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Spontaneous regeneration of keratinized tissue at implants and teeth

Jean-Claude Imber[1] | Andrea Roccuzzo[1] | Alexandra Stähli[1] | Dieter D. Bosshardt[1] | Fernando Muñoz[2] | Christoph A. Ramseier[1] | Niklaus P. Lang[1] | Anton Sculean[1]

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

[2]Department of Veterinary Clinical Sciences, University of Santiago de Compostela, iBonelab SL, Lugo, Spain

Correspondence
Jean-Claude Imber, Department of Periodontology, School of Dental Medicine, University of Bern, Freiburgstrasse 7, CH-3010 Bern, Switzerland.
Email: jean-claude.imber@unibe.ch

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Abstract

Aim: To investigate the spontaneous regeneration of the implanto-mucosal and dento-gingival unit after complete removal of keratinized tissue (KT).

Materials and Methods: One hemi-mandible per dog ($n = 4$) was allocated to receive three dental implants (test sites, premolar region), whereas three premolars on the contralateral side were controls. After osseointegration, the entire KT (buccal + lingual) was surgically excised on all test and control sites, leaving the bone exposed. Clinical measurements were performed before excision (T_0) and after 12 weeks (T_1). Following healing, the animals were euthanized, and the specimens were histologically processed. Descriptive statistical analyses were performed.

Results: Clinical measurements revealed that at T_1, on all teeth, a band of KT was spontaneously regenerated (mean width: 2.60 ± 0.66 mm), whereas on implants, KT was detected only occasionally at mesial or distal but not at buccal sites (mean total: 0.35 ± 0.53 mm; $p < .0001$). Histologically, spontaneous regeneration of the dento-gingival unit was evident, displaying masticatory mucosa. At the implant sites, on the other hand, the implanto-mucosal unit was characterized by a non-keratinized epithelium and elastic fibres, indicating the characteristics encountered in alveolar mucosa.

Conclusion: After excision of KT at implant sites, the spontaneous regeneration of the soft tissue is characterized by a non-keratinized epithelium typical for alveolar mucosa, while at tooth sites the spontaneous regeneration was characterized by soft tissue resembling gingiva.

KEYWORDS
gingiva, gingivectomy, keratinized mucosa, soft-tissue excision, soft-tissue regeneration

Jean-Claude Imber and Andrea Roccuzzo contributed equally to this study and share first authorship.

Clinical Relevance

Scientific rationale for study: The spontaneous regeneration of excised masticatory mucosa around teeth is dependent on the presence of gingival connective tissue or periodontal ligament cells. For implant sites, this process has not yet been studied in detail.

Principal findings: Following complete removal of keratinized mucosa around implants, the newly formed soft-tissue barrier was characterized by a non-keratinized epithelium and an underlying connective tissue with elastic fibres.

Practical implications: To maintain the integrity of the implanto-mucosal seal, efforts should be made to ensure the presence of keratinized mucosa when implants are placed.

1 | INTRODUCTION

Dental implant has become a frequent and predictable treatment for the replacement of missing teeth to improve the patient's masticatory function and the oral-health-related quality of life (Buser et al., 2017; Duong et al., 2022; Roccuzzo et al., 2022). Besides the functional aspects of implants supporting a prosthetic appliance, aesthetic aspects of the soft-tissue seal around implants have gained increasing attention in recent years (Schwarz & Ramanauskaite, 2022; Sculean et al., 2014). Moreover, as the mucosal seal around implants or the gingival seal around teeth provides predominantly the defence mechanisms against bacterial challenges, the maintenance of this biological seal has been shown to be essential for long-term peri-implant health (Lin et al., 2013; Ramanauskaite et al., 2022). As opposed to the peri-implant mucosal unit, the dento-gingival unit is not as susceptible to the same bacterial challenge (Wennström, 1983; Wennström & Lindhe, 1983a, 1983b). In this respect, it has been demonstrated that the complete surgical removal of the gingiva will result in the spontaneous regeneration of the dento-gingival unit with masticatory mucosa (gingiva) under the influence of the periodontal ligament (Wennström, 1983; Wennström & Lindhe, 1983a, 1983b).

The epithelial connective tissue interface has been studied extensively for the dento-gingival unit (Caffesse et al., 1977, 1979; Karring et al., 1971; Karring, Lang, & Löe, 1975). In essence, it has been demonstrated that tissue specificity was conserved even after heterotrophic transplantation of the gingiva into the alveolar mucosa (Karring et al., 1971). Moreover, the gingival connective tissue was found to be essential in determining epithelial differentiation (Karring, Lang, & Löe, 1975). Connective tissue grafts (CTGs) of gingiva became covered by the keratinized epithelium, displaying the clinical and histological characteristics of normal gingiva. On the other hand, alveolar connective tissue transplants became covered by the non-keratinized epithelium. Hence, it was evident that the gingival connective tissue was capable of inducing a masticatory mucosa (Karring, Lang, & Löe, 1975). Later, this conclusion was confirmed by two studies (Caffesse et al., 1977, 1979) in which the intrinsic potential for keratinization was revealed for the sulcular epithelium (Caffesse et al., 1977). Furthermore, the role of the sulcular environment in controlling keratinization was also demonstrated (Caffesse et al., 1979).

Most recently, CTGs were placed under a coronally positioned flap after excision of the keratinized tissue (KT) at teeth and implants (Liñares et al., 2022). As a control, no CTGs were applied. After surgical excision of the KT, new KT was always observed around teeth but not around implants, regardless of the placement of a CTG (Liñares et al., 2022). It is yet unknown why the CTG did not induce keratinization around the implants.

Obviously, the maintenance of the connective tissue seal displaying similar tissue characteristics as those around teeth would be desirable after the healing phase of an implant (Sculean et al., 2014). At tooth sites, the soft-tissue seal is composed of an intensely adhering masticatory mucosa (gingiva) bordered by a movable alveolar mucosa containing elastic fibres (Karring et al., 1971). At implant sites, however, it is desirable to mimic the same tissue arrangement as that of the dento-gingival unit to satisfy the requirement for a biological seal (Lin et al., 2013; Ramanauskaite et al., 2022; Warrer et al., 1995). Obviously, the establishment of the soft-tissue seal around implants depends on the origin of the granulation tissue (Karring, Cumming, et al., 1975), and hence the establishment of a masticatory mucosa would depend on tissue characteristics typical for keratinized mucosa prior to implant placement. It is to be demonstrated whether tissue characteristics are maintained around implants, as they are for teeth, or whether they are influenced by the environment in which the implant is installed. Hence, the present pre-clinical study aimed to elaborate on the specificity of soft-tissue characteristics and the dimensional changes of the tissue adjacent to osseointegrated implants after complete removal of the keratinized mucosa and relate those to the spontaneous regeneration at the dentition.

