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ORIGINAL ARTICLE

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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 71 landmark study

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Abstract

Aim: To compare clinical and histological keratinized tissue formation around teeth and implants following coronal repositioning of alveolar mucosa with or without a connective tissue graft (CTG).

Materials and Methods: In nine beagle dogs, the third and fourth premolars (P3 and P4) were extracted from one side of the maxilla. Three months after the tooth extraction, a full-thickness buccal flap was raised and two implants were placed in those healed areas. On the contra-lateral side, a buccal flap was also raised at the P3 and P4 areas. Before suturing, the dogs were randomly assigned to three study groups (control, non-keratinized tissue [NKT], and non-keratinized tissue CTG [NKT-CTG]). In the control group, the buccal flaps were re-positioned around the teeth (P3 and P4) on one side, and implants on the other side, presenting an adequate band of keratinized tissue (KT). For the NKT and NKT-CTG groups, this buccal KT was then excised. In the NKT group, the buccal flap without KT (alveolar mucosa) was repositioned around the teeth and implants. In the NKT-CTG group, a CTG taken from the excised KT was sutured to the buccal alveolar mucosa and then both were repositioned around the teeth and implants. The clinical height of the KT was measured at baseline and at 1, 2, and 3 months of healing. The animals were sacrificed at 3 months, at which point the KT height was measured histologically.

Results: The control group presented normal healing with a band of KT surrounding the teeth and implants. In the NKT and NKT-CTG groups, a new KT band approximately 2 mm in height (measured clinically and histologically) spontaneously formed around all teeth, regardless of whether a CTG had been placed. In the NKT implant group, no new KT was observed (clinically or histologically). Around the implants in the NKT-CTG group, a small amount of KT was formed in just two of the six implants.

Conclusions: After surgical excision of KT, spontaneous KT is formed around teeth but not around implants, regardless of the placement of a CTG.

KEYWORDS
connective tissue graft, implants, keratinized tissue, soft tissue healing, teeth

Clinical Relevance

Scientific rationale for study: Granulation tissue derived from the periodontal ligament is key for spontaneous new gingiva formation after KT excision. Since, this is not present around implants, the rational of this study is to analyze soft tissue healing after excision of KT at teeth and implants in a preclinical model.

Principal findings: After KT surgical excision at teeth and implants, new gingiva is formed at teeth but not at implants.

Practical implications: Soft tissue healing at implants with no KT is impaired with respect to teeth. Mucogingival procedures aiming KT increase at implants, are more challenging than at teeth.

1 | INTRODUCTION

In a landmark study, Karring et al. (1971) showed that spontaneous gingival regeneration could occur around teeth. In their experiment, the authors placed a non-keratinized alveolar mucosa pedicle in close contact with teeth, resulting in a new zone of keratinized tissue (KT), contiguous to the teeth, which formed spontaneously after 4 months of healing. The new gingival area was produced by connective tissue and was covered with keratinized epithelium. The development of this newly formed gingiva around teeth depends on the nature and origin of the granulation tissue, which is established during the post-surgical healing period. The connective tissue of this new gingiva develops from the connective tissue of the supra-alveolar area and periodontal ligament membrane. Even after full denudation of the gingiva, new KT even wider than the original tissue was generated (Wennström, 1983; Wennström & Lindhe, 1983). Experimental models have shown that, after periosteal exposure and denudation of alveolar bone, granulation tissue originates from the residual periosteal connective tissue, periodontal ligament, bone marrow spaces, and adjacent gingiva, thereby forming new attached gingiva (Staffileno et al., 1966; Karring, Cumming, et al., 1975; Kon et al., 1978).

Connective tissue plays an important role in facilitating and directing the phenotypic expression of the epithelium (Karring, Lang, & Löe, 1975; Ouhayoun et al., 1988). It has been hypothesized that transplanted sub-epithelial connective tissue from a keratinized mucosa into a non-keratinized area could induce keratinization. However, our understanding of how a band of KT around implants can be keratinized or augmented is limited. Moreover, the periodontal ligament, one of the sources of keratinization, is not present in dental implants.

Therefore, this study aimed to (1) compare the degree of spontaneous KT formation around teeth and implants in the absence of KT and (2) assess the possibility of increasing the band of KT around implants through the use of a transmucosal sub-epithelial connective tissue graft (CTG) in the absence of KT.

2 | MATERIALS AND METHODS

2.1 | Animals

This experimental model used nine healthy adult (3 years of age) female beagle dogs weighing 12–15 kg (Isoquimen, Barcelona, Spain) after obtaining approval from the regional ethics committee (Ministry of Rural Affairs, Government of Galicia, Lugo, Spain; approval number: 01/18/LU-001). The experimental site was the maxilla. The dogs' accommodation, daily monitoring, and experimental procedures were conducted in the Animal Experimentation Service of the Rof Codina Foundation (Cebiovet, Lugo, Spain) by veterinarians trained and accredited in laboratory animal science. During the experiment, the dogs were kept in a group in a kennel with an interior surface (15 m^2) and an exterior surface (20 m^2), with natural light, fresh air, and a controlled temperature of 18 ± 2°C in the interior area. The dogs were fed with pelleted dog food twice daily using individual bowls and a supply of water. All experiments were conducted in accordance with Spanish and European Union regulations regarding the care and use of research animals and following the latest guidelines for experimental surgical research related to implantology (Dard, 2012). During the preparation of the manuscript, the ARRIVE recommendations (Kilkenny et al., 2010) were taken into account.

2.2 | Study design and sample size

This study was designed as a randomized controlled experiment with one control group and two experimental groups for comparison of

three observation periods involving implants on one side of the mouth and teeth on the other.

Due to the limited number of pre-clinical studies on dogs with a comparable design and the primary outcome, no specific power analysis or sample calculation was performed. A recently comparable study (Thoma et al., 2020) used five dogs.

This study used nine dogs for the analysis, assigned randomly to control and experimental groups using a randomized computer-generated list. The allocation was concealed in sealed envelopes until the start of the procedure.

