Received: 13 February 2023 | Revised: 11 September 2023 | Accepted: 14 September 2023

DOI: 10.1111/clr.14184

ORIGINAL ARTICLE

CLINICAL ORAL IMPLANTS RESEARCH WILEY

Clinical and histologic evaluation of heterotopic mucosa transpositioning at teeth and dental implants

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, 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

Funding information
International Team for Implantology

Abstract

Aim: To investigate the healing after heterotopic mucosa transpositioning at dental implants and teeth.

Materials and Methods: One hemimandible per dog ($n=4$) was allocated to receive 3 implants (test), whereby 3 premolars on the contralateral side served as controls. After osseointegration, a Z-plasty was performed on the buccal aspect of the test and control sites to heterotopically move the zone of keratinized tissue (KT) into a region with non-keratinized tissue (nKT) and vice versa. Clinical measurements were performed before (T0) and at 12 weeks following heterotopic transposition (T1). Thereafter, specimens were processed for histological analysis.

Results: Clinical measurements revealed that at T1, a band of KT was reestablished at teeth (mean: 2.944 ± 1.866 mm), whereas at implants, the transpositioned nKT resulted in a mucosa without any signs of keratinization (mean: 0 mm; $p<.0001$). At implant sites, the probing attachment level loss was more pronounced compared to tooth sites (−1.667 ± 1.195 mm and −1.028 ± 0.878 mm, respectively; $p=.0076$). Histologically, the transpositioned nKT, was accompanied by the formation of KT at the tooth but not at implant sites. The supracrestal soft tissues were statistically significantly higher at tooth compared to implant sites (2.978 ± 0.483 mm and 2.497 ± 0.455 mm, $p=.0083$). The transpositioned KT remained mostly unaltered in its morphological characteristics.

Conclusions: The findings of this study indicate that: (a) transpositioned KT may retain its morphological characteristics; and (b) transpositioned nKM was accompanied by the formation of KT at the tooth but not at implant sites.

KEYWORDS
connective tissue, dental implants, gingiva, keratinized tissue, peri-implant mucosa, soft tissue healing, teeth, tissue characteristics

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

1 | INTRODUCTION

Around dental implants, some studies failed to reveal any difference in terms of healing or tissue stability related to the presence or absence of keratinized tissue (KT) (Wennström et al., 1994; Wennström & Derks, 2012). Other studies indicate that in the presence of a connective tissue seal covered by keratinized epithelium, the peri-implant mucosal seal was more stable than in the absence of a KT seal (Monje & Blasi, 2019; Roccuzzo et al., 2016). Findings from a preclinical study indicate that peri-implant tissues deprived of KT resulted in increased soft tissue recession and bone loss compared to pristine sites (Warrer et al., 1995). Moreover, the necessity of a certain width and thickness of the KT around implants has been demonstrated in recent systematic reviews (Giannobile et al., 2018; Lin et al., 2013; Ramanauskaite et al., 2022; Tavelli et al., 2021; Thoma et al., 2018). To maintain peri-implant health, it is therefore desirable to maintain a soft tissue seal with tissue characteristics similar to those around teeth (Sculean et al., 2014).

Compared to the peri-implant mucosal unit, the dento-gingival unit appears to be less susceptible to bacterial challenges (Wennström, 1983; Wennström & Lindhe, 1983a, 1983b). Interestingly, around teeth, a certain regeneration of KT (i.e., gingiva) was always observed following its complete excision and was attributed to the influence of the periodontal ligament (Wennström, 1983; Wennström & Lindhe, 1983a, 1983b).

Findings from an experimental study evaluating the heterotopic transpositioning of the gingiva into the alveolar mucosa (Karring et al., 1971) have shown that tissue specificities were preserved, revealing the characteristics of their original location. Experimental studies in preclinical models have indicated that the characteristic features of the epithelium are most likely determined by the underlying connective tissue (Caffesse et al., 1977, 1979; Karring et al., 1975). It has been shown that connective tissues (CTGs) harvested from the gingiva had the capacity to induce keratinization, whereas CTGs originating from non-keratinized tissue (nKT) were covered by a non-keratinized epithelium (Karring et al., 1975). However, until now, such an inductive effect of the connective tissue has not yet been demonstrated in peri-implant mucosal tissue.

In a very recent experimental study, CTGs derived from a keratinized site were placed under a coronally positioned flap after complete excision of the KT at teeth and implants (Liñares et al., 2022). On the control sites, no CTGs were applied. New KT reformed around teeth but not around implants, regardless of the placement of a CTG (Liñares et al., 2022). These findings are partially in agreement with those of Karring et al. (1975). However, the fact that no KT regenerated around implants in the presence of CTGs derived from a site with keratinization, is difficult to explain. At present, it is unknown to what extent, at dental implants, the tissue specificities of the KT remain the same following heterotopic transposition into the alveolar mucosa.

Hence, the purpose of the present preclinical study was to evaluate the tissue specificity after heterotopic transpositioning at implants and teeth.

2 | MATERIALS AND METHODS

2.1 | Animals

Four female Beagle dogs (20–22 months old, 12–15 kg in weight) were used in this study. The dentition of the animals was healthy and intact. Animals were housed at the animal facility of the Veterinary Faculty of the University of Santiago de Compostela (Lugo, Spain) under laboratory conditions, at a room temperature of 15–21°C and a humidity of >30%. They had access to tap water ad libitum and a laboratory diet.

The 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). 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

This study had a split-mouth design with one hemi-mandible with remaining teeth (control) and one hemi-mandible with dental implants (test). To ensure a balanced allocation, a colleague (A.St.), who was not involved in the surgeries, randomly assigned two test sites on the right and two on the left in the four animals. Premolars (PM) 2, 3, 4, and three dental implants per mandible were evaluated clinically and histologically. Thus, three test implants and three control teeth were available per animal, and a total of 24 sites were analyzed in four animals. After the final surgical procedure, a 12-week healing period was allowed. The timetable of the study is illustrated in Figure 1.

3 | SURGICAL PROCEDURE

3.1 | First phase—Tooth extraction

The animals were pre-anesthetized with medetomidine (20 µg/kg/IM, Domitor®, Orion Pharma) and morphine (0.4 mg/kg/IM., Morfina Braun 2%; B. Braun Medical). The anesthesia was initiated by propofol (2 mg/kg/IV; Propovet™, Abbott Laboratories) and maintained by inhalation of an O_2 and 2.5%–4% isoflurane mixture (Isobavet®, Schering-Plough). A local anesthesia composed of lidocaine and adrenaline (Anesvet®, Ovejero) was used to reduce perioperative pain and bleeding. According to the allocation, on each test hemi-mandible, PM2, 3, and 4 were extracted. After the surgical intervention, atipamezole (50 µg/kg/IM.; Antisedan®, Esteve) was administered to reverse the effects of the medetomidine. After extraction, the sites were allowed to heal for 12 weeks, and the remaining dentition received oral prophylaxis during this time.

