, 4.01 ± 1.40 mm for DCTG, and 4.21 ± 0.64 mm for CM). The biologic width (BW; cB-GM) was highest at the control teeth (5.14 ± 0.48 mm) where the crestal bone was located far apical to the CEJ. Not statistically significantly, but slightly lower

◂**Fig. 4** Graph representing mean and standard deviation of keratinized and non-keratinized epithelium (**a**) and of the histomorphometrically evaluated parameters around teeth (**b**). In **c**, the bars represent the median and the whiskers the interquartile range. Significance was set at $p < 0.005$. SCTG, superficial connective tissue graft; DCTG, deep connective tissue graft; CM, collagen matrix; BW(cB-GM), biologic width; aJE-GM, apical extent of the junctional epithelium – gingival margin; JE, junctional epithelium; cC-aJE; most coronal extent of new cementum — apical extent of the junctional epithelium; aN-cC, apical extent of the surgically exposed root surface — most coronal extent of new cementum; aN-cB, apical extent of the surgically exposed root surface — most coronal level of bone (bone crest). Graph illustrating the histomorphometrically evaluated parameters around implants (**d**) with means and standard deviations. In **e**, the bars represent the median and the whiskers the interquartile range. Significance was set at $p < 0.005$. SCTG, superficial connective tissue graft; DCTG, deep connective tissue graft; CM, collagen matrix; BW(cBI-PIMM), biologic width; cB-PIMM, bone crest — peri-implant mucosal margin; apical extent of the junctional epithelium — gingival margin; JE, junctional epithelium; cC-aJE; most coronal extent of new cementum — apical extent of the junctional epithelium; aN-cC, apical extent of the notch — most coronal extent of new cementum; aN-cB, apical extent of the notch — bone crest

BW values were measured for the experimental groups (4.22 ± 0.65 mm for SCTG, 4.24 ± 0.88 mm for DCTG, 4.04 ± 0.41 mm for CM). The biologic width comprised the epithelial attachment (the junctional epithelium JE plus sulcus depth), the connective tissue adhesion (cC-aJE), the gain of new cementum (aN-cC), and the distance aN-cB. Of all assessed parameters, only two (aJE-GM and the height of JE) reached a statistically significant difference between the control and the experimental groups. However, no statistically significant difference was observed between the experimental groups for any of the assessed parameters. The distance aJE-GM was smallest in the control group, while all experimental groups had a rather long JE including the sulcus depth (SCTG: 3.29 ± 0.76 mm, DCTG: 3.01 ± 0.73 mm, CM: 2.91 ± 0.77 mm, control: 1.55 ± 0.48 mm) reaching statistical significance only for the difference between control and each of the CTG groups ($p = 0.009$ and $p = 0.044$). The same was true for the height of the JE. The sulcus depth was smallest in the control group, while the other groups all showed a much greater sulcus depth associated with slight inflammation and pocket formation. The connective tissue adhesion (cC-aJE) was extremely small in all test groups, indicating that new cementum and the apical end of the JE were either confluent or in close proximity to each other. The mean vertical gain of new cementum (aN-cC) was highest in the CM group, followed by the SCTG and the DCTG groups. Of note, the distance between the apical end of the notch (i.e., former level of the gingival margin) to the bone crest reached a positive value in the CM group, whereas this distance was negative in the SCTG and DCTG groups. This implies that vertical bone growth was clearly greater in the CM group compared to the two CTG groups.

Implants

Descriptive histology

CAF + SCTG (Fig. 5a, b, c, d) The epithelium of the peri-implant mucosa facing the graft resembled a keratinized epithelium (Fig. 5a and b). There was a layer of soft connective tissue between the epithelium and the SCTG.

All 6 implants were non-submerged and demonstrated saucer-shaped bone defects both buccally and lingually (Fig. 5c). In 1 implant, advanced bone loss had occurred. Around another implant, dentin and cementum remnants were found. Small pocket formation, calculus, biofilm, and mild inflammation were observed in 4 implants (Fig. 5d). The vertical distance between the peri-implant mucosal margin and the most coronal level of the bone was conspicuously long. The junctional epithelium was long or very long, and its apical termination was always below the bone crest (Fig. 5c). The SCTG was found around all implants. It was big, round-shaped, and its localization in relation to the keratinized epithelium varied between implants (Fig. 5a).

CAF + DCTG (Fig. 5e, f, g, h) The epithelium of the peri-implant mucosa facing the graft resembled a keratinized epithelium (Fig. 5e and f). All 6 implants were non-submerged and demonstrated saucer-shaped bone defects both buccally and lingually (Fig. 5g). In 1 implant, advanced bone loss had occurred. Small pocket formation, calculus, biofilm, and mild inflammation were observed in 4 implants (Fig. 5h). The vertical distance between the peri-implant mucosal margin and the most coronal level of the bone was conspicuously long, and the junctional epithelium was very long and its apical termination always below the bone crest (Fig. 5g). The DCTG was found around all implants, was big, round-shaped, and its localization in relation to the keratinized epithelium varied between implants (Fig. 5e). A layer of soft connective tissue was interposed between the epithelium and the DCTG.