2.3 | Surgical procedures and study groups

All surgical procedures were performed under sterile conditions and general anaesthesia induced by intravenous propofol (3–5 mg/kg; Propovet, Abbott). Prior to the surgery, the dogs were sedated with intramuscular medetomidine (20 μg/kg; Domtor, Esteve), and pain was controlled with morphine (0.4 mg/kg; Morfina Braun 2%, B. Braun Medical) in combination with intramuscular meloxicam (0.2 mg/kg; Metacam, Boehringer) during the surgery and 5 days after surgery.

2.3.1 | Tooth extraction

At the end of the quarantine period, all dogs underwent extraction of the third and fourth premolars (P3 and P4) from a randomly selected hemimaxilla. The teeth were hemisected and carefully extracted with elevators and forceps in a flapless approach. To prevent infection, intravenous cefazolin (20 mg/kg; Kurgan, Normon, Madrid, Spain) and subcutaneous cefovecin (8 mg/kg SID; Convenia, Zoetis, Madrid, Spain) were administered intra-operatively. No soft tissue or bone grafting procedures were performed, and the ridges healed spontaneously.

2.3.2 | Implant placement and soft tissue procedures (study groups)

Three months after the tooth extraction, a full-thickness mucoperiosteal flap was raised in the extraction region of the maxilla of each dog to instal two implants (for a total of 18 implants in the 9 dogs). Two tissue-level standard implants (Straumann® AG, Basel, Switzerland), each with a 2.8-mm high polished neck and an intra-osseous part (3.3 mm in diameter and 8 mm in length), were placed in the

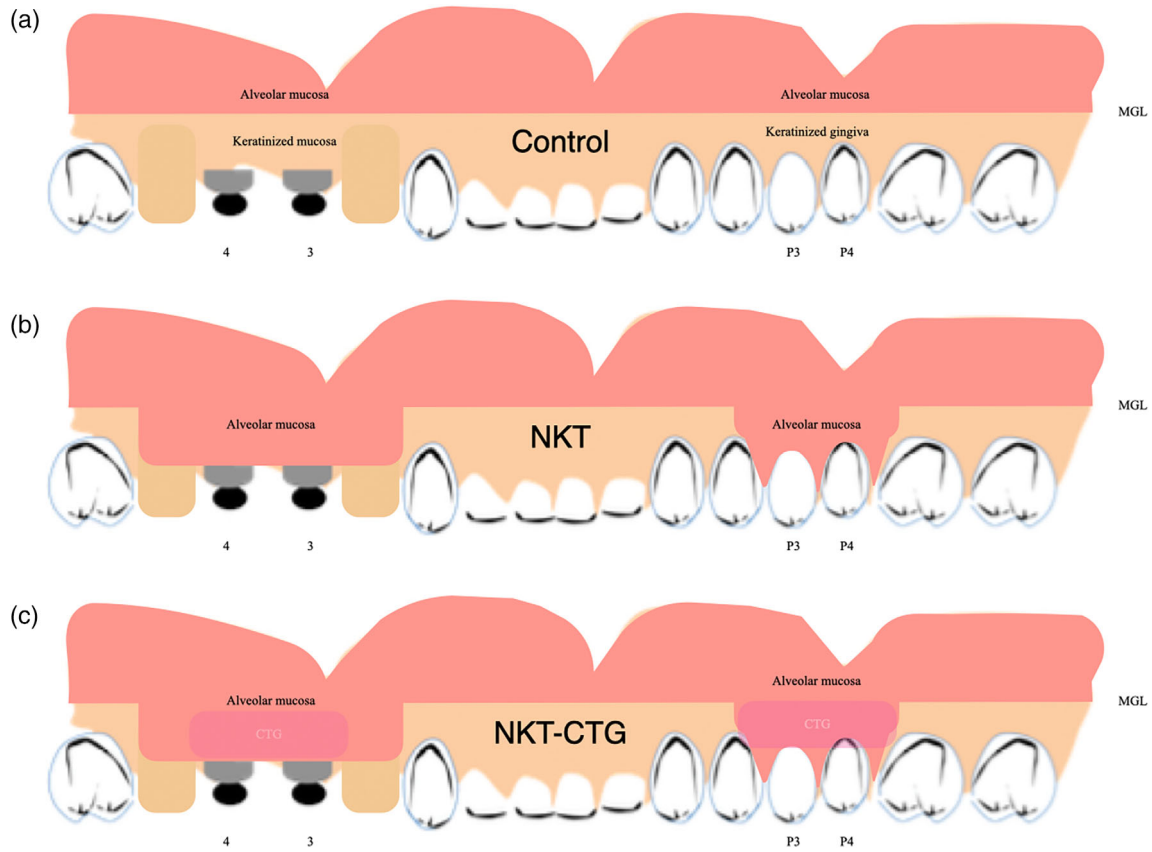

FIGURE 1 The three study groups: (a) control group, teeth and implants showing the presence of keratinized tissue (KT) on both sides; (b) non-KT [NKT] group, teeth and implants showing excised KT before suturing; (c) NKT-connective tissue graft (CTG) group, showing no KT around the implants and teeth but with a connective tissue graft under the alveolar mucosa on both sides. [Colour figure can be viewed at wileyonlinelibrary.com]

hemimaxilla where the previous tooth extraction had been performed (P3 and P4). The distance between the centres of each implant was 10 mm, and the implants were submerged to be flush with the rough/polished surface border with the bone crest. Next, closure caps were connected to the implants. On the contra-lateral hemimaxilla (tooth side), a buccal mucoperiosteal flap was raised from the first molar to the second premolar. Before suturing, each animal was randomly assigned to one of the three study groups (three dogs per study group, for six teeth and six implants per group): control, non-keratinized tissue (NKT), and non-keratinized tissue CTG (NKT-CTG) (Figure 1).

In the control group, the flaps were re-positioned around the teeth and implants at the cemento-enamel junction (CEJ) and implant shoulder (IS), respectively, in a non-submerged approach. Thus, all teeth and implants presented a buccal band of KT of at least 2 mm.

In the NKT group, all KTs were removed from the buccal flap on the teeth and implant sides. The buccal alveolar mucosa was sutured around the teeth and implants in a non-submerged manner. Therefore, there was no KT around the teeth (third and fourth premolar) or around the two implants.

In the NKT-CTG group, the same procedure was performed as in the NKT group; however, the removed KT was de-epithelialized, leaving a free CTG. This graft was then sutured to the alveolar mucosa flap, and both the graft and flap were sutured around the teeth and implants. Therefore, the NKT-CTG group had their alveolar mucosa sutured around the teeth and implants, with a buccal CTG transmucosally submerged (Figure 2).