3.2 | Second phase—Implant installment

In a second surgical phase, the animals were anesthetized identically to the first phase. All surgeries were performed by one experienced

periodontist (J.-C. I.). Mucoperiosteal flaps were elevated on the test side, and three dental implants (Straumann® Tissue Level, Ø 3.3 mm, length 8 mm, SP, SLActive®, Roxolid®, Straumann AG) were installed according to the manufacturer's instructions. The modified surface of all dental implants was fully inserted into the bone, 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 were closed tension-free by means of monofilament sutures (Stoma®-medilene 6–0 blue, Storz am Mark GmbH). Plaque control was performed three times a week to guarantee complication-free healing. Sutures were removed after 7 days.

3.3 | Third phase—Heterotopic positioning

The protocol for the third surgery was adapted from a previous study (Karring et al., 1975) and identical for the test and control sites. This surgical procedure is illustrated in Figure 2. On all buccal sites, a Z-plasty was performed to heterotopically move the zone of KT apically into the region with nKT, whereby the nKT was rotated to the region of the gingiva or peri-implant mucosa. First, a parallel incision to the mucogingival line was made approximately 1 mm below the mucogingival junction and an intrasulcular incision from PM1 distal to PM4 distal (Figure 2c,d). To raise two pediculated flaps, two vertical and an additional horizontal incision in the nKT were performed (Figure 2c,d). Both pediculated flaps were fixed in their heterotopic position (Figure 2e,f) with monofilament sutures (Stoma®-medilene 6–0 blue, Storz am Mark GmbH). The oral KT, papillae, and interimplant soft tissues were completely removed to exclude an influence of these tissues on buccal healing. Before the surgery, 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, whereby at the implant sites, the implant shoulder represented the reference.

Prior (timepoint T0) and after a healing period of 3 months after this surgery (timepoint T1, Figure 3), the following clinical measurements were obtained at all test implants and all control teeth (three sites per unit: mesio-buccal, buccal, and disto-buccal) 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 pocket depth (PPD, mm)
- Bleeding on probing (BOP, %)

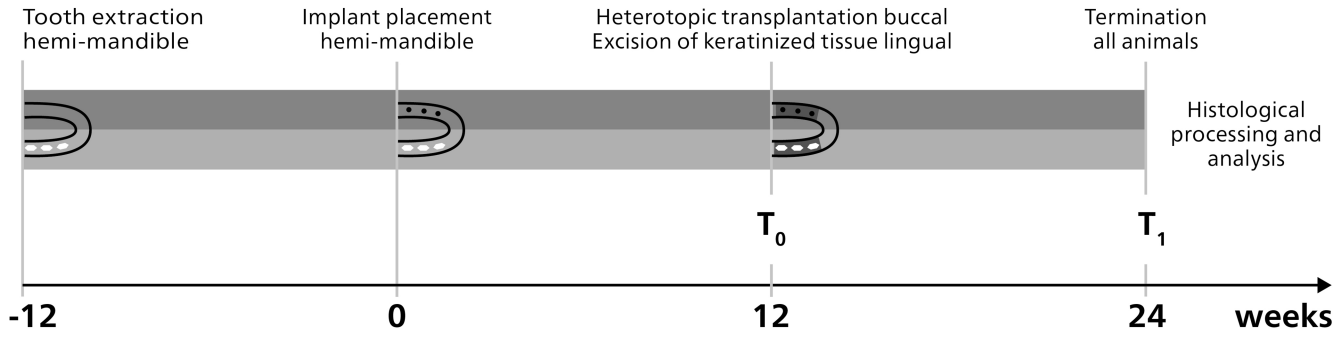

FIGURE 1 Timetable.

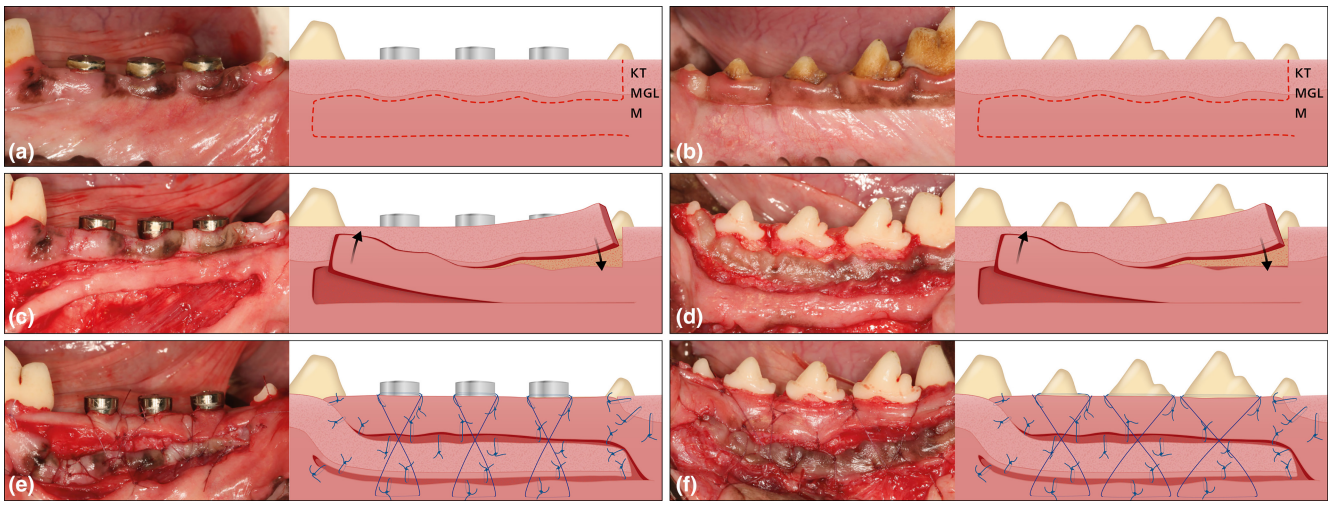

FIGURE 2 Clinical pictures and graphical illustrations in the test (a, c, e) and control group (b, d, f). Twelve weeks after implant placement (T0) in the test (a) and control group (b), after preparation of the keratinized tissue and non-keratinized mucosal graft in the test (c) and control group (d), and after heterotopic transposition in the test (e) and control group (f).