CAF + CM (Fig. 5i, j, k, l) The epithelium of the peri-implant mucosa facing the coronally located CM resembled a keratinized epithelium (Fig. 5i and j). One implant was lost in situ. Out of 5 implants, 2 implants were submerged, whereas 3 implants were non-submerged. Around all implants, saucer-shaped bone defects were observed both buccally and lingually (Fig. 5k). One implant showed a very small pocket formation. All other implants had no pocket formation (Fig. 5l). Healthy peri-implant soft tissue conditions with minimal (physiologically normal) inflammation were observed around all implants. Around all implants, the most coronal level of bone in contact with the implant (cBI) was located very apically (Fig. 5k). Likewise, the vertical

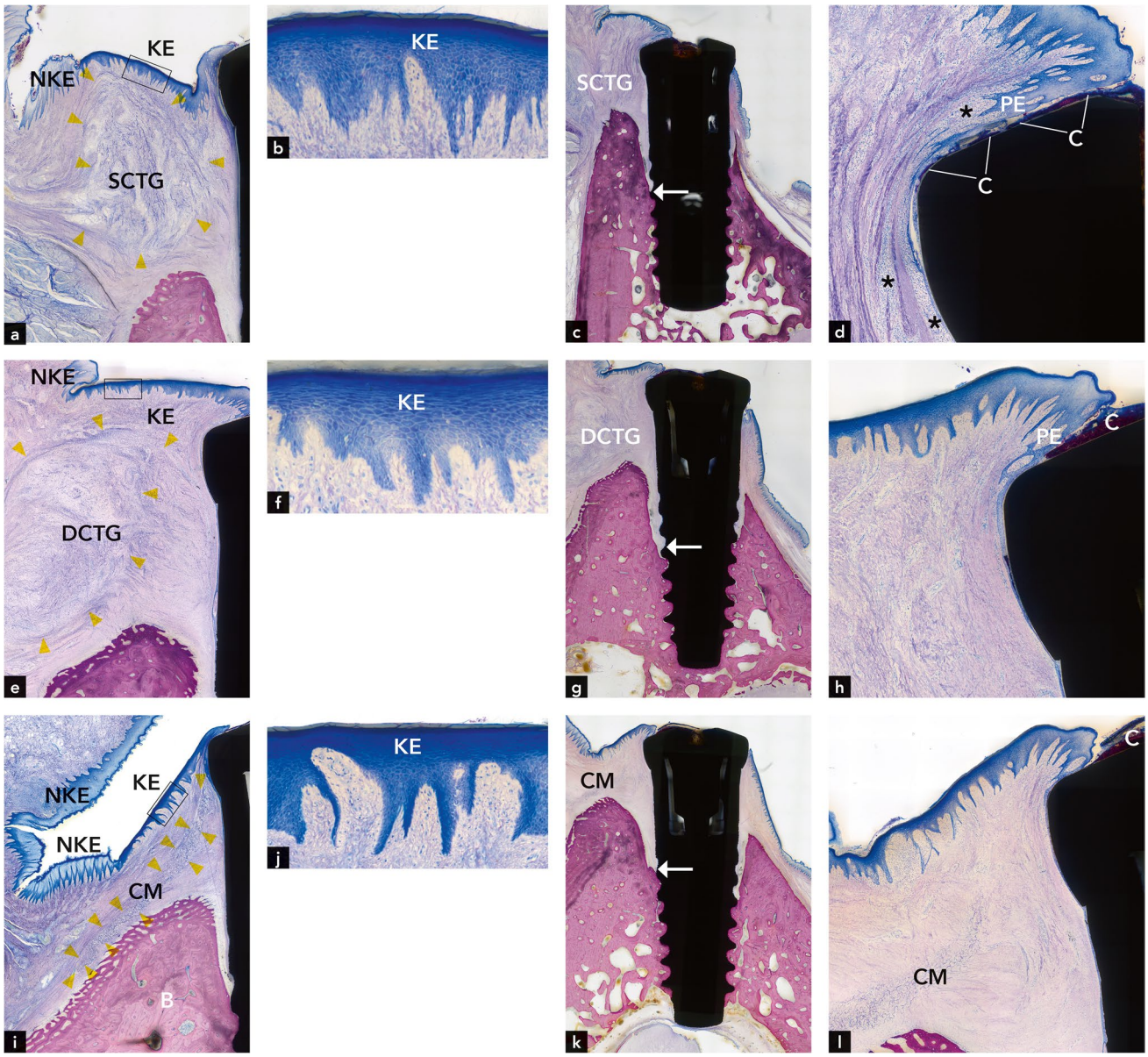


Fig. 5 Representative micrographs illustrating the grafting area at the 3 experimental groups at implants (**a**, **e**, **i**). **b** shows the marked region in **a**, **f** in **e**, and **j** in **i** of the keratinized epithelium in higher magnification. Representative micrographs illustrating the 3 experimental groups at implants in overview (**c**, **g**, **k**) and in higher magnification of the peri-implant mucosal margin (**d**, **h**, **l**). SCTG, superficial connective tissue graft; DCTG, deep connective tissue graft; CM, collagen matrix; KE, keratinized epithelium; PE, pocket epithelium; C, calculus