All groups had non-submerged healing with resorbable sutures (Coated VICRYL RAPIDE™, Ethicon, US, LLC).

The dogs were allowed to heal for 3 months, during which plaque control was applied with a chlorhexidine spray 2–3 times a week. After the 3 months, the animals were sacrificed using an overdose of intravenous pentobarbital (40–60 mg/kg; Dolethal, Vetoquinol) after being sedated.

2.4 | Histological preparation

Immediately after sacrifice, the maxillae were removed and immersed in buffered formalin for 2 weeks. Tissue blocks containing the implants/teeth and the surrounding soft and hard tissues were dissected and processed for ground sectioning according to the method described by Donath and Breuner (1982). From each tissue block, one buccopalatal section was prepared and reduced to a thickness of approximately 40 μm using a grinding machine (Exakt Apparatebau, Norderstedt, Germany). The slides were stained using the Levai-Laczko technique (Jenö & Géza, 1975).

2.5 | Outcome measures

The primary outcomes were the clinical and histological KT height in millimetres.

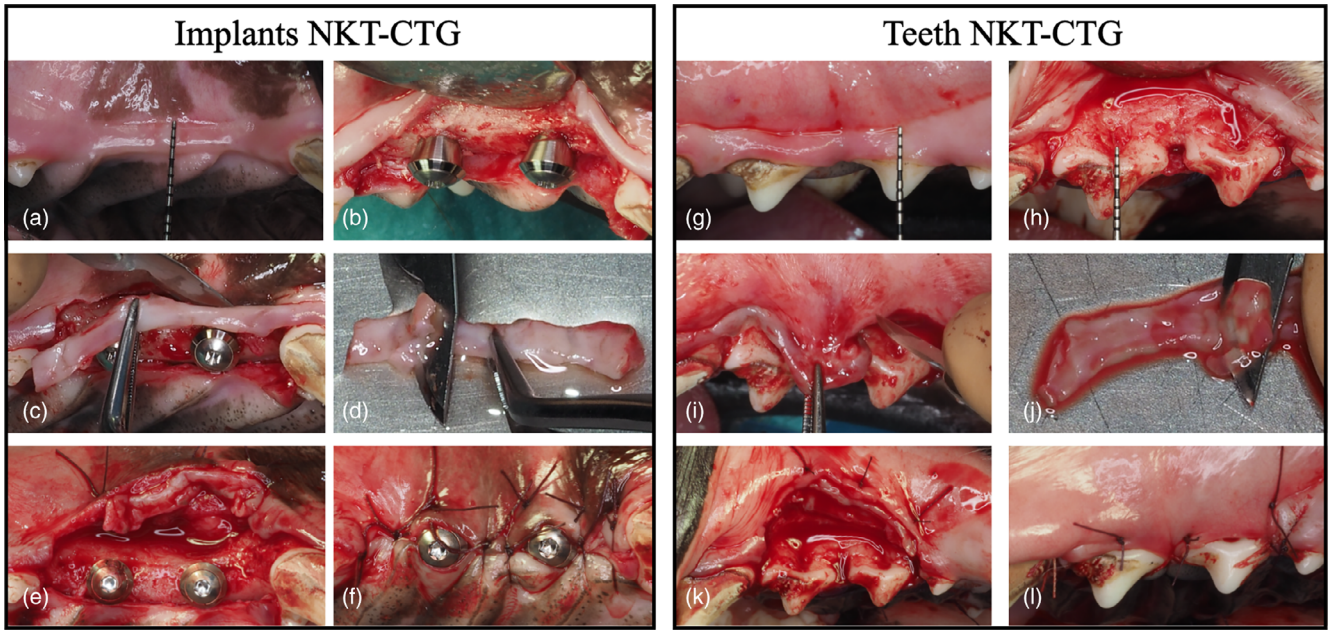

FIGURE 2 Photographs illustrating the surgical procedures for implants and teeth in the non-keratinized tissue connective tissue graft (NKT-CTG) group. (a) Baseline pre-surgical characteristics on the implant side and (g) tooth side. (b) Full-thickness flap elevation and implant installation; (h) flap elevation at the tooth side. (c) Excision of KT at the implant and (i) tooth sides. (d) De-epithelization of the graft at the implant site and (j) tooth site. (e) Suturing of the connective tissue graft to the marginal alveolar mucosa at the implant site and (k) tooth site. (f) Suturing of the alveolar mucosa plus the connective tissue graft around the implants and (l) at the buccal contra-lateral premolars 3 and 4. The NKT group underwent the same operations but without connective tissue grafts. [Colour figure can be viewed at wileyonlinelibrary.com]

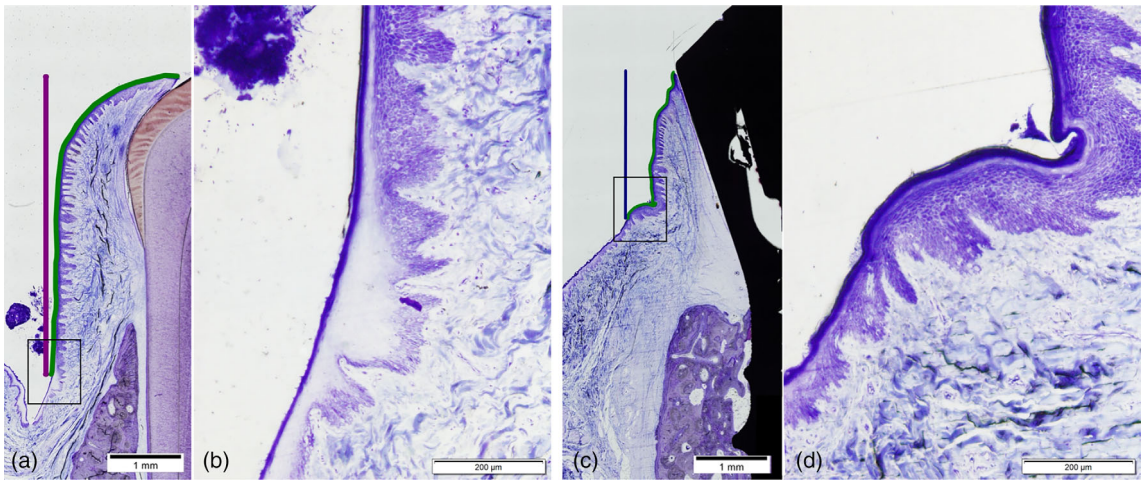

FIGURE 3 (a) Histological buccal sections showing linear (violet lines) and contour measures (green lines) of keratinized tissue height at a control tooth. (b) Higher magnification showing the transition zone between the gingiva and alveolar mucosa. (c) Control implant specimen with linear and contour measurements of keratinized height. (d) Higher magnification of (c) showing the transition zone between the peri-implant keratinized tissue and the alveolar mucosa. Levai-Laczko staining method; magnification indicated with bars. [Colour figure can be viewed at wileyonlinelibrary.com]