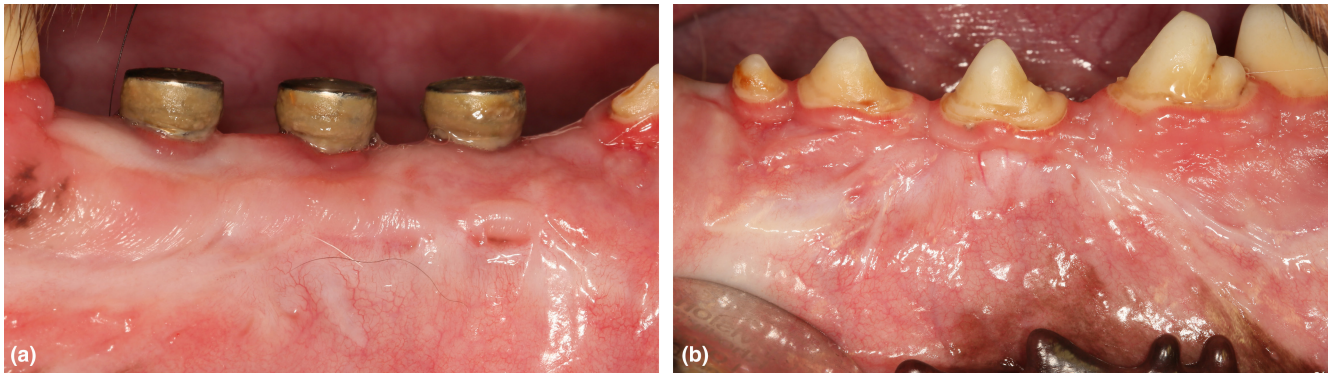

FIGURE 3 Clinical pictures illustrating the healing after heterotopic transposition (T1) in the test group (a) and control group (b).

- Probing attachment level (PAL, mm)
- Plaque and calculus (%)

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) for 4 days. Antibiotics (Cefovecin 8 mg/kg/SC, Convenia®, Zoetis) were administered for 7 days. The animals were controlled daily for health status using standardized scoring sheets. During the first two postoperative weeks, the teeth and implants were disinfected three times a week using gauzes soaked in chlorhexidine (0.12%, Perio-Aid Tratamiento®, Dentaid). Subsequently, a toothbrush with a chlorhexidine gel (0.2%; Chlorhexidine Bioadhesive Gel, Lacer) was used three times weekly for continued plaque control. The dogs were fed a soft-pellet diet for 1 week.

Following a healing period of 3 months, 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).

3.4 | Histological procedures

The soft and hard tissues were obtained and subsequently fixed in 10% formaldehyde. All 8 hemi-mandibles were dehydrated in an ascending series of ethanol, infiltrated, and embedded in methyl methacrylate (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). From every tooth root and every dental implant, three ground sections were produced. Thereafter, 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). 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) connected to a light microscope (Axio Imager M2; Carl Zeiss). To investigate elastic fibers, the remaining ground sections from four implant and four tooth sites were randomly chosen for a microtome procedure. Each section was trimmed as small as possible. Prior to the sectioning with the microtome, the implants were carefully removed from the ground sections with the use of a gentle heating and cooling procedure for the titanium implant. At tooth sites, only a very thin slice of the tooth root adjacent to the soft tissues was left. Thereafter, the undecalcified specimens were cut into approximately 5-µm thick sections with a microtome (Reichert-Jung). 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 fibers, resulting in blue/purple-black staining (Sheehan and Hrapchak, 1980).

3.5 | Histomorphometric analysis

The most central section of each implant or tooth root (i.e., the mesial and distal roots 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)
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 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 the sulcus (GM or MM—aJE)
3. Height of connective tissue at the teeth or implant 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—negative values representing a loss of tissue (GM—cN or MM—IS)

3.6 | Statistical analysis

Data analyses were performed using Prism v7 (GraphPad Software) and RStudio (version 4.22, 2022). RStudio: R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/). Descriptive statistics were used to calculate means, percentages, and standard deviations.

For between-group comparisons, categorical data from teeth versus implants were analyzed using Fisher's exact tests. This was done to discern differences in categorical outcomes between teeth and implants without considering the time of measurement. For within-subject comparisons, changes over time in both teeth and implants were evaluated by comparing baseline (T0) data to follow-up (T1) data using McNemar's chi-squared tests. This assessment aimed to detect significant changes in categorical outcomes from baseline to follow-up within each group.

Levene's tests were used to assess the equality of the variances of the measurements performed. To investigate the effect of time (T0, T1) and group (teeth, implants) on clinical measurements, we utilized a two-way ANOVA with two within-subject factors: "time" and "group" (implant vs. tooth), incorporating individual dogs as the random effect. To analyze between-group differences on histomorphometric measurements, repeated measures ANOVA tests with a random effect (animal) were conducted. Furthermore, a post hoc power analysis was performed on the basis of the primary outcome "Height of KT" to assess the power of our primary statistical analysis. The power analysis was conducted using an approximation based on the observed Cohen's dd and the sample size of the study. To account for multiple comparisons, we applied Holm corrections to the statistical analysis. Significance was set at $p < .05$.

4 | RESULTS

4.1 | Clinical findings

Following all surgical procedures, healing was uneventful without infections or other unusual complications. All 24 sites were available for further analysis. Clinically, the width of KT at T0 was smaller at implants compared to tooth sites (3.53 ± 0.85 mm and 4.31 ± 0.89 mm, respectively, $p < .0001$). At T1, at the soft tissue margin around teeth, a certain width of gingiva was reestablished (mean width of KT: 2.92 ± 1.83 mm), whereby at the mucosal margin around the implants, no tissue was formed, which showed clear signs of keratinization (mean width of KT: 0 mm). This was statistically significantly different between teeth and implants ($p < .0001$). Irrespectively of the group, the healing resembled, at some locations, scar-like tissues. In some cases, both at implants and teeth, the band of KT was maintained where the KT was apically transpositioned into the zone of previously nKM (Figure 3). In some cases where the KT was transpositioned apically, scar tissue was visible without obvious signs of keratinization. It may be speculated that the transpositioned flaps were partially lost during the healing.

The PPDs at tooth sites were statistically significantly reduced from T0 to T1 (2.06 ± 0.41 mm and 1.47 mm, $p = .0001$). At implant sites, the PPDs were reduced from T0 to T1 as well; this was statistically significant (2.00 ± 0.59 mm and 1.83 ± 0.81 mm, $p = .0001$). There were no statistically significant differences regarding the PPDs comparing implants and teeth at T0 ($p = .537$) but at T1 ($p = .0076$). The soft tissue reduction from T0 to T1 was higher for implants (−1.88 ± 0.81 mm) compared to tooth sites (−1.611 ± 0.45 mm, $p = .1719$). Moreover, the PAL loss was statistically significantly greater at the implant site (−1.67 ± 1.20 mm) compared to tooth sites (−1.03 ± 0.88 mm, $p = .0076$).