2 | MATERIALS AND METHODS

2.1 | Animals

Four 18- to 24-month-old beagle dogs, each weighing 12–15 kg, were used. The animals had an intact and healthy dentition. They were kept in the animal facility of the Veterinary Faculty of the University of Santiago de Compostela (Lugo, Spain). The dogs were housed under laboratory conditions, at room temperature (15–21°C) and a humidity of >30%. They had access to tap water ad libitum and laboratory diet.

The current study was conducted in accordance with the European Communities Council Directive 2010/63/EU, approved by the Ethics Committee of the Rof Codina Foundation, Lugo (03/19/

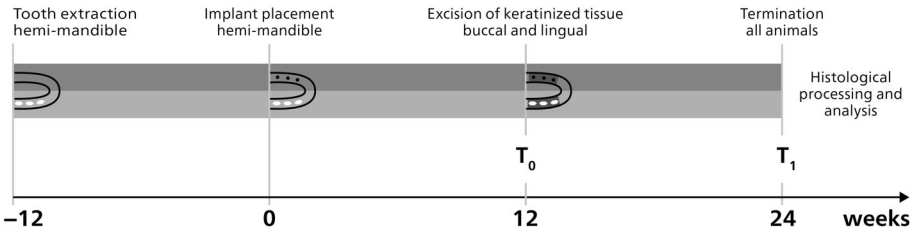

FIGURE 1 Timetable.

LU-001). In addition, the Guidelines for Animal Research: Reporting In Vivo Experiments (ARRIVE; Percie du Sert et al., 2020) have been followed.

2.2 | Study design and sample size

The study was designed as a split-mouth experiment with one test and one control hemi-mandible per animal. One co-worker (A.St.) who was not involved in the surgeries randomly allocated two right and two left test sites in the four animals. On the control side, premolars (PMs) 2, 3 and 4 were chosen as control teeth, whereas in the test hemi-mandible, PM 2, 3 and 4 were removed. After a 12-week healing time, three dental implants were installed on the test side. Consequently, three control teeth and three test implants per animal were available. With 4 animals, 3 teeth and 3 implants per animal, a total of 24 sites were studied. The defect was chosen as statistical unit. Given the paucity of similar pre-clinical studies in dogs, no power calculation was performed. The sample size calculation was based on a previous study from Thoma et al. (2020). The timetable of the study is shown in Figure 1.

2.3 | Surgical procedure

In a first phase, the animals were pre-anaesthetised with medetomidine (20 μg/kg/IM, Domitor, Orion Pharma, Espoo, Finland) and morphine (0.4 mg/kg/IM., Morfina Braun 2%; B. Braun Medical, Barcelona, Spain). The anaesthesia was initiated by propofol (2 mg/kg/IV; Propovet, Abbott Laboratories, Kent, UK) and maintained by inhalation of a mixture of O_2 and 2.5%–4% isoflurane (Isobavet, Schering-Plough, Madrid, Spain). A local anaesthesia composed of lidocaine and adrenaline (Anesvet, Ovejero, Leon, Spain) was used to reduce peri-operative pain and bleeding. After surgery, atipamezol (50 μg/kg/IM.; Antisedan, Esteve, Barcelona) was administered to revert the effects of medetomidine.

On each test hemi-mandible, PM 2, 3 and 4 were extracted, and the sites were allowed to heal for 12 weeks. The remaining dentition received oral prophylaxis after the extraction procedure.

In a second surgical phase, the animals were anaesthetised identical to the first phase. All surgeries were performed by one experienced periodontist (J.-C. I.). Muco-periosteal flaps were elevated on the test side, and three dental implants (Straumann Tissue Level, dia. 3.3 mm, length 8 mm, SP, SLActive, Roxolid, Straumann AG, Basel, Switzerland) were installed according to the manufacturer's instructions. The modified surfaces of all dental implants were fully inserted into the bone, thereby achieving primary stability. Special attention was given to soft-tissue management to establish a band of KT on the buccal and lingual sides. The flaps where closed tension-free by means of monofilament sutures (Stoma-medilene 6-0 blue, Storz am Mark GmbH, Emmingen-Liptingen, Germany) and the sites were allowed to heal in a non-submerged manner. Plaque control was performed three times a week to ensure complication-free healing. The sutures were removed after 7 days.

After an additional 3 months of healing (Figure 2a,b), a third surgery was performed (Figure 2c,d). Prior to this surgery (time point T_0), the following clinical measurements were obtained at all test implants and all control teeth (six positions per unit: mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, disto-lingual) with a periodontal probe (Stoma Perio probe, PCPNC North Carolina, Storz am Mark GmbH) by one examiner (A.R.).

- Width of the KT (mm)
- Probing depth (PD, mm)
- Bleeding on probing (BOP, %)
- Probing attachment level (PAL, mm)
- Plaque and calculus (%)

After the clinical examination, the entire masticatory mucosa was surgically removed on three teeth (PM2, 3 and 4) of the control side (Figure 2d) and on the three dental implants of the test side (Figure 2c). The elimination of the masticatory mucosa was performed on both buccal and lingual aspects, leaving the alveolar bone exposed. Before the surgical removal, a notch was placed around all teeth at the initial level of the gingival margin. The notch served as a reference point for histomorphometry and clinical measurements, whereas at the implant sites the implant shoulder represented the reference. After the surgeries, pain was controlled with morphine (0.3 mg/kg/IM/6 h) for 24 h and meloxicam (0.1 mg/kg/s.i.d/P.O.; Metacam, Boehringer Ingelheim, Barcelona, Spain) for 4 days. Antibiotics (Cefovecin 8 mg/kg/SC, Convenia, Zoetis, Louvain-La-Neuve, Belgium) were administrated for 7 days. The animals were monitored daily for health status using standardized scoring sheets. During the first two post-operative weeks, the teeth and implants were disinfected three times a week using gauzes soaked in chlorhexidine (0.12%, Perio-Aid Tratamiento, Dentaid, Barcelona). Subsequently, a toothbrush with a chlorhexidine gel (0.2%; Chlorhexidine Bioadhesive Gel, Lacer, Barcelona) was used