distance between the bone crest and the most coronal bone in contact with the implant was conspicuously long. The junctional epithelium was very long and its apical termination always below the bone crest (Fig. 5k). Residual CM was present in the soft connective tissue around all implants (Fig. 5i). It was thin and elongated and its localization in relation to the keratinized epithelium varied between implants. There was mostly a thick layer of connective tissue between the epithelium and the CM.

Although all implants were surrounded by a collar of keratinized mucosa (Fig. 5a, e, i), its length could not be determined histomorphometrically, since not all implants showed transmucosal healing and most implant healing caps were partially overgrown by peri-implant mucosa.

Histomorphometry

The histomorphometric data of the implants are shown in Table 2 and Fig. 4d and e. For none of the parameters, a statistically significant difference among the groups was achieved. The biologic width (cBI-PIMM) comprised of cBI-aJE and aJE-PIMM was very similar in all three groups. Likewise, no significant differences were seen for the distance between the bone crest and the peri-implant mucosal margin (cB-PIMM). The distance between the apical end of the junctional epithelium and the peri-implant mucosa (aJE-PIMM), which corresponds to the height of the junctional epithelium plus the sulcus depth, varied from 4.44 ± 1.24 mm to 5.35 ± 0.55 mm and was considerably longer around implants than around corresponding teeth. The distance between bone on the implant and the apical end of the junctional epithelium (cBI-aJE), corresponding to the connective tissue adhesion on the implant, was short in all 3 groups. The height of the saucer-shaped bone deficiency (cB-cBI) was greatest in the.

CM group followed by DCTG and SCTG, albeit without statistical significance.

Discussion

This animal study investigated the healing characteristics around teeth and implants after recession coverage using either a superficial or deep connective tissue graft from the palate or a collagen matrix. We applied descriptive histological and histomorphometrical analyses to evaluate whether differences among the groups exist regarding the healing pattern, epithelial keratinization, and dimensions of soft and hard tissues around teeth and implants.

In terms of keratinization, all groups demonstrated the formation of keratinized epithelium around both teeth and implants. In teeth, the 3 experimental groups obtained similar lengths of the keratinized epithelium, albeit significantly shorter compared to the group with the control teeth. The length of the non-keratinized epithelium was similar for the control and experimental groups. These results imply that the difference of the keratinized epithelium between control and experimental teeth might be strongly influenced by the recession defect that was surgically created. The length of the keratinized tissue around implants could not be determined due to the fact that not all implants demonstrated complete transmucosal healing and thus not equal healing conditions.

Also, in the minipig model, other studies evaluated the amount of keratinized tissue in response to treatment of gingival recession defects. CAF alone yielded about 1 mm greater width of keratinized tissue compared to CAF + CM [15]. The amount of keratinized tissue averaged 2.66 ± 0.42 mm before CAF + CTG and 3.83 ± 0.47 mm 12 weeks afterwards [14]. In our study, the keratinized epithelium at the experimental teeth measured 0.86 ± 0.92 mm (SCTG), 1.13 ± 0.62 mm (DCTG), and 1.44 ± 0.76 mm (CM). This might be partly due to differences in the histometric evaluation and to the fact that no baseline measurements (i.e., before CAF preparation) of the keratinized epithelium were taken; instead, the values after 8 weeks were compared with a control tooth. Furthermore, in the present study, only mandibular teeth and sites for implant installation were used, whereas the other studies used both maxillary and mandibular sites [14, 15].

The observation that CAF + CTG and CAF + CM resulted in an equivalent amount of keratinized tissue gain is in agreement with clinical studies [19, 20] where keratinized tissue gain averaged 1.26 mm for CAF + CTG and 1.34 mm for CAF + CM [12, 21].

The present study has failed to show that superficial and deep connective tissues display different inherent characteristics to induce keratinization at the recipient site as was suggested by Ouhayoun et al. (1988). However, when interpreting the here presented results, it must be kept in mind that the connective tissue grafts were covered with a rather thick layer of flap which might have hindered the direct influence of cells within the grafts onto the epithelium. Indeed, the results of Ouhayoun et al. (1988) showed that deep connective tissue grafts had not the same ability to induce keratinization as connective tissue grafts that were harvested closer to the epithelium [4]. A recent review with meta-analysis corroborated the superior outcome of superficial grafts, reporting a mean recession coverage of 89.3% for deeper