Plaque and calculus were clinically observed in both test and control sites. Additionally, BOP was a frequent finding at implants (T0: 52.8%, T1: 77.8%, respectively) and tooth sites (T0: 66.7%, mean T1:52.8%, respectively). The BOP increase at implants from T0 to T1 was statistically significant ($p = .02$). All clinical measurements are presented in Table 1.

4.2 | Descriptive histology

Representative histological sections are presented in Figure 4. All implants ($n = 12$) showed histologically successful osseointegration. Mild inflammation was observed in the soft tissues surrounding the teeth and implants, with biofilm and/or calculus formation.

At the implant sites, the peri-implant mucosal margin after transposition of nKM was characterized by a non-keratinized epithelium, indicating the characteristics typically encountered in the alveolar mucosa (Figures 4a–c and 5a,b). The non-keratinized zone directly at implant sites was mostly followed by a zone of KT originating from the transpositioned KT (Figures 4d and 5b). On the other hand, at the tooth sites, a regeneration of the gingival unit was evident at the location of the transposition of nKM, displaying the characteristics of KT (Figures 4e–g and 5c,d). Keratinization and rete peg formation were clearly visible on all buccal aspects at tooth sites. At a distance from the gingival margin, in most of the cases, the nKM transposition maintained a non-keratinizing epithelium (Figure 5d). In most of the cases, this band of non-keratinized epithelium was very small (Figure 5d) and apically followed by a keratinized band from the heterotopic transpositioned KT (Figure 5c,d).

On the microtome sections, elastic fibers were absent in the connective tissue below the keratinized epithelium (Figure 6a,b), whereas they were numerous in the connective tissue under the non-keratinized epithelium (Figure 6c,d).

TABLE 1 Clinical measurements.

	Implants Mean (SD) Median (IQR)	Teeth Mean (SD) Median (IQR)	*p*-value[a] teeth vs. implants
Width of KT (mm) T0	3.528 (0.845) 3.0 (1.0)	4.306 (0.889) 4.0 (1.0)	<.0001
Width of KT (mm) T1	0.000 (0.000) 0.0 (0.0)	2.944 (1.866) 2.0 (1.0)	<.0001
p-value[a] T0 vs. T1	<0.0001	<0.0001	
PPD (mm) T0	2.000 (0.586) 2.0 (0.0)	2.056 (0.410) 2.0 (0.0)	.1182
PPD (mm) T1	1.833 (0.811) 2.0 (1.25)	1.472 (0.609) 1.0 (1.0)	.0173
p-value[a] T0 vs. T1	0.0005	0.0001	
Soft tissue reduction (mm) T0–T1	−1.883 (0.811) −2.0 (1.0)	−1.611 (0.445) −2.0 (1.0)	.1719
Probing attachment level (mm) T0–T1	−1.667 (1.195) −2 (1.0)	−1.028 (0.878) −1 (0.5)	.0076
	% of positive sites	**% of positive sites**	
Plaque T0	100.0	100.0	>.9999
Plaque T1	100.00	83.3	.0249
p-value[a] T0 vs. T1	>0.9999	0.0143	
Calculus T0	100.0	100.0	>.9999
Calculus T1	97.2	69.4	.0030
p-value[a] T0 vs. T1	0.3173	0.0009	
BoP T0	52.8	66.7	.3366
BoP T1	77.8	52.8	.0466
p-value[a] T0 vs. T1	0.0201	0.2253	

Abbreviations: BoP, bleeding on probing; CI, confidence interval; KT, keratinized tissue; mm, millimeter; PAL, probing attachment level; PPD, probing pocked depth; SD, standard deviation; T0, before excision of KT; T1, after excision of KT.

[a]Two-way ANOVA with two within-subject factors.

4.3 | Histomorphometry

All 24 sites (12 teeth and 12 implants) were available for histomorphometrical analysis. Since the central section of every root was chosen, the number of analyzed sections was 24 for all teeth. For implants, only the most central section was chosen, and consequently, the number of analyzed sections was 12. The histomorphometric results are presented in Table 2 and Figure 7. The height of the KT was 2.06 ± 1.58 mm at the tooth and 0 mm at the implant sites ($p = .0066$). Thus, no KT directly at the mucosal margin was detected at any implant site. The height of the JE, including the sulcus depth, was similar for teeth and implants (1.19 ± 0.36 mm vs. 1.17 ± 0.42 mm, respectively; $p = .7176$). Furthermore, the vertical distance of soft connective tissue was statistically significantly higher at tooth (1.78 ± 0.51 mm) compared to implant sites (1.32 ± 0.29 mm, $p = .0144$). Moreover, the height of supracrestal tissues was larger for teeth (2.98 ± 0.48 mm) than that for implants (2.50 ± 0.67 mm; $p = .0083$). The vertical soft tissue loss (mucosal or gingival recession) was similar for tooth and implant sites (1.12 ± 0.48 mm vs. 1.03 ± 0.67 mm, respectively, $p = .1531$).

5 | DISCUSSION

The present investigation was performed to study the characteristics of oral mucosal tissues following their heterotopic transpositioning around dental implants and teeth.

The findings revealed that at both teeth and implants, the KT positioned into nKT may result in the formation of KT and confirm the results obtained for teeth in studies by Karring and co-workers published more than 50 years ago (Karring et al., 1971, 1975). Most importantly, the present study has, for the first time, provided histologic evidence for the maintenance of KT characteristics originating from peri-implant locations positioned into nKT. On the other hand, the heterotopic transpositioning of nKT into a region previously occupied by KT failed to result in the formation of KT at implant sites, while at tooth sites, the formation of a new band of gingiva was consistently observed after heterotopic transpositioning. Since keratinization of the mucosa may only be expected in the presence of connective tissue originating from KT, it can be assumed that the induction of KT adjacent to teeth must have come from cells originating from the periodontal ligament (Karring et al., 1975). Heterotopic