Clinical measures

The clinical KT height (the distance from the soft tissue margin at the teeth and implants to the mucogingival line) was measured by the same calibrated examiner (Antonio Liñares) at the mid-buccal area of the teeth and implants using a PCP UNC 15 periodontal probe to the nearest 0.5 mm (Hu-Friedy®, Chicago, IL). The mucogingival line was identified at those areas (if present), and the probe was placed parallel to the long axis of the teeth and implants, measuring the distance from the soft tissue margin to the mucogingival line. This distance was measured at baseline (after suturing) and at 1, 2, and 3 months.

The clinical secondary outcome was gingival/mucosal recession. The position of the gingival/mucosal margin was recorded after the suturing. The reference point was the IS at the implants and the CEJ at the teeth in the mid-buccal area on the distal roots. At the implant sites, the flaps were re-positioned to the level of the IS. Thus, the distance from the gingival/mucosal margin to the CEJ or IS was measured at baseline and at 3 months using the PCP UNC 15 periodontal probe.

Histometric analysis (keratinized tissue height)

Images were analysed and captured using a motorized light microscope and a digital camera connected to a PC-based image capture system (BX51, DP71, Olympus). KT was identified from the images and measured using a digitizing pad and image analysis system (cellSens, Olympus) by two calibrated investigators (Fernando Muñoz and Juan Blanco).

First, the soft tissue margin was identified at the teeth and implants. Using a 20× magnification, the apical end of the KT was assessed as a combination of epithelial layer structure (number and length of rete pegs) and underlying connective tissue. The alveolar mucosa region shows a thin NKT epithelial layer and underlying loose connective tissue. Based on these specific histological features, a transition zone around the mucogingival junction could be distinguished, and the KT height/length could be measured using two methods: (1) straight/linear parallel to the long axis of the implant/tooth as it is recorded clinically and (2) continuing the soft tissue profile (Figure 3).

Descriptive observations of the soft tissue healing around the implants and teeth were recorded.

2.6 | Statistical analysis

The quantitative variables are expressed as means and standard deviations and as medians and quartiles. The data were tested for normality by employing a Shapiro–Wilk test using SigmaPlot 12.5 software (Systat Software Inc., Chicago, IL). Comparisons between the teeth, implants, and observation periods were analysed using one-way analysis of variance. Data were analysed using a Bonferroni *t*-test for all pairwise multiple comparison procedures. $p < .05$ was considered statistically significant.

3 | RESULTS

After 3 months of healing, no dental implants were lost, and healing occurred uneventfully.

3.1 | Clinical keratinized tissue height

Table 1 displays the clinical KT heights at each time interval for each group. The control group showed a stable KT height at the teeth after suturing, with almost no reduction in height during the 3 months (−0.3 ± 0.5 mm). However, the KT height at the implants decreased each month, with a reduction of −1.1 ± 0.7 mm in 3 months.

TABLE 1 Height of clinical and histological keratinized tissue (KT) (in millimetres)

	Clinical KT height (mm)									Histological KT height (mm)			
Groups	**Initial**		**1 Month**		**2 Months**		**3 Months**		**0–3 Months**	**3 Months linear**		**3 Months contour**	
Control													
Teeth ($N = 6$)	**3.5 ± 0.5** 3.5 (3.0, 4.0)	***p* = .004**	**3.2 ± 0.4** 3.0 (3.0, 3.3)	$p = .252$	**3.3 ± 0.4** 3.0 (3.0, 3.6)	***p* < .001**	**3.2 ± 0.4** 3.0 (3.0, 3.3)	***p* < .001**	**−0.3 ± 0.5** 0 (−1.0, 0)	**3.1 ± 0.4** 3.2 (2.0, 3.9)	***p* = .004**	**3.7 ± 0.4** 3.8 (2.9, 4.7)	***p* = .001**
Implants ($N = 6$)	**2.8 ± 0.4** 3.0 (2.8, 3.0)		**2.3 ± 0.8** 2.3 (1.8, 2.8)		**1.9 ± 0.5** 2.0 (1.8, 2.1)		**1.8 ± 0.4** 2.0 (1.4, 2.0)		**−1.1 ± 0.7** −1.0 (−1.6, −0.8)	**1.7 ± 0.2** 1.8 (1.4, 2.1)		**2.0 ± 0.3** 2.0 (1.6, 2.6)	
NKT													
Teeth ($N = 6$)	**0 ± 0** 0 (0, 0)	$p = 1.000$	**1.8 ± 0.8** 1.8 (1.0, 2.3)	***p* = .001**	**2.0 ± 0.5** 2.0 (1.5, 2.3)	***p* < .001**	**2.2 ± 0.4** 2.0 (2.0, 2.3)	***p* < .001**	**2.2 ± 0.4** 2.0 (2.0, 2.3)	**1.7 ± 0.2** 1.7 (1.4, 2.1)	***p* < .001**	**2.1 ± 0.1** 2.1 (1.9, 2.4)	***p* < .001**
Implants ($N = 6$)	**0 ± 0** 0 (0, 0)		**0 ± 0** 0 (0, 0)		**0 ± 0** 0 (0, 0)		**0 ± 0** 0 (0, 0)		**0 ± 0** 0 (0, 0)	**0 ± 0** 0 (0, 0)		**0 ± 0** 0 (0, 0)	
NKT-CTG													
Teeth ($N = 6$)	**0 ± 0** 0 (0, 0)	$p = 1.000$	**2.1 ± 0.6** 2.0 (1.5, 2.6)	***p* = .002**	**2.0 ± 0** 2.0 (2.0, 2.0)	***p* < .001**	**2.0 ± 0** 2.0 (2.0, 2.0)	***p* < .001**	**2.0 ± 0** 2.0 (2.0, 2.0)	**2.1 ± 0.3** 2.1 (1.5, 2.8)	***p* < .001**	**2.3 ± 0.3** 2.3 (1.7, 3.0)	***p* < .001**
Implants ($N = 6$)	**0 ± 0** 0 (0, 0)		**0.5 ± 0.8** 0.3 (0, 0.9)		**0.3 ± 0.4** 0 (0, 0.6)		**0.4 ± 0.8** 0 (0, 0.9)		**0.4 ± 0.8** 0 (0, 0.9)	**0.2 ± 0.2** 0 (0, 0.7)		**0.3 ± 0.2** 0 (0, 0.8)	