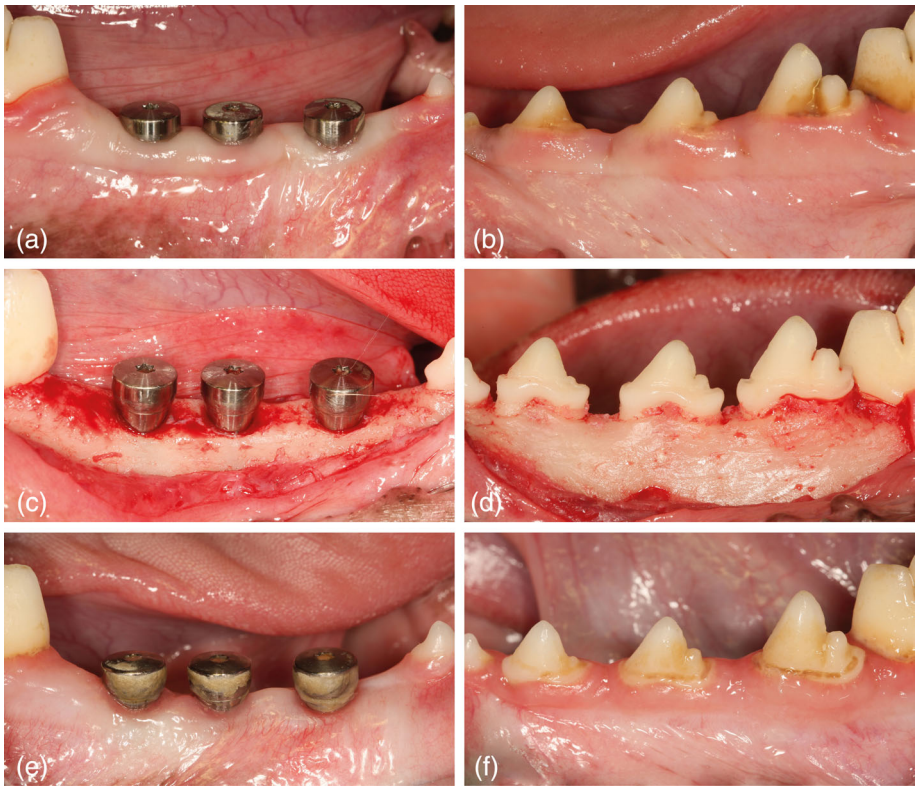

FIGURE 2 Clinical pictures illustrating the procedure in the test group (a, c, e) and control group (b, d, f). Twelve weeks after implant placement (T_0) in the test (a) and control group (b), after surgical excision of the keratinized tissue (KT) in the test (c) and control group (d) and 12 weeks after the surgical excision of KT in the test (e) and control group (f). [Colour figure can be viewed at wileyonlinelibrary.com]

three times a week for continued plaque control. The dogs were fed a soft-pellet diet for 1 week.

After a healing period of 3 months (time point T_1, Figure 2e,f), the clinical measurements were repeated; additionally, PAL loss (T_0–T_1) and soft-tissue reduction (gingival or mucosal recession from T_0 to T_1) were calculated. Following this, the animals were euthanized by sedation with medetomidine (30 µg/kg/IM; Esteve) and subsequently sacrificed with an overdose of sodium pentobarbital (60 mg/kg/IV, Dolethal, Vetoquinol, France).

2.4 | Histological procedures

After euthanization, the mandibles of the animals were removed, and individual blocks including the soft and hard tissues were obtained and subsequently fixed in 10% formaldehyde.

All eight hemi-mandibles were dehydrated in an ascending series of ethanol and infiltrated and embedded in methylmethacrylate (MMA). After polymerization, the specimens were sectioned in a bucco-oral plane along their longitudinal axis with a slow-speed diamond saw with a coolant (Varicut VC-50; Leco, Munich, Germany). From each tooth root and each dental implant, three ground sections were produced. Thereafter, always two approximately 800-µm-thick ground sections per tooth root or implant were mounted on Plexiglas slabs and ground to a final thickness of 150 µm (Knuth-Rotor-3; Struers, Rodovre/Copenhagen, Denmark). Finally, the sections were superficially stained with toluidine blue/McNeal combined with basic fuchsin. Photography was performed using a digital camera (AxioCam MRc; Carl Zeiss, Oberkochen, Germany) connected to a light microscope (Axio Imager M2; Carl Zeiss). To investigate elastic fibres, from the remaining group section per site, four implant and four tooth sites were selected for a microtome procedure. Before sectioning with the microtome, the implants were carefully removed out of the ground sections. The undecalcified specimens were cut into approximately 5-µm-thick sections with a microtome (Reichert-Jung, Heidelberg, Germany). Thereafter, the polymethylmethacrylate was removed, and the sections were stained with resorcin-fuchsin and Masson–Goldner trichrome and digitized. Weigert's resorcin-fuchsin is a common stain for elastic fibres, resulting in blue/purple-black staining (Sheehan D. and Hrapchak B., *Theory and Practice of Histotechnology*, 2nd ed., 1980, pp. 194–195, Battelle Press, OH, USA).

2.5 | Histomorphometric analysis

The most central section of each implant or tooth root (i.e., mesial and distal root of each PM) was chosen for histomorphometric analysis. Regions of interest were digitized with a computer connected to a light microscope (Axio Imager M2; Carl Zeiss). Thereafter, the following histomorphometric landmarks were identified and discussed by two investigators (D.D.B. and J.-C.I.):

1. Gingival margin (GM) or mucosal margin (MM)
2. Apical termination of the KT (aKT)
3. Apical termination of the junctional epithelium (aJE)
4. Apical end of the coronal notch (cN) or implant shoulder (IS)

TABLE 1 Multi-level comparisons of clinical data at baseline (T_0) and after keratinized tissue (KT) excision (T_1).