Table 2 Assessed parameters at implants

	BW(cBI-PIMM) in mm	cB-PIMM in mm	aJE-PIMM In mm	cBI-aJE in mm	cB-CBI mean ± SD
SCTG	5.38 ± 0.90	3.17 ± 0.62	4.44 ± 1.24	0.63 ± 0.48	− 2.16 ± 0.80
DCTG	5.90 ± 0.97	3.17 ± 0.43	5.23 ± 0.79	0.66 ± 0.89	− 2.68 ± 1.04
CM	5.82 ± 0.51	2.93 ± 0.56	5.35 ± 0.55	0.47 ± 0.32	− 2.89 ± 0.27

SCTG superficial connective tissue graft, *DCTG* deep connective tissue graft, *CM* collagen matrix;, *BW* biologic width, *cBI* most coronal level of bone in contact with the implant, *PIMM* peri-implant mucosal margin, *cB* the most coronal level of the bone (crest), *aJE* most apical part of the junctional epithelium

connective tissue grafts and 94.0% for de-epithelialized superficial connective tissue grafts (Travelli et al., 2019). In terms of keratinized tissue gain and recession reduction, better results were found in favor of the superficial graft [22].

Whether inflammatory processes may affect tissue keratinization is still a matter of discussion. Chronic or acute inflammation, experimentally induced in animals, was not able to convert tissue keratinization [23, 24]. On the other hand, a reduction of gingival inflammation allowed sulcular keratinization to occur [25]. In the present study, pocket formation with subgingival calculus formation and inflammatory processes were observed at nearly all (experimental) teeth and around the implants receiving a CTG. In contrast, the implants that received a CM showed no pocket formation and healthy peri-implant soft tissue conditions with minimal (physiologically normal) inflammation. Nevertheless, no difference was observed among inflamed and non-inflamed conditions in terms of epithelial keratinization. One possible explanation for the difference in pocket formation between the CM and the CTG groups at the implants is that the rather voluminous, spherical CTGs substantially lifted the bottom of the vestibulum and may have hampered tight sealing between flap and teeth/implants thus favoring plaque-induced inflammation. Conversely, the less voluminous and rather flat CMs did not result in an elevation of the bottom of the vestibulum and around implants allowed for a undisturbed healing.

One interesting finding was that after 8 weeks of healing, both superficial and deep connective tissue grafts hardly showed signs of degeneration or integration into the surrounding tissues. This observation was made for both teeth and implants. So far, little is known about the temporal sequence of tissue degradation/integration of transplanted connective tissue grafts from the palate. The seminal studies of Karring et al. (1971) in monkeys not only first addressed the question of the specificity of the epithelium but also described healing from a few days up to 12 months [26]. After 3 months of healing, the transposed tissues had partly degenerated [27, 28]. But here it has to be kept in mind that the surgical techniques and species differed in the latter and the present study.

In the present study, all experimental groups yielded similar results in terms of biologic width. Of note, at control teeth the BW averaged 5.1 mm which is considerably higher than in other species or in humans [29]. In the SCTG and DCTG groups around teeth, the JE measured 2.51 ± 0.72 mm and 2.21 ± 0.81 mm, what is significantly longer than at control teeth. These results strongly suggest that the surgical manipulation of the soft tissue resulted in a repair process with an apical migration of the JE. Nevertheless, these results are comparable with previous findings in dogs [30] and minipigs, where treatment with CAF alone resulted in 2.79 ± 0.77 mm and CAF + CM in 2.26 ± 0.23 mm of JE [15]. At the implants, the JE was even longer. Also, at the implants, the distance cB-PIMM averaged 3.17 ± 0.62 mm and 3.17 ± 0.43 mm for SCTG and DCTG, while a bit less for CM. Here, it might be possible that the connective tissue grafts may induce some kind of bone resorption likewise to root resorptions that have rarely been described [31–33].

Much can be discussed about the limitations of this model. The miniature pig model might not be perfectly suitable for this research question considering that it displays a different and for this type of surgical procedure more challenging anatomy of the vestibulum compared to humans. Other researchers have performed coronally advanced flap surgeries after connective tissue or biomaterial transplantations in the minipig in both the mandible and maxilla [14, 15] or in a more anterior position [15]. Consequently, the 3 experimental groups resulted in a deep (CM group), very shallow, missing, or directly rising vestibulum (CTG groups). The thickness of the transplanted materials together with the anatomy at these sites may account for the differences between CM and the two CTG groups. Furthermore, harvesting superficial and deep connective tissue from the palate is difficult to standardize. Implant placement and positioning in relation to hard and soft tissues had to be adapted to the anatomical situation and do not fully correspond to the situation in humans which might have been one reason for the saucer-shaped defects to occur. Furthermore, some of the implants resulted in a submerged or semi-submerged healing, while few healed fully transmucosally. During healing, adequate measures of plaque control and postoperative care were not feasible in this animal model. Consequently, tissues around all the teeth and most of the implants showed signs of inflammation and calculus formation on teeth and implants. Horizontal measurements along any level for both teeth and implants were not doable for all samples. The control teeth were not planned but then included in order to have a comparison with normal histomorphometric parameters around teeth (i.e., JE, soft connective tissue height, bone level). However, while the control teeth were molars, all experimental teeth were premolars and thus not fully comparable. Finally, a rather small number of teeth and implants were treated by two surgeons. This might have caused some inter-operator variation.