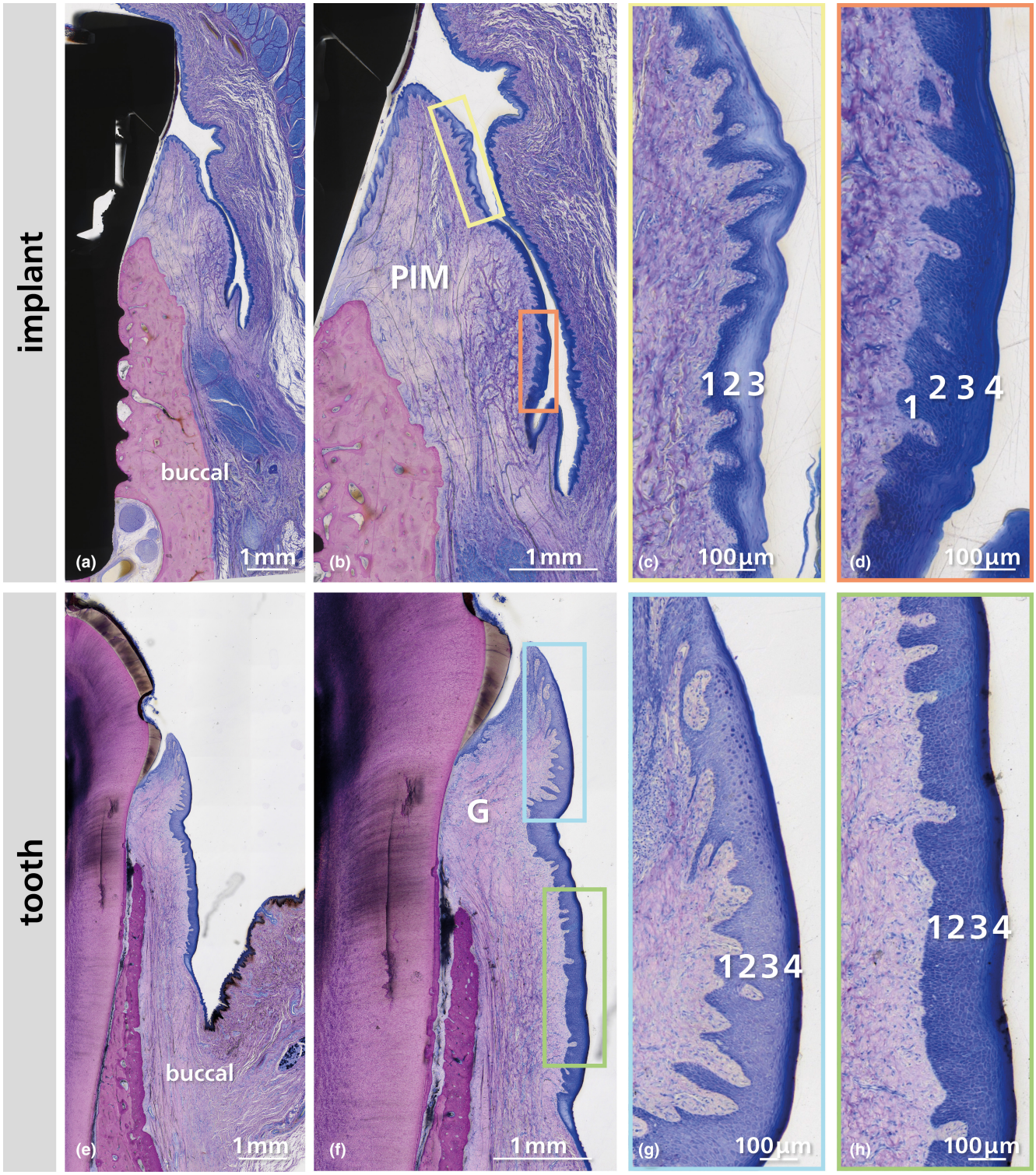

FIGURE 4 Representative overview of histological sections of the test group (a, b) and control group (e, f). Higher magnification of the buccal epithelium near the peri-implant mucosal (b) and gingival sulcus (f) without keratinization at the implant site (c) and with keratinization at the tooth site (g). Higher magnification of the mucosa with keratinization at the implant (d) and tooth site (h) after heterotopic transposition of keratinized tissue. Staining: toluidine blue/McNeal + basic fuchsin. G, gingiva; PIM, peri-implant mucosa.

transpositioning of nKT to a region adjacent to implants did not result in the formation of KT. Therefore, our results lend further support to the need for soft tissue derived from an anatomical site with KT to induce the formation of a keratinized epithelium.

Interestingly, a recently published experimental study (Liñares et al., 2022) showed that a CTG derived from a location with KT did not initiate KT formation when grafted below a coronally positioned flap without KT at implant sites. The coronally positioned flap with