	Teeth buccal Mean, SD	Implants buccal Mean, SD	*p*-Value	Teeth lingual Mean, SD	Implants lingual Mean, SD	*p*-Value	Teeth total Mean, SD	Implants total Mean, SD	*p*-Value
Width of KT T_0	5.111	3.278	<.0001	6.027	4.027	<.0001	5.569	3.653	<.0001
	1.008	0.815		0.810	0.971		1.019	0.966	
Width of KT T_1	2.722	0.250	<.0001	2.472	0.444	<.0001	2.597	0.347	<.0001
	0.615	0.439		0.696	0.607		0.664	0.534	
p-Value T_0 versus T_1	<.0001	<.0001		<.0001	<.0001		<.0001	<.0001	
PD T_0	2.167	2.389	.102	2.361	2.111	.101	2.264	2.250	.883
	0.447	0.599		0.487	0.465		0.474	0.550	
PD T_1	1.194	1.583	.009	1.528	1.694	.191	1.361	1.639	.007
	0.401	0.770		0.609	0.577		0.538	0.677	
p-Value T_0 versus T_1	<.0001	<.0001		<.0001	<.0001		<.0001	<.0001	
MM, GM T_0	2.750	1.306	<.0001	2.833	0.889	<.0001	2.792	1.097	<.0001
	0.554	0.710		0.447	0.887		0.502	0.825	
MM, GM T_1	1.306	−1.194	<.0001	1.694	−1.389	<.0001	1.500	−1.292	<.0001
	0.668	0.624		0.624	0.803		0.671	0.721	
p-Value T_0 versus T_1	<.0001	<.0001		<.0001	<.0001		<.0001	<.0001	
Soft tissue reduction T_0–T_1	−1.291	−2.389	<.0001	−1.139	−2.278	<.0001	−1.292	−2.389	<.0001
	0.568	0.815		0.593	0.882		0.567	0.814	
Probing attachment level T_0–T_1	−0.472	−1.694	<.0001	−0.306	−1.861	<.0001	−0.3889	−1.778	<.0001
	0.560	0.856		0.525	1.046		0.5453	0.952	
	***n* (%)**	***n* (%)**		***n* (%)**	***n* (%)**		***n* (%)**	***n* (%)**	
Plaque-positive T_0	31 (86.1)	36 (100.0)	.0539	26 (72.2)	36 (100.0)	.0001	57 (79.2)	72 (100.0)	<.0001
Plaque-positive T_1	31 (86.1)	35 (97.2)	.1987	26 (72.2)	31 (86.1)	.2454	57 (79.2)	66 (91.7)	.0570
p-Value T_0 versus T_1	1.0000	1.0000		1.0000	.0539		1.0000	.0280	
Calculus-positive T_0	31 (86.1)	24 (66.7)	.0942	26 (72.2)	18 (50.0)	.0898	57 (79.2)	42 (58.3)	.0114
Calculus-positive T_1	32 (88.9)	27 (75.0)	.2196	16 (44.4)	27 (75.0)	.0156	48 (66.7)	54 (75.0)	.3594
p-Value T_0 versus T_1	1.0000	.6047		.0307	.0505		.1330	.0513	
BoP-positive T_0	24 (66.7)	26 (72.2)	.7985	23 (63.9)	29 (80.6)	.1877	47 (65.3)	55 (76.4)	.1991
BoP-positive T_1	17 (80.6)	29 (80.6)	.0064	15 (41.7)	28 (77.8)	.0036	32 (44.4)	57 (79.2)	<.0001
p-Value T_0 versus T_1	.1528	.5798		.097	1.0000		.0187	.8414	

Abbreviations: BoP, bleeding on probing; GM, gingival margin; MM, mucosal margin; PD, probing depth; SD, standard deviation; T_0, before excision of KT; T_1, after excision of KT.

5. First bone to implant contact (fBIC)
6. Bone crest (BC)

The following vertical measurements were performed buccally and lingually along the axis of each implant or tooth root by one experienced investigator (J.-C.I.) using the software Zeiss Efficient Navigation Pro (Zen Pro, Carl Zeiss):

1. Height of the KT (GM or MM–aKT)
2. Height of the junctional epithelium (JE) including sulcus (GM or MM–aJE)
3. Height of connective tissue at teeth or implants surface (aJE–BC or aJE–fBIC)
4. Height of supracrestal tissues (height of JE + sulcus + connective tissue) at teeth and implants (GM–BC or MM–fBIC)
5. Vertical soft-tissue loss, with negative values representing a loss of tissue (GM–cN or MM–IS)

2.6 | Statistical analysis

Data analysis and visualization were performed using Prism v7 (GraphPad Software, La Jolla, CA) and RStudio (version 1.3.1093, RStudio Team, (2020). RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/). Descriptive statistics were used to calculate means, percentages and