To better understand the characteristics and effects of superficial and deep connective tissue grafts, further studies and more suitable models are warranted.

Conclusion

Around both teeth and implants, CAF + SCTG/DCTG/CM resulted in the formation of keratinized epithelium with no differences between SCTG and DCTG. The length of the keratinized epithelium was conspicuously shorter at the

experimental teeth compared to the control teeth. All experimental teeth and implants receiving SCTG or DCTG showed pocket formation with subgingival calculus and inflammation, whereas implants receiving CM displayed healthy periimplant soft tissue conditions what implies that CAF + CM was superior to CAF + SCTG/DCTG regarding this aspect. All procedures (CAF + SCTG/DCTG/CM) resulted in a long JE that was even longer at the implants. After 8 weeks of healing, both SCTG and DCTG hardly showed any signs of degeneration or integration into the surrounding tissues.

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Data Availability Data are available on request.

Declarations

Competing interests The authors declare no competing interests.

Ethics approval All procedures performed in this study involving animals were conducted in accordance with the ARRIVE guidelines for animal pre-clinical studies. The study was approved by the local Committee for Animal Research, University of Szeged, Hungary No 1-74-2/2015 MAB.

Conflict of interest Sofia Aroca, Anton Sculean, and Frank Schwarz received a grant from the Osteology Foundation (13-101). The other authors declare that they have no conflict of interest.

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SZTE, Fogorvostudományi Kar, Parodontológiai Tanszék*
SZTE Fogorvostudományi Kar, Konzerváló és Esztétikai Fogászati Tanszék**

Feszes íny szélesítés implantátumok körül – „H” technika esetismertetés

DR. PÁRKÁNYI LÁSZLÓ*, DR. FRÁTER MÁRK**

Míg a természetes fogak körül a gingiva vastagságának és a feszes íny jelenlétének inkább csak esztétikai jelentősége van, addig a koronai restaurátummal ellátott implantátumok körül a lágyszöveti viszonyok nem csak az implantációs pótlások esztétikai megjelenését, hanem akár az implantátumok hosszú távú sikerességét is befolyásolhatják. Implantátumok mellett hagyományosan a biotípus-módosítás, illetve keratinizált szövetszélesítés külön sebészi beavatkozás eredménye. Ez megtörténhet az implantátum felszabadítása előtt vagy azt követően. A beavatkozáshoz alkalmazott graft rendszerint szabad íny graft (Free Gingival Graft, FGG), mely jelentős donor morbiditást jelenthet, valamint az esztétikájában, megjelenésében eltér a környező lágyszövetektől. Jelen technika célja a biotípus módosítása és keratinizált szövet szélesítésének elvégzése egyazon lépésben az implantációval, kötőszövet-helyettesítő kollagén mátrix (Acellular dermal matrix, ADM) alkalmazásával, nyitott gyógyulás során. Ezzel egyszerre csökkenthető a donor morbiditás, a sebészi beavatkozások száma (ezáltal a gyógyulási idő), valamint javítható az esztétikai végeredmény.

Kulcsszó: feszes íny szélesítés, ADM, kollagén mátrix, tunnel-technika

Bevezetés

Napjainkban bizonyított tény, hogy implantációs helyreállítások esetében kiemelt jelentőséggel bírnak az implantátumok körüli lágyszövetek mind mennyiségi, mind minőségi szempontból [15]. Bár a megfelelő esztétikához is nagymértékben hozzájárul az ideális lágyszövet környezet [4, 9], az implantátum sikeressége szempontjából ennél jóval lényegesebb befolyásuk is van, ezáltal az esztétikai zónán kívül is nagy fontossággal bírnak. A periimplantáris mucosa vastagságának közvetlen hatása van a marginális lágyszövet stabilitására, valamint az alatta levő csont megtartásában is [6, 8]. A keratinizált, és ezen belül is legfőképpen a feszes mucosa ugyancsak nagyban hozzájárul az egészséges periimplantáris szövetek fenntartásához, többek között a marginális csontszint stabilizálásával [2, 11]. Bár a keratinizált mucosa hiánya nincsen egyértelműen bizonyított összefüggésben a periimplantáris gyulladásos kórképek kialakulásával, az individuális szájhigiéne befolyásolásán keresztül azonban mindenképpen összefügg ezen folyamatokkal is [17, 3, 14].