Note: Data are presented as mean and SD, median, and inter-quartile range. *p*-Values between teeth and implants for each study group at each time point are presented; *p*-values <.05 were considered statistically significant.

Note: The significant of bold is p values *p* <.05

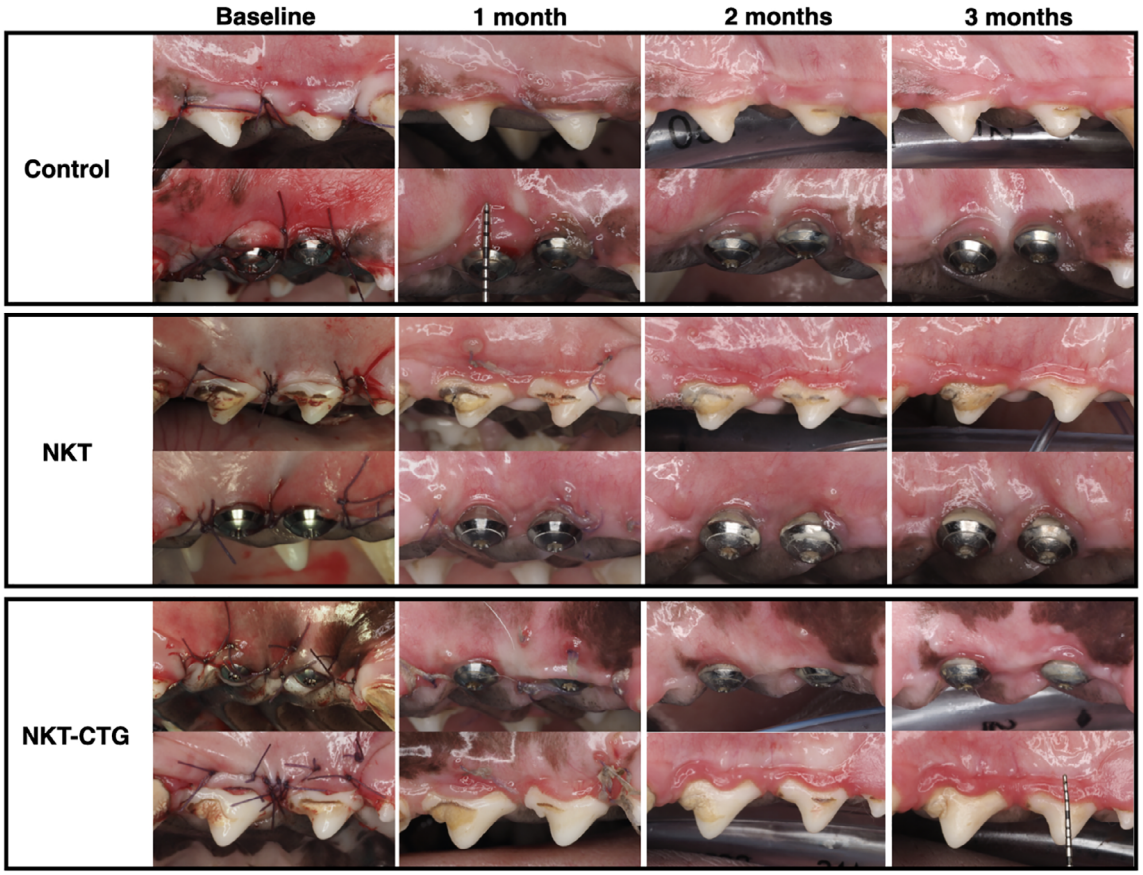

FIGURE 4 Representative images of the study groups for the teeth and implants (control, non-keratinized tissue [NKT], and NKT-connective tissue graft [CTG]), at baseline (after suturing) and at 1, 2, and 3 months on the same animal in each group. Note that new gingiva formed within 1 month in the excised groups at teeth (NKT and NKT-CTG). No new KT was observed in the NKT group and minimal KT was observed in the NKT-CTG group at implants. [Colour figure can be viewed at wileyonlinelibrary.com]