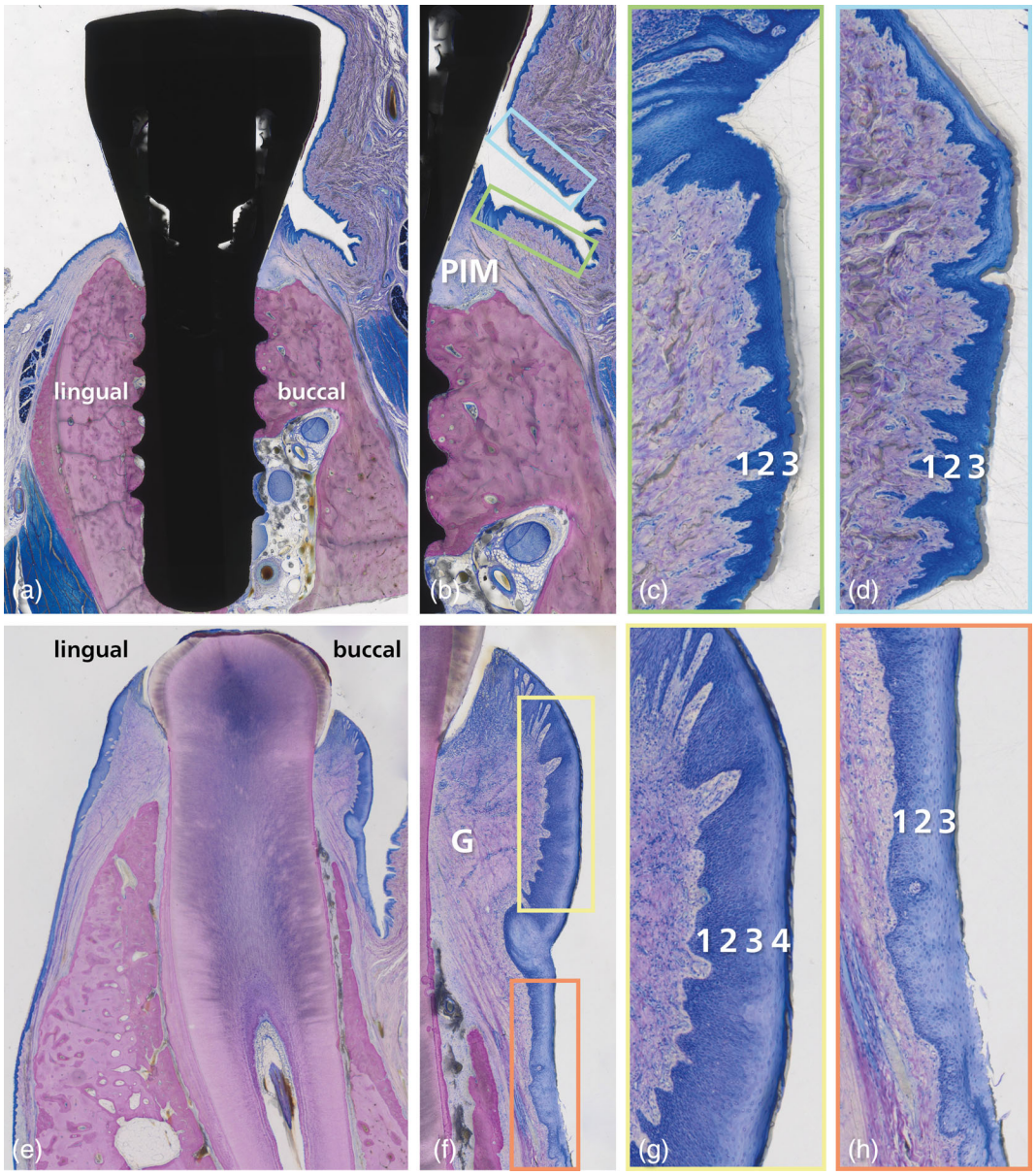

FIGURE 3 Representative overview of histological sections of the test (a, b) and control group (e, f). Higher magnification of the buccal epithelium near the peri-implant mucosal (PIM) (b) and gingival sulcus (f) without keratinization at the implant site (c) and with keratinization at the tooth site (g). Higher magnification of the mucosa without keratinization at implant (d) and tooth site (h). Keratinizing epithelium (gingiva) around tooth consisting of four strata, that is, stratum basale (1), stratum spinosum (2), stratum granulosum (3) and stratum corneum (4). Non-keratinizing epithelium around implant consisting of three strata, that is, stratum basale (1), stratum filamentosum (2) and stratum distendum (superficiale) (3). G, gingiva. Staining: toluidine blue/McNeal + basic fuchsin. [Colour figure can be viewed at wileyonlinelibrary.com]

standard deviations. Categorical data were analysed using Fisher's exact tests. Levene's tests were performed to assess the equality of variances of the measurements performed. To investigate the effect of time (T_0, T_1) and group (teeth, implants) on clinical measurements, two-way analysis of variance (ANOVA) tests with a random effect (animal) were used. To analyse between-group differences on histomorphometric measurements, one-way ANOVA tests with a random effect (animal) were conducted. To account for multiple comparisons, Holm corrections were applied to the statistical analysis. Significance was set at $p < .05$.

3 | RESULTS

3.1 | Clinical findings

Following all surgical procedures, healing was uneventful. No infections or other complications were noted. This, in turn, meant that all 24 sites were available for further analysis. The clinical measurements for buccal, lingual and total measurements (six positions per tooth) at T_0 and T_1 are reported in Table 1. After tooth extraction on the test side, a certain loss of the width of KT was evident. Consequently, the