Háttér

Fogak elvesztését követően a gyógyulás során az állcsontgerinc változó mértékű resorptión megy keresztül, mely főleg a horizontális dimenziót érinti [13]. Az állcsont involutio sohasem korlátozódik a keménységövetekre, minden esetben a lágyszövetekben is volumen-, illetve keratinizáció-csökkenés történik [1]. A fogvesztések (akár kariológiai, akár parodontális okokból) legtöbb esetben elsőként a moláris régióban következnek be [12]. A keratinizált szövet csökkenése különösen kifejezett a moláris régióban, így moláris implantátumon rögzülő restaurátumok körül többször figyelhető meg annak jelentős hiánya [5]. A moláris régió a páciens számára nehezebbem hozzáférhető, így eleve a legnehezebben tisztítható terület. Implantátumok esetében adott mennyiségű plakk hatására könnyebben indul el gyulladásos folyamat a periimplantáris mucosa mentén, mint megtartott fogazat mellett a gingiva területén [17, 16].

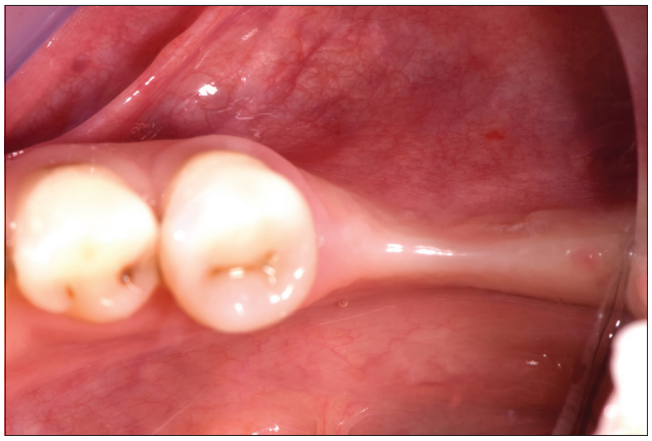
Kezelési koncepció

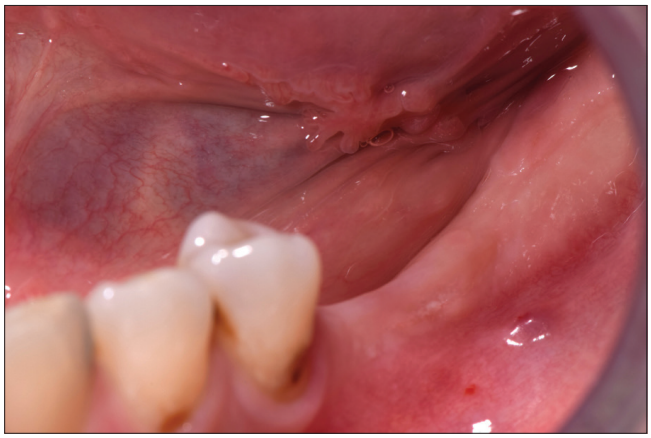
Az alább ismertetett kezelés lényege a lágyszövetek vastagságának és keratinizációjának helyreállítása implantátumok körül, az implantációval egyazon sebészi lépésben [7]. Az invazivitást csökkentve, nyitott sebgyógyulás mellett, a gyógyulási felépítmények aktív szerepet játszanak a sebészi technikában. Továbbá, a műtét morbiditását csökkentve, saját kötőszövet nem kerül felhasználásra, a mucosa vastagítása és a keratinizáció növelése térhálós kollagén mátrixszal történik [10].

Esetismertetés

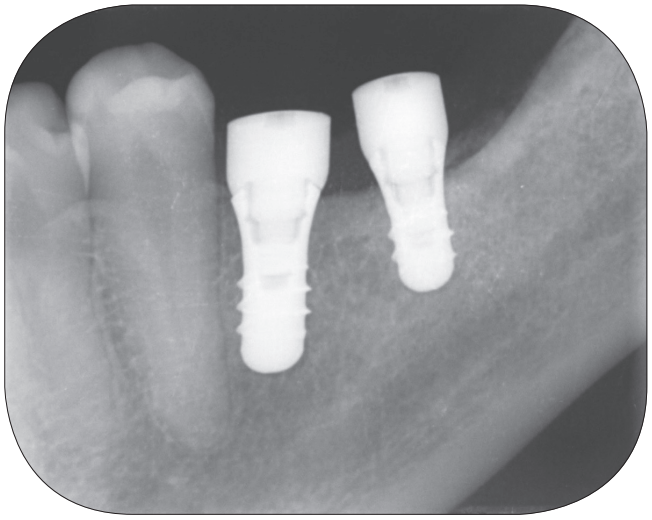
A középkorú hölgy páciensünk azért érkezett rendelőnkbe, hogy bal alsó laterális foghiányát pótoltassa. A páci-

Érkezett: 2017. december 10.
Elfogadva: 2018. január 11.

