J Clin Periodontol 2011; 38: 1063–1070 doi: 10.1111/j.1600-051X.2011.01786.x

Journal of Clinical Periodontology

Soft tissue volume augmentation by the use of collagen-based matrices in the dog mandible – a histological analysis

Daniel S. Thoma[1], Christoph H. F. Hämmerle[1], David L. Cochran[2], Archie A. Jones[2], Christoph Görlach[3], Lorenz Uebersax[3], Stephanie Mathes[4], Ursula Graf-Hausner[4] and Ronald E. Jung[1]

[1]Clinic of Fixed and Removable Prosthodontics and Dental Material Science, Center of Dental Medicine, University of Zurich, Zurich, Switzerland; [2]Department of Periodontics, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; [3]Geistlich Pharma AG, Wolhusen, Switzerland; [4]Zurich University of Applied Sciences, Wädenswil, Switzerland

Thoma DS, Hämmerle CHF, Cochran DL, Jones AA, Görlach C, Uebersax L, Mathes S, Graf-Hausner U, Jung RE. Soft tissue volume augmentation by the use of collagen-based matrices in the dog mandible – a histological analysis. J Clin Peridontol 2011; 38: 1063–1070. doi: 10.1111/j.1600-051X.2011.01786.x.

Abstract

Objectives: The aim was to test, whether or not soft tissue volume augmentation with a specifically designed collagen matrix (CM), leads to ridge width gain in chronic ridge defects similar to those obtained by an autogenous subepithelial connective tissue graft (SCTG).

Material and Methods: In six dogs, soft tissue volume augmentation was performed by randomly allocating three treatment modalities to chronic ridge defects [CM, SCTG and sham-operated control (Control)]. Dogs were sacrificed at 28 ($n = 3$) and 84 days ($n = 3$). Descriptive histology and histomorphometric measurements were performed on non-decalcified sections.

Results: SCTG and CM demonstrated favourable tissue integration, and subsequent re-modelling over 84 days. The overall mean amount of newly formed soft tissue (NMT) plus bone (NB) amounted to 3.8 ± 1.2 mm (Control), 6.4 ± 0.9 mm (CM) and 7.2 ± 1.2 mm (SCTG) at 28 days. At 84 days, the mean NMT plus NB reached 2.4 ± 0.9 mm (Control), 5.6 ± 1.5 mm (CM) and 6.0 ± 2.1 mm (SCTG). Statistically significant differences were observed between CM/SCTG and Control at both time-points ($p < 0.05$).

Conclusion: Within the limits of this animal model, the CM performed similar to the SCTG, based on histomorphometric outcomes combining NB and NMT.

Key words: collagen-based matrix; soft tissue augmentation; soft tissue volume; subepithelial connective tissue graft

Accepted for publication 23 July 2011

Conflict of interest and source of funding statement

This study was funded by a grant from the Swiss Confederation's innovation promotion agency (CTI), by Geistlich Pharma AG, Wolhusen, Switzerland and by the Clinic of Fixed and Removable Prosthodontics and Dental Material Science, University of Zurich, Switzerland. Christoph Görlach and Lorenz Uebersax are employees of Geistlich Pharma AG. All other authors do not report any conflict of interest for this study.

Immediately following tooth extraction, biological processes are initiated, which lead to bone resorption and may result in localized alveolar ridge defects (Cardaropoli et al. 2003, Schropp et al. 2003, Araujo et al. 2005). Untreated localized alveolar ridge defects can cause aesthetic limitations in implant dentistry and in conventional prosthetics. Several techniques have been proposed to correct these defects using soft tissue augmentation procedures (Seibert 1983, Studer et al. 2000). Currently used techniques are associated with several disadvantages and limitations, mainly due to the second surgical site needed to harvest autogenous tissue from the patient's palate (Rossmann & Rees 1999, Del Pizzo et al. 2002, Soileau & Brannon 2006). However, literature is scarce with regard to alternative materials for soft tissue volume augmentation (Thoma et al. 2009). Any potential device intended to be used as a replacement for autogenous subepithelial connective tissue grafts, needs