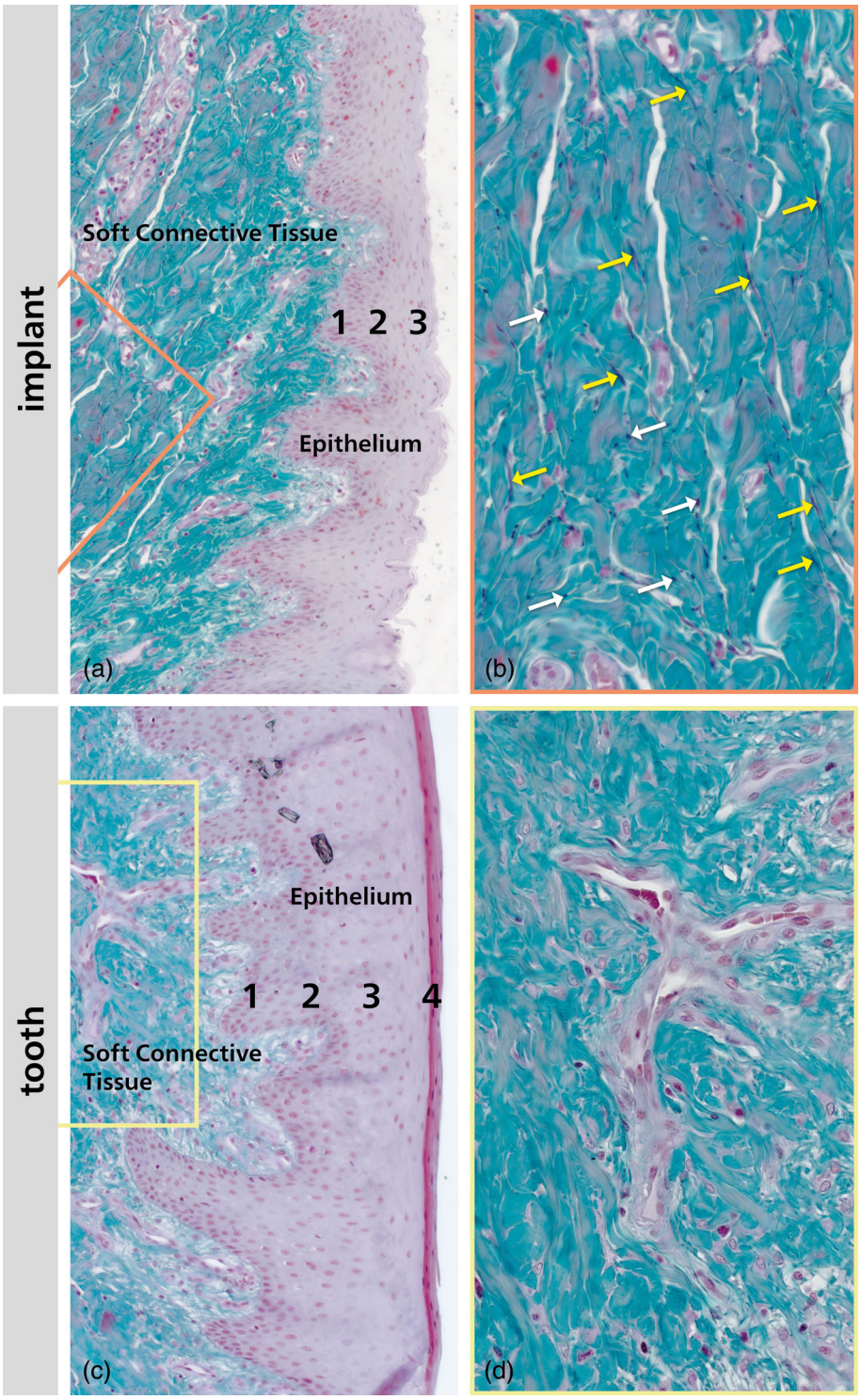

FIGURE 4 Microtome sections near the mucosal margin at implant site (a, b) and near the gingival margin at tooth site (c, d). The gingival epithelium consists of four strata, that is, stratum basale (1), stratum spinosum (2), stratum granulosum (3) and stratum corneum (4). The epithelium of the peri-implant mucosa consists of three strata, that is, stratum basale (1), stratum filamentosum (2) and stratum distendum (superficiale) (3). Yellow arrows indicate longitudinally cut elastic fibres, whereas white arrows indicate cross-cut elastic fibres. Magnification 20× (a, c), 40× (b, d). Staining: resorcin-fuchsin and Goldner. [Colour figure can be viewed at wileyonlinelibrary.com]

width of KT at T_0 was higher at the teeth (total mean: 5.56 mm, SD: 1.02) than at implants (total mean: 3.65 mm, SD: 0.96; $p < .0001$). At T_1, on all teeth, a band of KT was spontaneously regenerated (total mean: 2.60 mm, SD: 0.66). In contrast, at implant sites, only occasionally was KT detected (mean total: 0.35 mm, SD: 0.53). This was statistically significantly different comparing tooth and implant sites ($p < .0001$). The KT observed at implant sites was located between the implants or near the teeth PM1 and molar 1. On the mid-buccal or mid-lingual location of all implants, no band of KT was observed (mean: 0 mm).

The PD at tooth sites (total mean T_0: 2.26 mm, SD: 0.47; total mean T_1: 1.36 mm, SD: 0.54) and implant sites (total mean: 2.25 mm, SD: 0.55; total mean T_1: 1.64 mm, SD: 0.68) was significantly reduced from T_0 to T_1 ($p < .0001$). The soft-tissue reduction from T_0 to T_1 at implant sites was statistically significantly higher (total mean: −2.39 mm, SD: 0.81) compared to that at tooth sites (total mean:

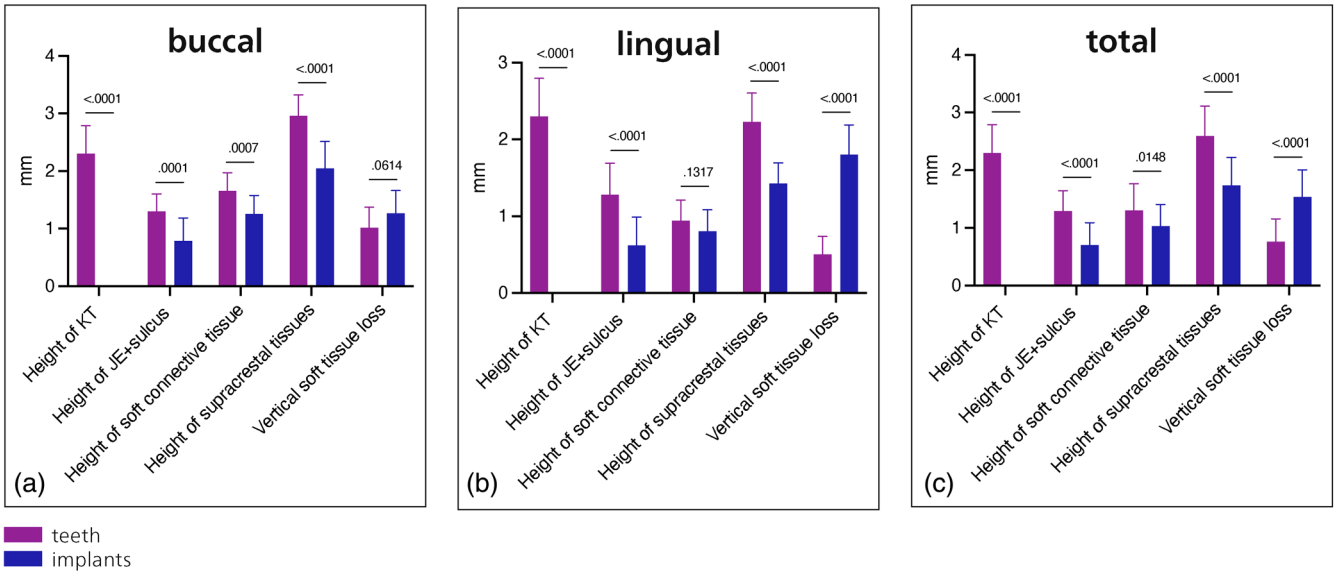

FIGURE 5 Histomorphometric measurements on the buccal (a) or lingual (b), and total sites (c, buccal + lingual). JE, junctional epithelium; KT, keratinized tissue. [Colour figure can be viewed at wileyonlinelibrary.com]

−1.29 mm, SD: 0.57; $p < .0001$). Soft-tissue loss in combination with the reduction of PD resulted in a small amount of PAL loss at tooth sites (total mean: −0.39 mm, SD: 0.55) and a statistically significant higher loss at implant sites (total mean: −1.78, SD: 0.95; $p < .0001$).

Plaque and calculus were clinically observed in both test and control sites. Additionally, BOP was a frequent finding at implant sites (mean T_0: 76.4%, mean T_1: 79.2%) and tooth sites (mean T_0: 65.3%, mean T_1: 44.4%). The BOP values at teeth were statistically significantly improved from T_0 to T_1 ($p = .019$). Moreover, the BOP values at tooth sites were statistically significantly lower than those at implant sites at T_1 ($p < .0001$).