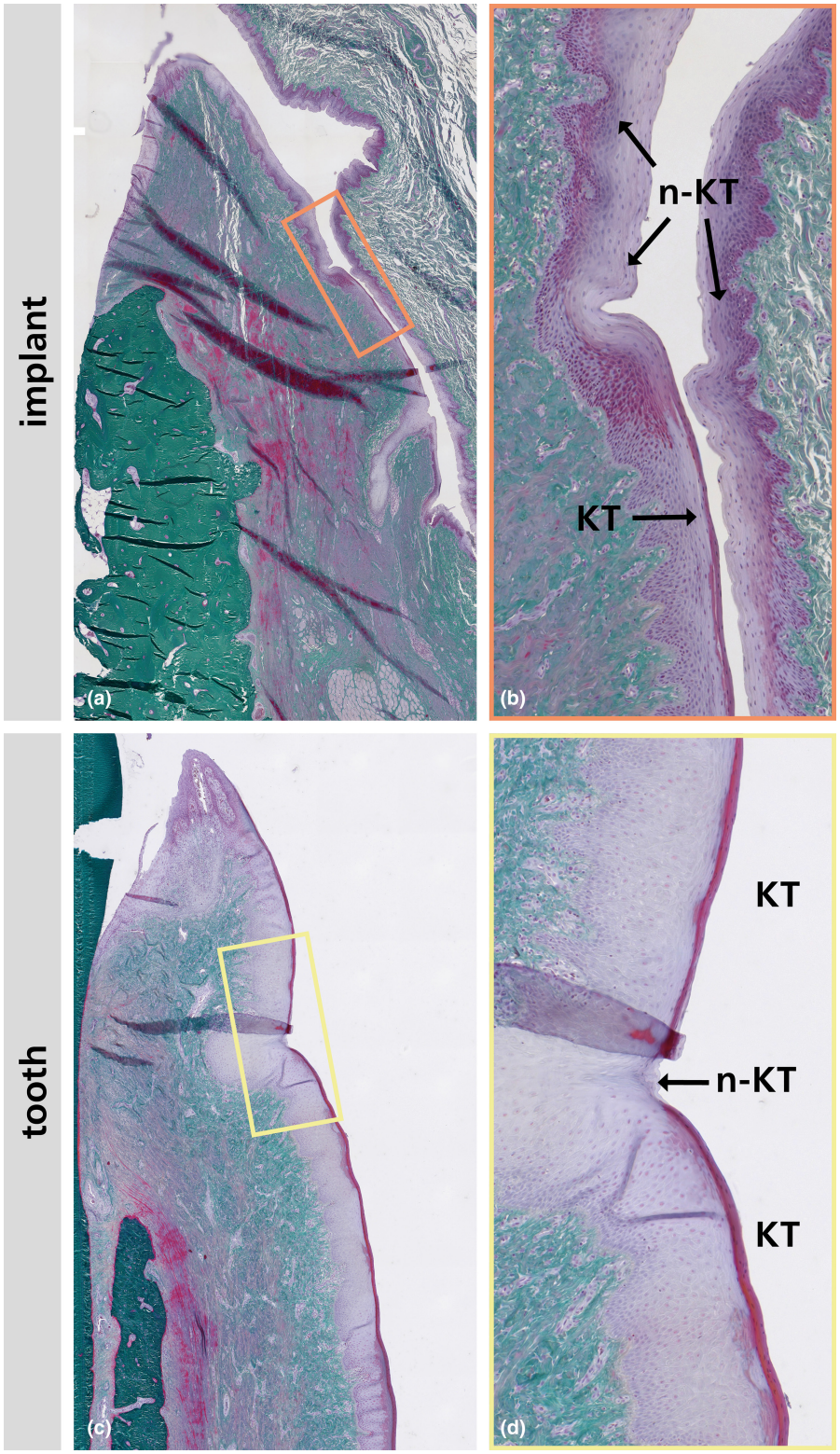

FIGURE 5 Microtome sections of the test group (a, b) and control group (c, d). Staining: resorcin-fuchsin and Goldner. KT, keratinized tissue; N-KT, non-keratinized tissue.

its connective tissue with a nonkeratinized epithelium may have influenced the healing pattern. Additionally, it may be speculated that only an exposed graft originating from a location with KT may induce the formation of KT. Contrarily, it had been previously established that the differentiation pattern of epithelialization is conditioned by the connective tissue originating from KT applied to the recipient site (Bernimoulin & Schroeder, 1980). In that respect, CTGs have been used clinically to increase the width of KT (Edel, 1974; Stähli et al., 2022).

The analysis of the clinical parameters before and after the heterotopic transposition indicated a complete loss of the KT at implants concomitant with a reduction of the supracrestal soft tissue compartment. On the other hand, at tooth sites, the gingiva reformed with a KT compartment that was reduced in size. This, in turn,

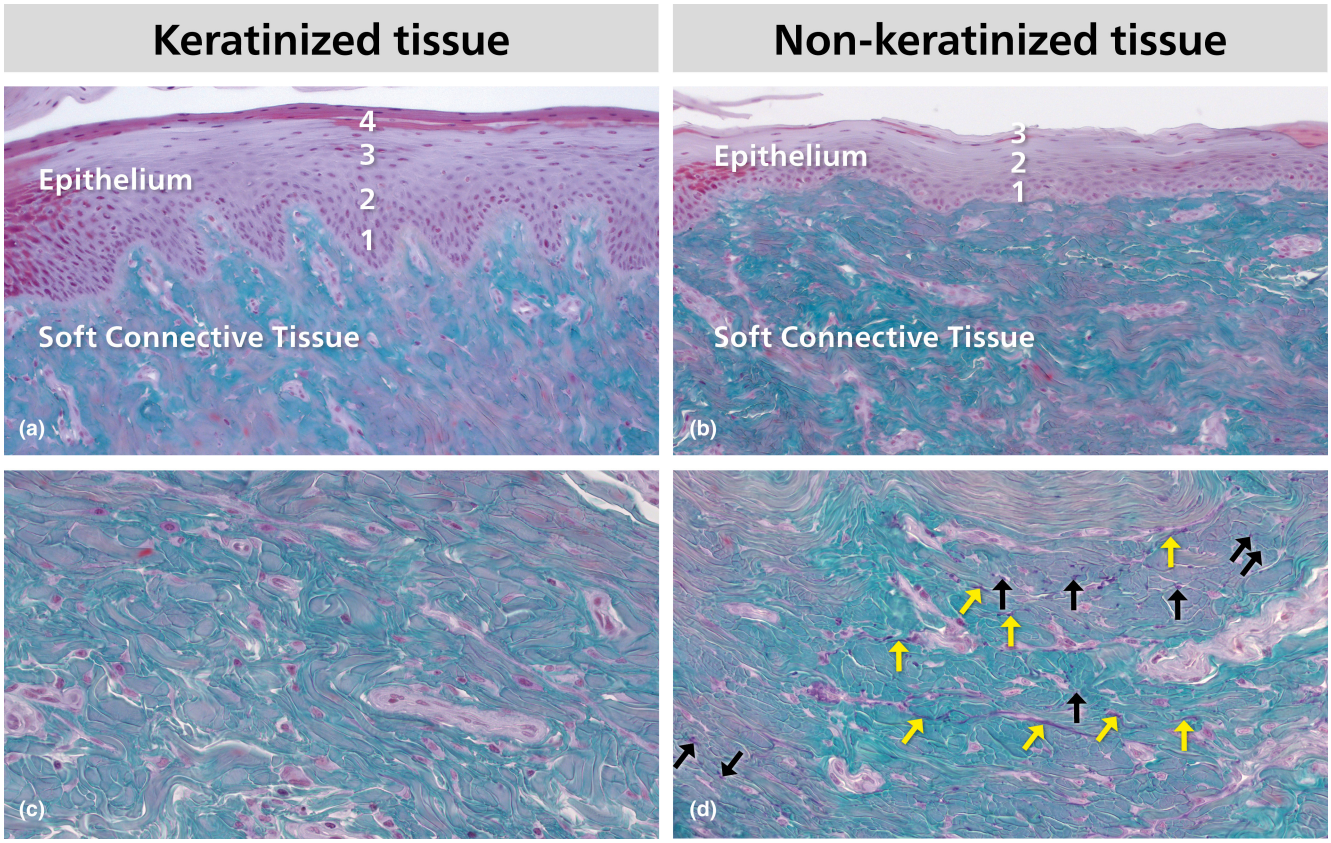

FIGURE 6 Microtome sections showing a keratinized epithelium (a) and a soft connective tissue underneath without any elastic fibers (c); and a non-keratinized epithelium (b) with a soft connective tissue with elastic fibers (d). Arrowheads point to elastic fibers in the connective tissue. Magnification 20× (a, b), 40× (c, d). Staining: resorcin-fuchsin and Goldner. Staining: resorcin-fuchsin and Goldner.

TABLE 2 Histomorphometrical results.