to fulfil a number of criteria: (i) successful integration of the device/graft into the surrounding tissue, (ii) ability to degrade and being replaced by soft connective tissue, and (iii) three-dimensional volume stability over time as during regular function, compression and shear forces are constantly applied in the augmented area. Recently, collagen-based matrices were developed as alternatives to autogenous soft tissue grafts. It has been demonstrated, based on pre-clinical and clinical studies, that these devices may serve as alternatives to free gingival grafts, to increase the width of keratinized tissue (Sanz et al. 2009, Jung et al. 2011). However, for soft tissue volume augmentation, these collagen matrices are associated with limitations, as they may not increase the desired volume to correct ridge deficiencies. Therefore, potentially volume-stable collagen matrices with a three-dimensional network based on a modified cross-linking protocol were developed. In vitro experiments demonstrated that these prototype collagen matrices were able to comply with simulated compression and shear forces similar to those of a healing wound in the oral environment (Mathes et al. 2010). Cultivation in a specifically designed bioreactor and perfusion with human fibroblasts, resulted in a stiffening of the material. Hence, these prototype collagen matrices rendered mechanical volume stability and favourable biological attributes over time (Mathes et al. 2010). Based on these favourable outcome measures in vitro, one of the prototype collagen matrices was chosen to be tested in a pre-clinical trial. The chosen collagen matrix was characterized by a loose network and subsequently used in a clinically more relevant chronic ridge defect canine model. Volumetric measurements of augmented areas with either an autogenous subepithelial connective tissue graft (SCTG) or the collagen matrix demonstrated no significant differences rendering similar soft tissue volume augmentation, and stability over an observation period of 3 months (Thoma et al. 2010). However, histological and histomorphometric results still have to confirm the results of the volumetric measurements.

The aim of the present study was to test whether or not soft tissue volume augmentation with a newly developed collagen matrix leads to ridge width gain in alveolar ridge defects similar to those obtained by an autogenous subepithelial connective tissue graft based on histological and histomorphometrical analyses.

The hypothesis of the study was that a similar gain in ridge width will be obtained in sites treated by either an autogenous connective tissue graft or a newly developed collagen matrix in the dog mandible.

Material and Methods

Animals

Following the approval of this randomized controlled experimental study by the local ethical committee (Institutional Use and Care of Animals Committee at the University of Texas Health Science Center at San Antonio (UTHSCSA), USA), six male large hound type dogs were used. Initially, the dogs were > 2 years old, weighing between 60 and 70 kg and were kept in a purpose-designed room for experimental animals at UTHSCSA.

Surgeries

All surgical procedures and materials were described previously in detail (Thoma et al. 2010). In brief, all mandibular P2, P4 and the distal roots of M1 were extracted on both sides of the mandible. The mesial root of each M1 was root canal treated. Thereafter, the buccal plate of the extraction sites was removed and the three defect sites (anterior, middle, posterior) enlarged (Fig. 1A). Subsequently, primary wound closure was obtained. After a healing period of 2 months (Fig. 1B), full-thickness mucoperiosteal flaps were elevated. A titanium pin (Frios®, Dentsply, Konstanz, Germany) was placed on top of the bone crest in the middle of each chronic defect (anterior, middle, posterior) to serve as a reference marker for the histological processing (Fig. 1C). Three treatment modalities were randomly applied to the chronic ridge defects (Fig. 1D):

Group A: Prototype collagen matrix made of porcine collagen (CM) (Geistlich Pharma AG, Wolhusen, Switzerland)
Group B: Autogenous subepithelial connective tissue graft (SCTG)
Group C: Sham-operated site (Control)