3.2 | Descriptive histology

All 36 sites (12 teeth with 2 roots each and 12 implants) were available for descriptive analysis. In total, 108 ground sections were produced and 72 were mounted on Plexiglas and further processed. Eight sites (4 implants and 4 teeth) were investigated with 4 microtome sections per unit (total: 32 microtome sections). Representative sections are shown in Figure 3. Processing artefacts were very rare and never compromised the assessments. All 12 implants were successfully osseointegrated. Minor amounts of inflammatory infiltrates were observed, both in the peri-implant mucosae and the gingivae. On tooth sites, small root resorptions filled with reparative cementum (i.e., mixed stratified cementum) were occasionally observed (Figure 3e,f). Biofilm and/or calculus deposits were recognized in most cases supragingivally or supramucosally. Occasionally, biofilm and/or calculus was found as well subgingivally or submucosally. At the tooth sites, a spontaneous regeneration of the dento-gingival unit was evident, displaying the characteristics of masticatory mucosa (Figure 3e–g). Keratinization and rete peg formation were clearly visible on all buccal and lingual aspects. At a distance from the gingival margin, the keratinized epithelium had transformed into a non-keratinized epithelium (Figure 3h). On the microtome sections, elastic fibres were absent in the connective tissue bellow the keratinized epithelium (Figure 4c,d), whereas they were numerous in the connective tissue under the non-keratinized epithelium (Figure 4a,b). At the implant sites, on the other hand, the implanto-mucosal unit was characterized by a non-keratinized epithelium, indicating the characteristics typically encountered in alveolar mucosa (Figure 3a–d). Elastic fibres were abundant in the connective tissue near and far away from the mucosal margin (Figure 4a,b). The soft tissues were closely adapted to the surface of the implants but, obviously, the buccal alveolar mucosa directly inserted towards the implants. On the buccal side, the depth of the vestibular fold was shorter at implant sites compared to tooth sites, resulting in a distance from the gingival or mucosal margin to the vestibular sulcus that was clearly shorter at implant sites (Figure 3a,e).

3.3 | Histomorphometry

All 24 sites (12 teeth and 12 implants) were available for histomorphometric analysis. Since the central section of every root was chosen, the number of analysed sections was 24 for teeth. For implants, only the most central section was chosen and, consequently, the number of analysed sections was 12. The histomorphometric results are presented in Figure 5 and Table S1 for buccal, lingual and total (buccal + lingual) measurements. The height of the KT (total mean) was 2.30 mm (SD: 0.48) at tooth sites and 0 mm at implant sites ($p < .0001$). Thus, no KT was detected at any implant site. The height of the JE, including the sulcus depth, was higher for teeth (total mean: 1.30 mm, SD: 0.36) than for implants (total mean: 0.71 mm, SD: 0.38; $p < .0001$). Furthermore, the height of soft connective tissue was 1.30 mm at teeth and 1.03 mm at implants ($p = .0148$). Consequently, the height of supracrestal tissues

was larger for teeth (total mean: 2.59 mm, SD: 0.52) than that for implants (total mean: 1.74 mm, SD: 0.49; $p < .0001$). The vertical soft-tissue loss (mucosal or gingival recession) was higher for implants (total mean: 1.54 mm, SD: 0.47) than for teeth (total mean: 0.76 mm, SD: 0.39, $p < .0001$).

4 | DISCUSSION

The aim of this study was to investigate the specificity and the dimensions of the soft tissues during the spontaneous regeneration of the dento-gingival and implanto-mucosal units after the elimination of adjacent masticatory mucosa. The excision of the masticatory mucosa around implants resulted in healing without masticatory mucosa and with reduced soft-tissue barrier height and vertical soft connective tissue loss (recession). In contrast, at teeth the dento-gingival unit was spontaneously regenerated. The regeneration was accompanied by gingival recession.

In the original study by Karring, Lang, and Löe (1975), it was demonstrated that the underlying connective tissue influenced tissue specificity for the spontaneous regeneration of a dento-gingival unit after gingivectomy. The presence of tissue proliferating from the periodontal ligament always mediated the establishment of a masticatory mucosa characterized by rete peg formation, epithelial keratinization and absence of elastic fibres when the gingiva had been excised completely by gingivectomy. Hence, it was concluded that the dento-gingival unit always spontaneously regenerated to a certain extent when it had been excised experimentally. This formed the basis of the healing concept following gingivectomies in clinical periodontology. However, in that study (Karring, Lang, & Löe, 1975), the implanto-mucosal unit was not included. Because implant dentistry has become very important, it was necessary to study this biological concept also in relation to dental implants.

While the results of the present study confirmed those obtained previously, the hypothesis of the connective tissue influencing tissue specificity in the spontaneous regeneration of the implanto-mucosal unit, likewise, was established. Indeed, at the implant sites, there was no positive influence of masticatory mucosa on keratinization, and hence the spontaneous regeneration of the implanto-mucosal unit healed without the formation of a masticatory mucosa. As a consequence, this unit spontaneously regenerated after excision of the masticatory mucosa with tissue adjacent to the implant that was not covered by keratinized mucosa. Rather, tissue characteristics typical for alveolar mucosa were established. This, in turn, means that the formation of an implanto-mucosal barrier around an osseointegrated implant is dependent on the presence of keratinized mucosa. If the latter is absent, a tissue structure would result that may not resist the challenges encountered and usually coped with by the dento-gingival unit.

Similar results in terms of KT heights following excision of masticatory mucosa were obtained in a recently published article (Liñares et al., 2022). Unfortunately, that study did not report on clinical parameters other than the extent of the KT. Nevertheless, it had established that tissue characteristics were maintained if the soft-tissue barriers were left untreated. Obviously, this negative control shed some light on the maintenance of tissue specificity if an implant was placed and subsequently surrounded by keratinized mucosa. In the present study, no such negative control situation had been generated. Moreover, the histomorphometric measurements presented in the study of Liñares et al. (2022) were similar to those obtained in the present study regarding the height of the KT. It has to be realized that the study mentioned did not consider the palatal aspects of the soft-tissue barrier at all. This means one cannot exclude that some influence in determining the tissue characteristics was originating from the remaining palatal KT. Consequently, the model applied cannot be compared with that of the present study, as the experiment had been performed in the maxilla rather than the mandible.