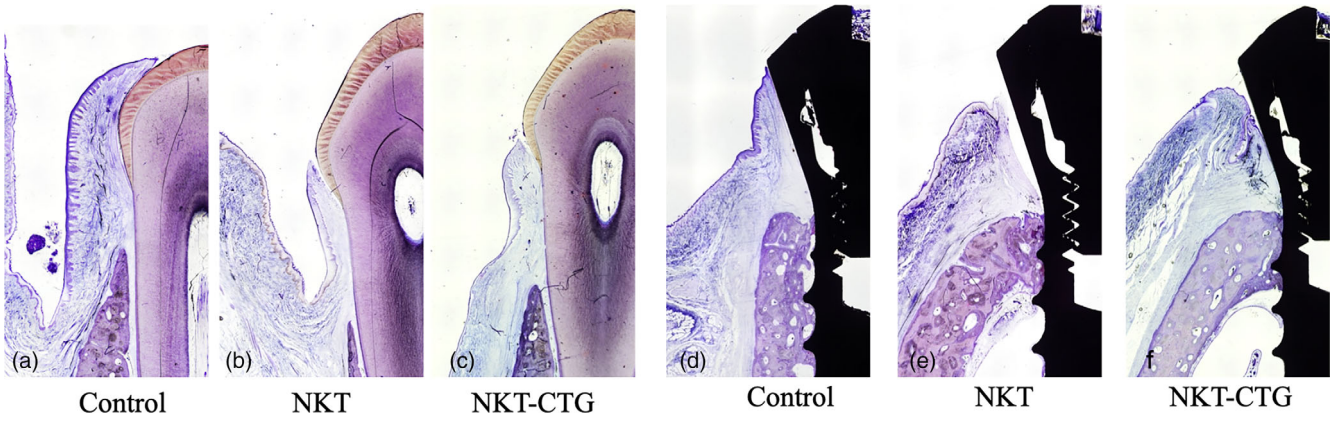

FIGURE 5 Histological buccal sections of teeth from (a) the control, (b) non-keratinized tissue (NKT), and (c) NKT-connective tissue graft (CTG) groups. Implants from (d) the control, (e) NKT, and (f) NKT-CTG groups. Levai-Laczko staining method, original magnification 4×. [Colour figure can be viewed at wileyonlinelibrary.com]

In the NKT group, new gingiva formed spontaneously after the first month around all teeth. This new KT increased in height each month, from 0 to 1.8 ± 0.8 mm during the first month and from 1.8 to 2.2 ± 0.4 mm in the 1–3-month interval. Conversely, no new KT was detected at any of the implants during the experimental period (Figures 4 and 5). Thus, the KT height in the NKT group was significantly greater at the teeth than at the implants by the first month (1.8 ± 0.8 mm vs. 0 ± 0 mm), a difference that continued during the 3 months (2.2 ± 0.4 mm vs. 0 ± 0 mm).

In the NKT-CTG group, healing around the teeth occurred in a similar manner to the NKT group. In all dogs, a new KT band (measuring 2.1 ± 0.6 mm) formed around the teeth within 1 month, and its

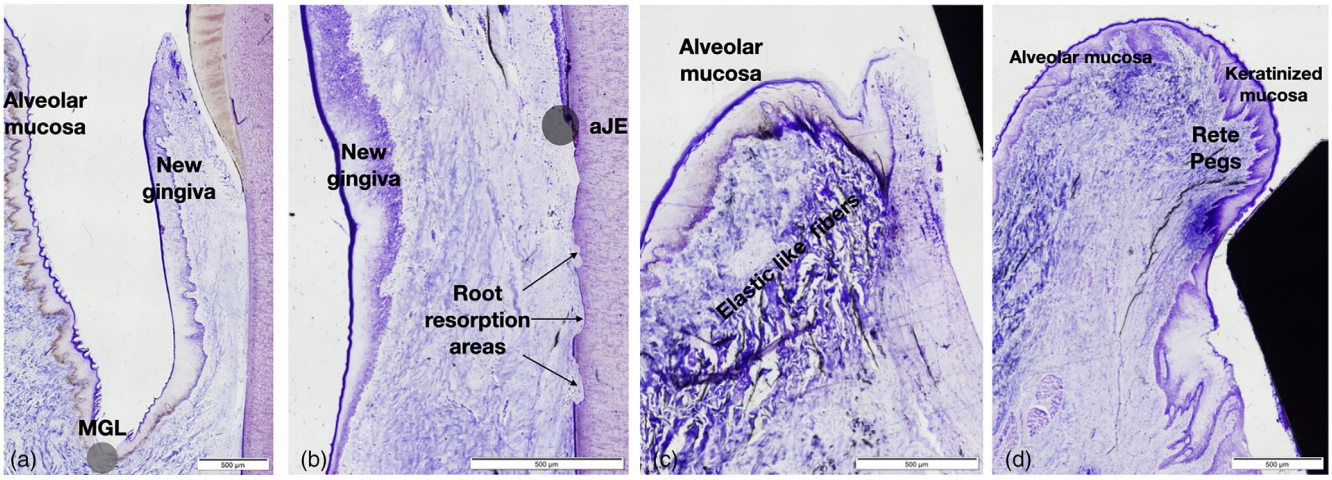

FIGURE 6 Histological buccal sections with higher magnification. (a) Tooth from the non-keratinized tissue (NKT) group showing new keratinized tissue limited apically by the mucogingival junction (MGL) and continuing with the alveolar mucosa. (b) Close-up of tooth from the NKT-CTG group showing areas of root resorption apical to the junctional epithelium (aJE), also showing the area with new keratinized tissue. (c) Implant specimen from the NKT group showing alveolar mucosa and a dense network of elastic-like fibres. (d) Implant specimen from the NKT-CTG group showing marginal mucosa with areas of alveolar mucosa (left) and keratinized epithelium (right). This turns into a barrier epithelium with the appearance of keratinized tissue and invaginations. Levai-Laczko staining method; magnification indicated with bars. [Colour figure can be viewed at wileyonlinelibrary.com]

height remained constant during the experimental period. At 3 months, the KT height was similar to that of the KT height in the teeth of the NKT group (2.2 ± 0.4 mm vs. 2.0 ± 0.0 mm, respectively), with no statistically significant differences. Moreover, the placement of the CTG at the implants did not significantly increase the KT height after 3 months (0.4 ± 0.8 mm). In fact, just two of the six implants in the NKT-CTG group presented KT tissue by the end of the study (KT height of 0.5 mm and 2 mm). In the other four implants, no KT tissue was detected (Figures 4 and 5).

3.2 | Histological keratinized tissue height

One buccopalatal section was analysed for each specimen. Thus, 32 sections (16 implants and 16 teeth) were measured. Table 1 lists the final histological KT heights, measured linearly and in contour.

In the control group, the final linear histological KT height was similar to that obtained clinically (3.1 ± 0.4 mm and 3.2 ± 0.4 mm for the histological and clinical measurements, respectively, at the teeth; 1.7 ± 0.2 mm and 1.8 ± 0.4 mm, respectively, at the implants). The KT height measured after the soft tissue contour was slightly longer than the linear KT height.

In the NKT group, no KT was found at any implants on the histological sections. The linear histological KT height at the teeth (1.7 ± 0.2 mm) was similar to the height obtained clinically.