Group A: The CM (dimensions: width 10 mm, length 12 mm, thickness 5 mm) having been soaked in sterile saline for 60 s was folded once and positioned in the pouch underneath the elevated buccal flap. The CM was secured using a hori-

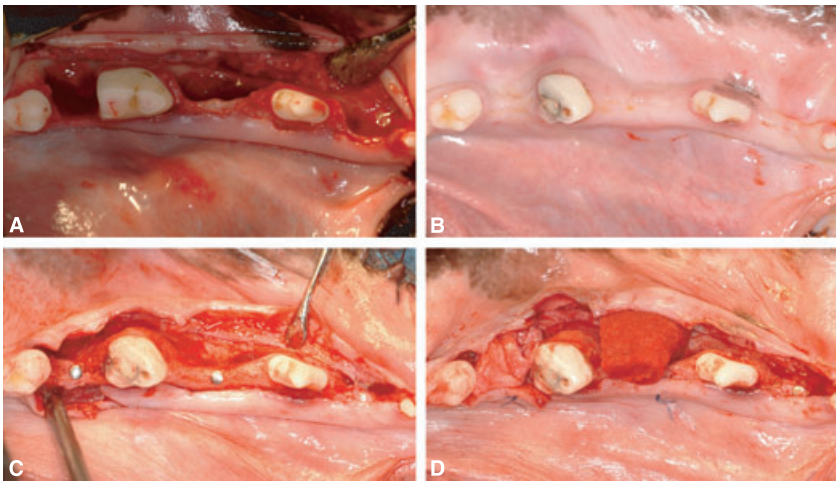

Fig. 1. Clinical pictures representing the two performed surgeries. (A) Surgery 1 with extracted distal root of M1, P3, P2. (B) Prior to surgery 2 with chronic ridge defects. (C) After flap reflection and placement of pins. (D) All three treatment modalities applied (SCTG distal; CM middle; Control mesial).

zontal mattress suture connecting it to the lingual flap (Dafilon® 5-0, B. Braun Melsungen AG, Melsungen, Germany).

Group B: The autogenous SCTG was harvested from the lateral part of the palatal vault. Fatty tissue, glandular tissue and remnants of the epithelium were removed, resulting in a SCTG with the same dimensions as the CM (width 10 mm, length 12 mm, thickness 5 mm). The SCTG was then folded once and positioned similar to the CM in the buccal pouch under the elevated flap. Immobilization of the SCTG was obtained using a horizontal mattress suture, connecting it to the lingual flap.

Group C: No further treatment was applied to the sham-operated sites.

Periosteal releasing incisions were then made to allow a tension-free primary wound closure using one horizontal mattress suture (Gore Tex 5-0®, W.L.Gore & Associates, Inc, Putzbrunn, Germany) and four to five single sutures (Gore Tex 5-0®). The dogs were maintained on a soft diet for the remainder of the study. Suture removal was performed 14 days later.

Sacrifice

At 28 days ($n = 3$ animals) and 84 days ($n = 3$) following soft tissue augmentation surgery, euthanasia was performed on all animals with an overdose of intravenous pentobarbital sodium (100 mg/kg body weight). The mandibular segments with the augmented sites were resected and immersed in a solution of formaldehyde 4% combined with $CaCl_2$ 1% for histological analysis.

Histological preparation, descriptive histology

The 36 samples (six samples per animal; two samples per group) were dehydrated in a series of graded alcohol solutions and embedded in PMMA (polymethylmetacrylate, Merck AG, Darmstadt, Germany). From each specimen, one central orofacial section through the pin, one mesial (at a distance of 2 mm from the pin) and one distal (at a distance of 2 mm from the pin) section were prepared for histological and histomorphometric assessments. The longitudinal sections of 50–60 μm thickness were obtained by a microcutting and grinding technique adapted by Donath (Donath & Breuner 1982). Thereafter, the sections were stained with Giemsa. A qualitative analysis was performed with a stereoscope (Nikon Eclipse 90i, Nikon, Egg, Switzerland), evaluating the different components and parameters (old bone, newly formed bone, non-mineralized bone, collagen matrix, vascularization of the matrix, tissue integration and inflammatory reaction) according to the standard nomenclature of the International Society for Stereology (Exner 1987).