In the study by Karring, Lang, and Löe (1975), the role of the gingival connective tissue in determining epithelial differentiation had been evaluated by grafting connective tissue without epithelium removed from either the gingiva or from the non-keratinized mucosa into the alveolar mucosa. The grafts were allowed to heal into connective tissue pouches prepared close to the overlying epithelium. Once the transplants had healed, they were exposed to the oral environment by removal of the overlying tissue. Subsequently, the transplants were allowed to epithelialize with cell proliferation from the surrounding non-keratinized alveolar mucosa. The transplants were examined clinically and histologically at various time intervals up to 12 months. All the gingival CTGs had become covered by a keratinized epithelium, displaying the typical characteristics of normal masticatory mucosa. However, the alveolar mucosa transplants had been covered with non-keratinized epithelium. This clearly indicated that gingival connective tissue was capable of inducing the formation of a keratinized barrier. The biological principle revealed in that study was confirmed in the present experiment for tooth sites. Likewise, the absence of gingival connective tissue around implants deprived of their soft-tissue barrier yielded the healing of the implant-mucosal unit without keratinization of the epithelium and, hence, a soft tissue presenting with characteristics of the alveolar mucosa.

The above-mentioned principles of healing indicate that a functional peri-implant soft-tissue seal is dependent on the preservation of masticatory mucosa following implant installation.

If implants are placed exclusively into alveolar mucosa, the soft-tissue barrier may be jeopardized. A number of clinical studies (Bonino et al., 2018; Kabir et al., 2021; Monje & Blasi, 2019; Perussolo et al., 2018; Roccuzzo et al., 2016) have attempted to study the influence of such a situation on the longevity of the implants, but the results are still controversial and non-conclusive. A recent systematic review (Ramanauskaite et al., 2022), however, indicated that a reduced KT width is associated with an increased prevalence of peri-implantitis, plaque accumulation, soft-tissue inflammation, mucosal recession, marginal bone loss and greater patient discomfort.

Moreover, an experimental study in monkeys (Warrer et al., 1995) attempted to evaluate the potential of inducing peri-implant infection at implants with or without keratinized mucosa by placing biofilm-retaining ligatures. In sites without keratinized mucosa,

the recession was significantly more marked than at sites with keratinized mucosa. Likewise, the amount of bone loss encountered was greater at sites without keratinized mucosa, indicating that an implanto-mucosal barrier with KT may represent a protective mechanism if the sites are challenged with biofilm accumulation (Warrer et al., 1995).

Interestingly, the supracrestal tissue complex was buccally and lingually statistically significantly larger for teeth (2.96 mm, SD: 0.36 and 2.23 mm, SD: 0.38, respectively) compared to implants (2.05 mm, SD: 0.46; $p < .0001$ and 1.43 mm, SD: 0.27, $p < .0001$, respectively) after excision of KT. Both the sulcular and the soft connective tissue compartments were larger at tooth sites. This is in contrast with findings from previous studies where the supracrestal complex was larger for implants than for teeth (Berglundh et al., 1991; Berglundh & Lindhe, 1996; Ivanovski & Lee, 2018). Berglundh et al. (1991) investigated the supracrestal complex for teeth and implants in healthy conditions in the same species as used in the present study. The supracrestal complex in the present experiment after the excision of KT appeared to be shorter for both tooth and implant sites. Moreover, at implant sites, this difference appeared to be much more pronounced when comparing healthy sites in the study by Berglundh et al. (1991) (3.80 mm, SD: 0.65) with sites without keratinization in our study (1.74 mm, SD: 0.49). One may speculate that these differences are due to an irregular connective tissue seal after excision of the KT.

The histological assessment of the vertical soft-tissue loss (recession) was more accurate at tooth sites compared to implant sites, as a clear reference (i.e., notch at the initial gingival margin at T_0) was established. At implants, this was technically not feasible and the implant shoulder acted as the reference line. Since the initial mucosal margin at implant sites was slightly above the implant shoulder (clinical measurement, mean total: 1.10 mm), the loss of soft-tissue height was slightly underestimated. This, in turn, means that recession around implants is even more pronounced as indicated by histometry.

The results of the present study provide additional evidence to support the biological rationale to preserve KT prior to implant placement. Clinical procedures for implant installation should consider these findings because, in many instances, keratinized mucosa may be excised through (or by) the uncovering of second-stage surgery of a submerged implant healing or by punch soft-tissue excision prior to guided implant placement. Consequently, after excision of KT, the spontaneous regeneration of the soft tissue at implant sites was characterized by a non-keratinized epithelium and a diminished supracrestal tissue dimension resembling alveolar mucosa, whereas at tooth sites the gingiva was spontaneously regenerated.

AUTHOR CONTRIBUTIONS

Jean-Claude Imber: conception, surgeries, histological processing, histomorphometric analysis, data collection and interpretation, manuscript drafting. **Andrea Roccuzzo**: conception, surgeries, clinical measurements, histological processing, histomorphometric analysis, data collection and interpretation, manuscript drafting. **Alexandra Stähli**: data collection and interpretation, manuscript approval. **Dieter D. Bosshardt**: histological processing, histomorphometric analysis, data collection and interpretation, manuscript approval. **Fernando Muñoz**: surgeries, clinical measurements, data collection and interpretation, manuscript approval. **Christoph A. Ramseier**: data interpretation, statistics, manuscript correction and approval. **Niklaus P. Lang**: conception, planning, supervision, manuscript drafting. **Anton Sculean**: conception, grant acquisition, planning, supervision, manuscript drafting.

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CONFLICT OF INTEREST STATEMENT

The authors report no potential conflict of interest related to this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The current study was conducted in accordance with the European Communities Council Directive 2010/63/EU, approved by the Ethics Committee of the Rof Codina Foundation, Lugo, Spain (03/19/LU-001).

ORCID

Jean-Claude Imber https://orcid.org/0000-0001-6690-5249
Andrea Roccuzzo https://orcid.org/0000-0002-8079-0860
Alexandra Stähli https://orcid.org/0000-0002-5631-3300
Dieter D. Bosshardt https://orcid.org/0000-0002-2132-6363
Fernando Muñoz https://orcid.org/0000-0002-4130-1526
Christoph A. Ramseier https://orcid.org/0000-0002-5110-2539
Niklaus P. Lang https://orcid.org/0000-0002-6938-9611
Anton Sculean https://orcid.org/0000-0003-2836-5477

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Additional supporting information can be found online in the Supporting Information section at the end of this article.

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