In the NKT-CTG, the histometric outcomes revealed a similar increase in KT at the implant side as did the clinical measurements (0.2 ± 0.2 mm when measured linearly and 0.3 ± 0.2 mm when following the implant contour). Only two of the six implants presented a minor increase in histological KT at the soft tissue margin. One implant showed 0.7 mm of KT and another showed 0.99 mm. Similar to the NKT group, statistically significant differences between the teeth and implants in the NKT-CTG group could be observed during the 1–3-month interval.

3.3 | Gingival/mucosal recession

At 3 months, almost no gingival recession was observed around the teeth in the three groups. Conversely, mucosal recession was observed around the implants in the three groups. In the control group, we observed a recession of −0.75 ± 1.1 mm, similar to the NKT-CTG group (−0.9 ± 0.25). In the NKT group, greater mucosal recession (−1.75 ± 0.5 mm) was observed than in the other groups.

3.4 | Histological observations

3.4.1 | Teeth

Around the teeth, a normal soft tissue healing with buccal KT was observed. This presented stratified squamous epithelial cell layers with numerous rete pegs and underlying dense connective tissue. When this epithelium reached the gingival margin, it turned into the sulcus as a non-keratinized sulcular and junctional epithelium (Figure 5a). In terms of the buccal aspect, this keratinized epithelium continued apically to an area with narrower epithelial layers and lack of rete pegs, the mucogingival junction area, where the alveolar mucosa begins. The alveolar mucosa region showed a thin epithelial layer and underlying loose connective tissue. Although this mucosa can be described as NKT, a thin layer of keratin could be appreciated on the outer surface in most of the specimens.

In the NKT group, new gingiva with KT formed around the teeth (Figure 5b). This new gingiva presented an "immature" epithelium with normal thickness but less pronounced rete pegs. The characteristics of the alveolar mucosa and sulcular/junctional epithelium were similar to those of the control group.

The NKT-CTG group had similar histological observations (Figure 5c); however, areas of root resorption could be detected apically to the end of the junctional epithelium (Figure 6b) in five of the six specimens.

3.4.2 | Implants

In the control group, a normal KT with a dense keratin layer, as well as rete pegs, was observed. When this tissue reached the marginal mucosa, it turned into the non-keratinized barrier epithelium (Figure 5d). Similar to the teeth side, the oral epithelium continued apically to an area with narrower epithelial layers and lack of rete pegs, the mucosal junction area, where the alveolar mucosa begins. This alveolar mucosa region showed a thin epithelial layer and underlying loose connective tissue, with dark elastic-like fibres. Although this mucosa can be described as NKT, a thin layer of keratin was observed, as was the case with the teeth side.

In the NKT group, the buccal alveolar mucosa showed a thin epithelial layer and loose underlying connective tissue (Figure 5e). Below the basal lamina, we observed a dense network of elastic-like fibres typical of this type of elastic mucosa (Figure 6c). The alveolar mucosa turned at the top margin into normal non-keratinized barrier epithelium (Figures 5e and 6c).

As with the NKT group, the alveolar mucosa in the NKT-CTG group showed a thin epithelial layer and loose underlying connective tissue (Figure 5f). Interestingly, when the alveolar mucosa reached the implant, it turned into a sulcular epithelium with an "atypical" appearance. This marginal alveolar mucosa changed into an area with a thicker epithelium layer and rete pegs that continued as a barrier epithelium with the appearance of "keratinized-like tissue" (Figure 6d).

4 | DISCUSSION

The main purpose of the present study was to assess whether the potential of KT regeneration is similar for teeth and implants. To our knowledge, this is the first pre-clinical study whose main aim was to analyse spontaneous KT regeneration around teeth and implants measured clinically and histologically. The main finding was that the degree of keratinization differs between teeth and implants.

In the control group, a buccal band of normal KT was observed around teeth and implants, although the stability of this tissue was more evident around teeth. The KT was reduced around the implants by 1 mm at 3 months.

In the NKT group (no baseline KT), spontaneous regeneration of new gingiva by the first month was observed around all teeth (approximately 2 mm). Conversely, no KT was detected around the implants in the areas where KT was removed during surgery. The finding of spontaneous KT regeneration around the teeth agrees with the study by Karring et al. (1971) who showed that new KT could be formed around teeth. The authors placed a non-keratinized alveolar mucosa pedicle in close contact with teeth and observed a new zone of KT contiguous to the tooth forming spontaneously after 4 months of healing. Smith (1970) performed a similar surgical approach to the present study in mongrel dogs, with the aim of coronally advancing alveolar mucosa into a site previously occupied by gingiva and determining (by clinical and histological criteria) the effect of such transplantation on the final form of the transplants. The full thickness of the buccal gingiva over the first and second premolar teeth was removed, and the buccal alveolar mucosa was advanced coronally to occupy this position. The animals were sacrificed at 1 week, 2 weeks, 1 month, 3 months, 6 months, and 1 year. During the 1–2-week observation period, the appearance of the transplanted mucosa resembled that of the original mucosa; at 1 month, however, a soft tissue collar was observed around the teeth. When compared with the controls, this collar resembled gingiva more than mucosa. The author reported that this "new gingiva" had a mean height of 2 mm after 1 month and was stable at 3, 6, and 12 months. These findings are in complete agreement with the findings of the present study, where the KT height was 1.8 mm at 1 month in the teeth areas where all KT had been excised. Another study in humans (Wennström, 1983) showed that new gingiva formed by the first month, after a flap procedure and excision of the entire KT, similar to the present study.

Non-keratinized epithelium is formed from granulation tissue emerging from the alveolar sub-mucosa, whereas the keratinized epithelium develops from granulation tissue found in the periodontal ligament and in supra-alveolar connective tissue (Karring et al., 1971; Karring, Lang, & Löe, 1975). The periodontal ligament might therefore play an important role in the formation of new KT. It can therefore be hypothesized that the same procedures for implants might not result in healing in the same manner as with teeth, given that implants lack periodontal ligament. In fact, this investigation has shown that no new KT formed after the surgical excision of KT around the implants, in contrast to the teeth. This finding is in agreement with the study by Bengazi et al. (2014), in which no spontaneous KT was observed after removing all KT during implantation surgery.