	Teeth Mean (mm) SD	Implants Mean (mm) SD	*p*-value[a]
Height of KT (mm)	2.061 1.584	0.000 0.000	0.0066*
Height of JE + sulcus (mm)	1.193 0.359	1.173 0.417	0.7176
Height of soft connective tissue (mm)	1.784 0.510	1.324 0.294	0.0144*
Height of supracrestal tissues (mm)	2.978 0.483	2.497 0.455	0.0083*
Vertical soft tissue loss (mm)	1.122 0.482	1.032 0.671	0.1531

Abbreviations: CI, confidence interval; JE, junctional epithelium; KT, keratinized tissue; mm, millimeter; SD, standard deviation;

[a]Repeated measures ANOVA. Post hoc power analysis for height of the KT = 0.492.

*Statistically significant.

indicates that the establishment of the connective tissue seal was jeopardized at implant sites, most likely due to the fact that a physiological sulcus could not be developed at implant sites. Moreover, probing attachment level loss was significantly more pronounced at implant sites than at tooth sites. Obviously, the establishment of a functional connective tissue seal at implants sites represents a challenge and is unpredictable in the absence of KT. Thus, it may be assumed that KT around implants may only be generated by soft tissues originating from a keratinized area.

The clinical results were basically confirmed by the histologic analysis. However, it has to be kept in mind that the histological analysis only represents a status after a well-defined healing

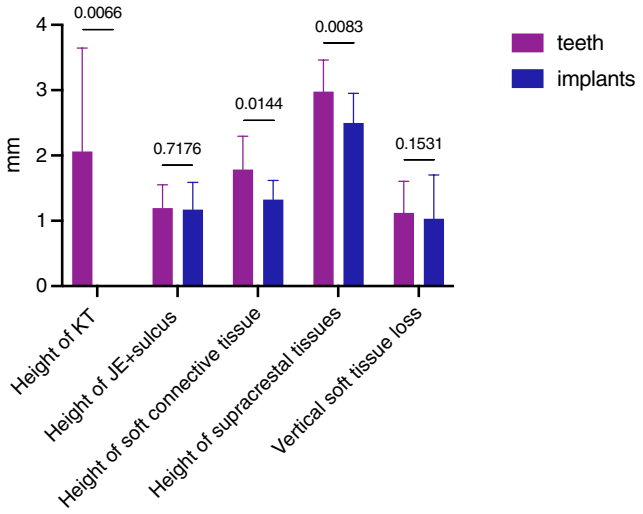

FIGURE 7 Histomorphometrical measurements. JE, junctional epithelium; KT, keratinized tissue; mm, millimeter.

period, and no preoperative values are available to assess the dynamics of the tissue changes encountered during heterotopic transposition. Nevertheless, the data also suggest that the KT at implants has been completely removed. Moreover, the height of the reestablished supracrestal tissues is significantly lower at implant sites than at tooth sites. This is also reflected in the vertical dimension of the soft connective tissue component. It thus appears that the establishment of the soft tissue seal around implants in the absence of KT is more difficult to achieve than at tooth sites.

As opposed to the studies of similar nature performed over 50 years ago (Karring et al., 1971, 1975), in which the cynomolgus monkey (*Macaca fascicularis*) had been treated, the present study used a beagle dog model. This model offers an improved possibility of controlling oral hygiene. Furthermore, the beagle dog model has extensively been used in periodontal etiology, pathogenesis, and treatment studies. Their microbiome (Syed et al., 1981) and the pathogenesis of periodontal diseases as a result of the bacterial challenge (Lindhe et al., 1975) are well established. In the present study, all the procedures were performed without losing any of the animals or implants. In general, wound healing was without any adverse events, pointing to optimal animal care during the entire study period.

The post-hoc power analysis of our primary statistical analysis has revealed an estimated power of approximately 0.492. This observation underscores the potential limitations stemming from our study's sample size, suggesting that smaller effects may not have been effectively detected. As we advance our research, we acknowledge the delicate balance between enhancing statistical power and minimizing the utilization of animals. While future studies with larger sample sizes are advised to better explore and validate our findings, ethical considerations should keep guiding our scientific practices.

Within the limits of the present study, the present findings indicate that: (a) the KT transpositioned into nKT remained unaltered in its morphological characteristics; and (b) the transpositioned nKT was accompanied by the formation of KT at the tooth but not at implant sites.

AUTHOR CONTRIBUTIONS

A.S. and N.P.L. conceived the ideas; J.-C.I. and F.M. performed the clinical procedures; J.-C.I., A.R., and D.D.B. collected the data; C.A.R. performed the statistical analysis; C.A.R., J.-C.I., D.D.B., and A.St. interpreted the data; and J.-C.I., N.P.L., and A.S. led the writing. All authors read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

The authors express their special thanks to the staff at the Veterinary Faculty Lugo, University of Santiago de Compostela, Spain for excellent handling of the animals, Silvia Owusu (laboratory technician, Robert K. Schenk Laboratory of Oral Histology at the School of Dental Medicine, University of Bern) for the competent help during histological processing. For the financial support, the ITI Foundation, Basel, Switzerland is acknowledged. Open access funding provided by Universitat Bern.

FUNDING INFORMATION

This study was funded by a grant from the ITI Foundation (ITI Grant number 1365_2018).

CONFLICT OF INTEREST STATEMENT

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

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials. Further data that support the findings of this study are available from the corresponding author [J.-C.I.], 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

REFERENCES

Bernimoulin, J. P., & Schroeder, H. E. (1980). Changes in the differentiation pattern of oral mucosal epithelium following heterotopic

connective tissue transplantation in man. *Pathology, Research and Practice*, *166*(2–3), 290–312. https://doi.org/10.1016/s0344-0338(80)80136-1

Caffesse, R. G., Karring, T., & Nasjleti, C. E. (1977). Keratinizing potential of sulcular epithelium. *Journal of Periodontology*, *48*(3), 140–146. https://doi.org/10.1902/jop.1977.48.3.140

Caffesse, R. G., Nasjleti, C. E., & Castelli, W. A. (1979). The role of sulcular environment in controlling epithelial keratinization. *Journal of Periodontology*, *50*(1), 1–6. https://doi.org/10.1902/jop.1979.50.1.1

Edel, A. (1974). Clinical evaluation of free connective tissue grafts used to increase the width of keratinised gingiva. *Journal of Clinical Periodontology*, *1*(4), 185–196. https://doi.org/10.1111/j.1600-051x.1974.tb01257.x

Giannobile, W. V., Jung, R. E., & Schwarz, F. (2018). Evidence-based knowledge on the aesthetics and maintenance of peri-implant soft tissues: Osteology Foundation Consensus Report Part 1-Effects of soft tissue augmentation procedures on the maintenance of peri-implant soft tissue health. *Clinical Oral Implants Research*, *29*(Suppl 15), 7–10. https://doi.org/10.1111/clr.13110