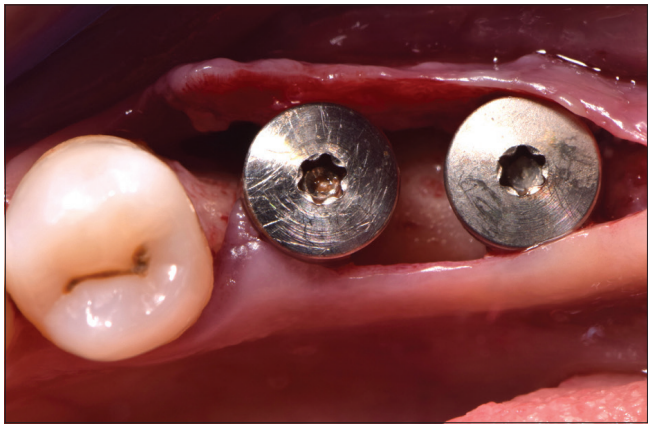




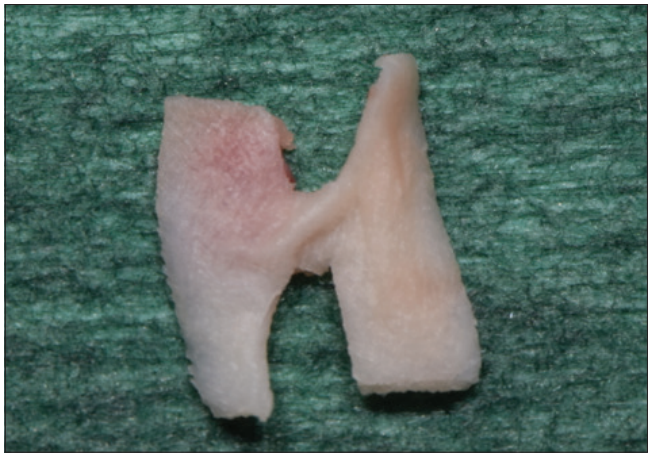
1. A és B kép: Kiindulási klinikai szituáció



2. kép: Postoperativ periapicalis rtg.



3. kép: Gerincéli metszés
és gyógyulási csavarral ellátott implantátumok,
behelyezést követően



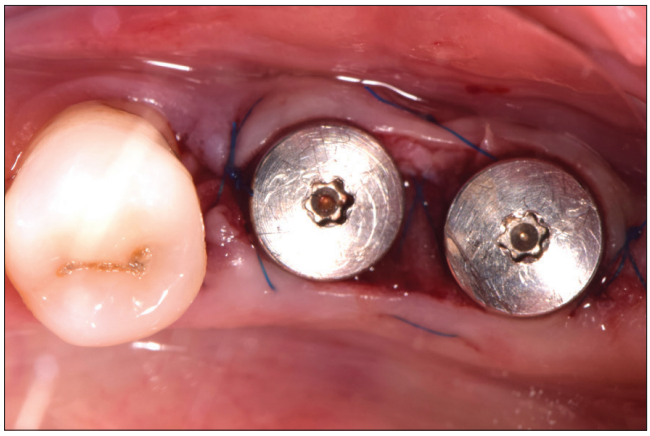
4. kép: „H” alakban megformázott kollagén mátrix

ens nem dohányzó, szisztémásan egészséges, kimondottan jó szájhigiénével rendelkezik, fogágybetegségtől mentes. A CT-vizsgálat eredménye alapján a bal alsó hiányzó molárisok (36, 37) területére standard átmérőjű (d: 4,1 mm) rövid (36: 8 mm; 37: 6 mm) (Straumann Roxolid) implantátumokat terveztünk behelyezni csavarozott korona felépítménnyel.

Ezzel a megoldással a nehezen kivitelezhető, hosszú gyógyulási időt igénylő csontpótlás elkerülhetővé vált. A kiindulási szituációban jól látszik a kimondottan vékony mucosa a fogatlan állcsontgerincen, valamint a vékony, nagyjából 2 mm széles keratinizált mucosa. *(1. kép)*

A gerincéli metszés a vékony keratinizált sáv közepén történt, hogy mindkét lebenyszél tartalmazza – ha kis mennyiségben is – a keratinizációhoz szükséges sejteket. Az implantátumok behelyezését követően a nagy primer stabilitásnak (35 Ncm) köszönhetően az implantátumokat gyógyulási felépítményekkel láthattuk el a műtét során. *(2., 3. kép)*

A buccalis, illetve lingualis lebeny nyálkahártyájának megvastagítására, továbbá a lebenyszélek közti keratinizáció elősegítésére kollagén mátrixot (Mucograft®, Geistlich Pharma AG, Wolhusen, Switzerland) alkalmaztunk, melyet „H” alakban formáztunk meg. *(4. kép)*



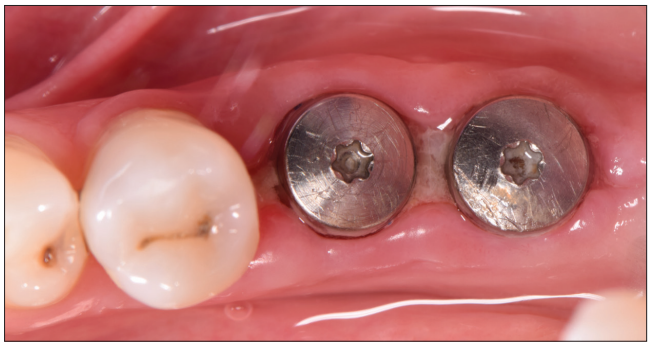
5. kép: Kollagén mátrix, gyógyulási csavarok és matracöltés által immobilizálva

A H alakú kollagén összekötő része fedte a két implantátum között szabadon maradt denudált gerincfelszínt, míg a „H" forma szélső részeit a lebenyszélek alá helyeztük be (tunnel-technikával).