The placement of a CTG under the alveolar mucosa flap in our study did not increase the new KT band around teeth. It therefore appears that the CTG does not induce new KT around teeth per se but that the recipient site might induce the formation. This finding contrasts with a systematic review on human studies on treating single-tooth recessions with coronally advanced flaps (CAF) or CAF + CTG (Cairo et al., 2014). A 0.7-mm increase in the KT band was reported for the grafted group compared with the flap alone. However, in this study, no KT was present at baseline around the teeth in the NKT and NKT-CTG groups, while the above-mentioned systemic review included studies that started with certain amounts of KT at baseline. Another recent systematic review on the treatment of localized and multiple tooth recessions reported a mean KT width change ranging from −0.30 to −0.53 mm for CAF and from −0.15 to −3.30 mm for CAF + CTG (Chambrone et al., 2018). Thus, the KT

around teeth might be increased with just a flap procedure and without CTG.

In five of the six specimens, areas of root resorption apical to the junctional epithelium could be observed, a finding that agrees with the 1980 study by Nyman et al. who showed that the connective tissue in contact with a root deprived of periodontal ligament might induce root resorption in a submerged environment. This situation usually does not occur in clinical practice, because mucogingival procedures on teeth are performed in a non-submerged manner, and healing usually occurs with a long junctional epithelium that protects the denuded root surface from the CTG (McGuire et al., 2016). Nevertheless, a number of case reports have shown areas of root resorption after mucogingival procedures with sub-epithelial CTG (Carnio et al., 2003; Cizza & Migues, 2010). It therefore appears that new gingival formation around teeth can be expected at 1 month after surgical excision of KT, regardless of the placement of a CTG.

At the implant sites, the placement of a CTG under the alveolar mucosa flap did not induce new KT formation. This is in agreement with another pre-clinical study (Bengazi et al., 2015), where marginal bone levels on implants were analysed after placing a CTG under alveolar mucosa in areas with no KT. After 3 months of healing, no KT was observed around the implants. Based on these observations and those of the present study, the placement of sub-epithelial CTG might not significantly induce new KT formation at implant sites without baseline KT. In fact, a prospective study on single soft tissue dehiscences on implants (Zucchelli et al., 2013) showed an improvement of just 0.5 mm in KT height following CTG plus CAF after 1 year of healing. This finding is in contrast to the results reported on teeth with the same procedure in terms of KT height increase (Cairo et al., 2014; Chambrone et al., 2018). Moreover, it has been shown that if the connective tissue is exposed, it will be covered by new epithelium that resembles that of its origin at the donor area (Karring, Lang, & Löe, 1975), which might explain why a minimal amount of KT could be detected at the border of the marginal mucosa in two cases of the present study, given that the grafts were placed in a transmucosal manner and not completely submerged, as described by Karring, Lang, and Löe (1975). In fact, the "barrier epithelium" that formed along the implant surface did not have a normal appearance and presented keratinized-like tissue with invaginations.

It can therefore be speculated that a free gingival graft might be the most predictable way to increase KT height around implants with a lack of KT (Thoma et al., 2018). A recent pre-clinical study (Thoma et al., 2020) analysed three therapeutic methods (apically re-positioned flap, free gingival graft, and a xenogeneic collagen matrix) to increase KT height around teeth and implants in areas with minimal or "normal" KT. After KT excision, spontaneous KT re-growth was greater around teeth than at implant sites. However, it should be emphasized that the surgical implant sites and the tooth sites were adjoining, as indicated by the authors, and therefore the healing of the implant sites might have been affected by the tooth sites and vice versa. Therefore, certain improvements in KT at the implant sites without grafts could be due to the proximity to the teeth. In fact, the present investigation applied a split-mouth design, with teeth on one side and implants on the other, in order to avoid that limitation. A recently published prospective clinical study (Golmayo et al., 2021) showed that the percentage of free gingival graft shrinkage was almost double at implants (62%) compared with teeth (37%) at 12 months. Again, the presence of the periodontal ligament around teeth might improve the stability of those grafts compared with implants.

The placement of the CTG reduced the amount of mucosal recession at the implant sites with no KT. The study by Bengazi et al. (2014) showed greater recession where the KT was excised compared with the control group (Bengazi et al., 2014). Moreover, Bengazi et al. (2015) reported that submerging a gingival graft in those areas devoid of KT adjacent to implants reduced the degree of recession, showing similar results to the group where the KT had been preserved (Bengazi et al., 2015), a finding that is also in agreement with the present study.

This investigation presents a number of limitations. The study used a pre-clinical model, with its inherent limitations when extrapolating the results to humans. In fact, the degree of keratinization of the gingival and oral mucosa varies between humans and dogs, oscillating between keratinized and parakeratinized depending on the presence of cell nuclei in the superficial layers (Sa et al., 2016). In humans, stippling of the attached gingiva has been associated with the inter-digitation of the rete pegs and dermal papillae, which appears not to be the case in the canine model (Kyllar et al., 2010). The microscopically detectable pits in the canine gingiva do not correspond to the structures of the rete pegs and dermal papillae. Moreover, certain areas where a non-keratinized alveolar mucosa has been reported, a thin keratin layer could be observed, which can also be related to the thickness of the histological sections. Paraffin-embedded slides might have ensured a detailed description of the tissues in thin histological sections.

Moreover, the clinical KT height could be measured with more accurate tools, such as callipers, or even with intra-oral scanners (Lee et al., 2020). Nevertheless, this study employed standard methods (such as periodontal probes) commonly used in daily practice and in previous studies. However, histological measurements could be retrieved, which showed similar results to those obtained clinically, thereby supporting those results.

5 | CONCLUSIONS

- After total surgical excision of buccal KT, spontaneous new keratinized tissue is formed around teeth but not around implants.
- The use of sub-epithelial CTGs in the absence of KT does not induce new keratinized tissue around implants and does not increase the keratinized height around teeth.

AUTHORS CONTRIBUTIONS

Antonio Liñares contributed to conceptualization, research protocol development, surgical procedures, clinical measurements, data interpretation, and manuscript writing. Ana Rubinos contributed to data collection, data processing, and statistical analysis. Ana Puñal contributed to data collection, data processing. Fernando Muñoz contributed to animal

care, histological processing, writing M&M section, and histological analysis. Juan Blanco contributed to research protocol development, surgical procedures, and histological measurements. All authors gave final approval and agree to be accountable for all aspects of the work.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

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

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