Karring, T., Lang, N. P., & Löe, H. (1975). The role of gingival connective tissue in determining epithelial differentiation. *Journal of Periodontal Research*, *10*(1), 1–11. https://doi.org/10.1111/j.1600-0765.1975.tb00001.x

Karring, T., Østergaard, E., & Löe, H. (1971). Conservation of tissue specificity after heterotopic transplantation of gingiva and alveolar mucosa. *Journal of Periodontal Research*, *6*(4), 282–293. https://doi.org/10.1111/j.1600-0765.1971.tb00619.x

Lin, G. H., Chan, H. L., & Wang, H. L. (2013). The significance of keratinized mucosa on implant health: A systematic review. *Journal of Periodontology*, *84*(12), 1755–1767. https://doi.org/10.1902/jop.2013.120688

Liñares, A., Rubinos, A., Puñal, A., Muñoz, F., & Blanco, J. (2022). Regeneration of keratinized tissue around teeth and implants following coronal repositioning of alveolar mucosa with and without a connective tissue graft: An experimental study in dogs: Fifty years after Karring's landmark study: Fifty years after Karring's 71 landmark study. *Journal of Clinical Periodontology*, *49*, 1133–1144. https://doi.org/10.1111/jcpe.13673

Lindhe, J., Hamp, S. E., & Löe, H. (1975). Plaque induced periodontal disease in beagle dogs. A 4-year clinical, roentgenographical and histometrical study. *Journal of Periodontal Research*, *10*(5), 243–255. https://doi.org/10.1111/j.1600-0765.1975.tb00031.x

Monje, A., & Blasi, G. (2019). Significance of keratinized mucosa/gingiva on peri-implant and adjacent periodontal conditions in erratic maintenance compliers. *Journal of Periodontology*, *90*(5), 445–453. https://doi.org/10.1002/jper.18-0471

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M. T., Baker, M., Browne, W. J., Clark, A., Cuthill, I. C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S. T., Howells, D. W., Karp, N. A., Lazic, S. E., Lidster, K., MacCallum, C. J., Macleod, M., … Würbel, H. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biology*, *18*(7), e3000410. https://doi.org/10.1371/journal.pbio.3000410

Ramanauskaite, A., Schwarz, F., & Sader, R. (2022). Influence of width of keratinized tissue on the prevalence of peri-implant diseases: A systematic review and meta-analysis. *Clinical Oral Implants Research*, *33*(Suppl 23), 8–31. https://doi.org/10.1111/clr.13766

Roccuzzo, M., Grasso, G., & Dalmasso, P. (2016). Keratinized mucosa around implants in partially edentulous posterior mandible: 10-year results of a prospective comparative study. *Clinical Oral Implants Research*, *27*(4), 491–496. https://doi.org/10.1111/clr.12563

Sheehan, D., & Hrapchak, B. (1980). *Theory, and practice of histotechnology* (2nd ed., pp. 194–195). Battelle Press.

Sculean, A., Gruber, R., & Bosshardt, D. D. (2014). Soft tissue wound healing around teeth and dental implants. *Journal of Clinical Periodontology*, *41*(Suppl 15), S6–S22. https://doi.org/10.1111/jcpe.12206

Stähli, A., Duong, H. Y., Imber, J. C., Roccuzzo, A., Salvi, G. E., Katsaros, C., Ramseier, C. A., & Sculean, A. (2022). Recession coverage using the modified coronally advanced tunnel and connective tissue graft with or without enamel matrix derivative: 5-year results of a randomised clinical trial. *Clinical Oral Investigations*, *27*, 105–113. https://doi.org/10.1007/s00784-022-04691-8

Syed, S. A., Svanberg, M., & Svanberg, G. (1981). The predominant cultivable dental plaque flora of beagle dogs with periodontitis. *Journal of Clinical Periodontology*, *8*(1), 45–56. https://doi.org/10.1111/j.1600-051x.1981.tb02023.x

Tavelli, L., Barootchi, S., Avila-Ortiz, G., Urban, I. A., Giannobile, W. V., & Wang, H. L. (2021). Peri-implant soft tissue phenotype modification and its impact on peri-implant health: A systematic review and network meta-analysis. *Journal of Periodontology*, *92*(1), 21–44. https://doi.org/10.1002/jper.19-0716

Thoma, D. S., Naenni, N., Figuero, E., Hämmerle, C. H. F., Schwarz, F., Jung, R. E., & Sanz-Sánchez, I. (2018). Effects of soft tissue augmentation procedures on peri-implant health or disease: A systematic review and meta-analysis. *Clinical Oral Implants Research*, *29*(Suppl 15), 32–49. https://doi.org/10.1111/clr.13114

Warrer, K., Buser, D., Lang, N. P., & Karring, T. (1995). Plaque-induced peri-implantitis in the presence or absence of keratinized mucosa. An experimental study in monkeys. *Clinical Oral Implants Research*, *6*(3), 131–138. https://doi.org/10.1034/j.1600-0501.1995.060301.x

Wennström, J. (1983). Regeneration of gingiva following surgical excision. A clinical study. *Journal of Clinical Periodontology*, *10*(3), 287–297. https://doi.org/10.1111/j.1600-051x.1983.tb01277.x

Wennström, J., & Lindhe, J. (1983a). Plaque-induced gingival inflammation in the absence of attached gingiva in dogs. *Journal of Clinical Periodontology*, *10*(3), 266–276. https://doi.org/10.1111/j.1600-051x.1983.tb01275.x

Wennström, J., & Lindhe, J. (1983b). Role of attached gingiva for maintenance of periodontal health. Healing following excisional and grafting procedures in dogs. *Journal of Clinical Periodontology*, *10*(2), 206–221. https://doi.org/10.1111/j.1600-051x.1983.tb02208.x

Wennström, J. L., Bengazi, F., & Lekholm, U. (1994). The influence of the masticatory mucosa on the peri-implant soft tissue condition. *Clinical Oral Implants Research*, *5*(1), 1–8. https://doi.org/10.1034/j.1600-0501.1994.050101.x

Wennström, J. L., & Derks, J. (2012). Is there a need for keratinized mucosa around implants to maintain health and tissue stability? *Clinical Oral Implants Research*, *23*(Suppl 6), 136–146. https://doi.org/10.1111/j.1600-0501.2012.02540.x

How to cite this article: Imber, J.-C., Roccuzzo, A., Stähli, A., Bosshardt, D. D., Muñoz, F., Ramseier, C. A., Lang, N. P., & Sculean, A. (2023). Clinical and histologic evaluation of heterotopic mucosa transpositioning at teeth and dental implants. *Clinical Oral Implants Research*, *00*, 1–11. https://doi.org/10.1111/clr.14184