Ily módon a kollagén mátrix egyszerre biztosította a mucosa vastagítását (mind buccalisan, mind lingualisan), valamint a keratinizált szövet szélesítését. A kollagén immobilitását a formája által önmagában nagyrészt a gyógyulási csavarok adták, valamint egy keresztezett horizontális matracöltés az implantátumok között. *(5. kép)*

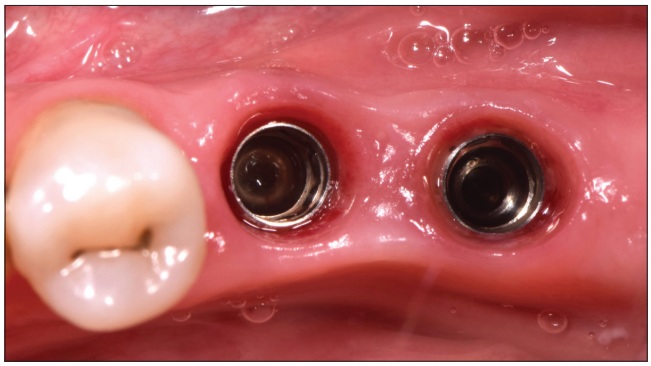
Eredmény

A rövid távú gyógyulás során (varratszedés 2 hét után) már látható a megvastagított biotípus, valamint a kezdődő keratinizáció a szabadon maradt kollagén mátrix felszíneken. *(6. kép)*



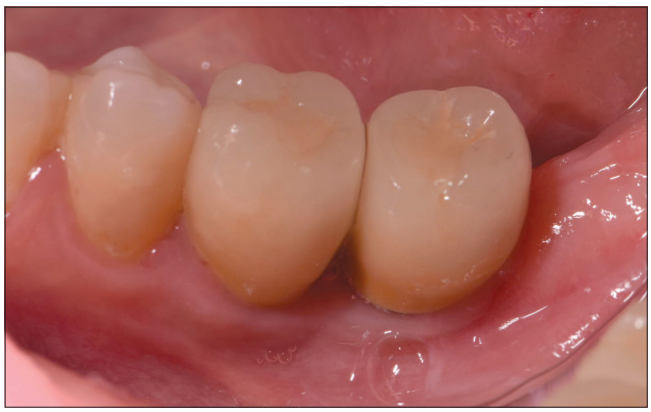
6. kép: Korai gyógyulás – 2 hét

A gyógyulás teljes befejeztével (2 hónap) a lágyszövet vastagodása és a keratinizáció kiszélesedése a területen egyértelműen látható, színben, textúrában eltérés a környező szövetektől nincsen, ellentétben a hagyományos szabad íny graft technikánál tapasztalható esetekkel. *(7. kép)*



7. kép: Teljes gyógyulás – 2 hónap

Az implantátumokra csavarral rögzített fix fogpótlás került,és 1 éves utánkövetés után is egészséges, stabil és esztétikus lágyszövet profilt tapasztaltunk, az egyéni szájhigiéne a nehezen elérhető terület ellenére is optimális volt. *(8. kép)*



8. kép: Utánkövetés – 1 év

Konklúzió

Bár ezen technika a bemutatott eseten keresztül ígéretesnek tűnik, létjogosultságának igazolásához hosszú távú utánkövetés, nagy esetszám és kontrollcsoporttal való összehasonlítás (ahol nem történt módosítás a periimplantáris lágyszöveteken) szükséges a jövőben.

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Párkányi L, Fráter M

Thickening the attached gingiva around implants – the "H-technique" – case presentation

The soft tissue environment around implants and around the related indirect restoration significantly influences not only the esthetic outcome of the implant-prosthetic solution, but may also influence long term stability of the dental implants. Traditionally biotype modification and thickening or widening the keratinized tissues around implants is carried out during a separate surgical procedure. This can be done either on the appointment of uncovering the implant and placing the healing abutment or later around the already placed healing abutments. The graft used for this specific procedure is usually a free gingival graft (FGG), which can cause significant morbidity to the donor site and also will differ in esthetic appearance from the surrounding soft tissues. The aim of the proposed technique is the simultaneous biotype modification and keratinized tissues augmentation with the aid of acellular dermal matrix (ADM) at the time of implant placement during an open healing. With this technique one should be able to decrease the morbidity of the donor site, reduce the number of surgical interventions and separate surgical steps (leading to reduced healing time) and also improve the esthetic outcome.

Keywords: thickening attached gingiva, ADM, collagen matrix, tunnel technique