Histometric assessment

Computer-assisted histomorphometric measurements were obtained using an automated image analysing system (Visiopharm Integrator System®, Visiopharm A/S, Hørsholm, Denmark), coupled with a video camera (Nikon Digital Sight DS-5Mc, Nikon) mounted on a light microscope (Nikon Eclipse 90i).

The augmented ridge width at four different levels was calculated (1.5, 3.5, 5.5, 7.5 mm below the crest) including measurements of native bone, newly formed bone and non-mineralized tissue (Fig. 2). Three sections were evaluated for each site and mean values calculated. In order to eliminate possible dependencies between sites the mean by treatment and dog was always used as statistical unit. These statistical units were averaged for each treatment group.

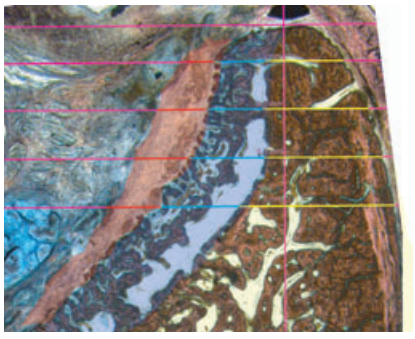

Fig. 2. Eighty-four days histological slide at 25× original magnification illustrating histomorphometric measurements. Green lines represent grid to perform measurements. Calculations were performed at 1.5, 3.5, 5.5 and 7.5 mm below bone crest level. Yellow = old bone; blue = new bone; red = regenerated soft tissue.

Statistical analysis

Data analyses were performed using a statistical software (SAS software, SAS Institute, Cary, NC, USA). The ridge width differences were assessed at 28 and 84 days for a defined region of interest. Based on the two site values by dog and treatment, the mean value was always used in the statistical description and analysis. Measured parameters were summarized in terms of means, standard deviations and confidence intervals. Explorative pre-analyses, (analyses of variance with higher models or nested designs) showed that the effects of the factors on the dog, side and site were of less importance and that the variable values within factor combinations were rather stable and uniformly distributed. Therefore, ridge width differences were analysed using analysis of variance (ANOVA) with one factor model to describe and compare the three treatment modalities. Multiple comparisons were performed with the procedure of Duncan and the paired *t*-test. The independent *t*-test was used to judge the mean differences between the two experimental periods. The level of significance was set at $p < 0.05$.

Results

Clinical findings

The dogs remained healthy, and neither systemic complications nor local intolerances at the augmented sites occurred during the entire study period. One site augmented with a SCTG demonstrated some swelling and a small wound dehiscence at the day of suture removal. This site healed without further treatment.

Descriptive histology

Control sites demonstrated limited new bone and non-mineralized tissue formation, revealing healed chronic ridge defects covered with a thin buccal flap at both time-points (Fig. 3).

At 28 days, SCTG sites revealed limited bone formation (Fig. 4). The augmented soft tissue could clearly be identified. Few inflammatory cells

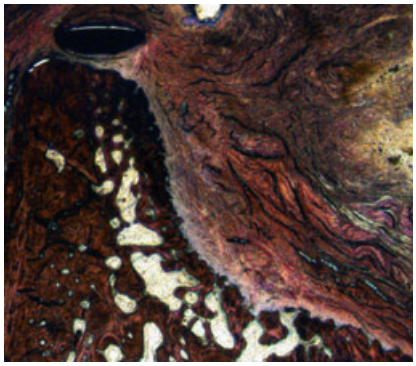

Fig. 3. Histological slide representing control site at 28 days at 25× original magnification.

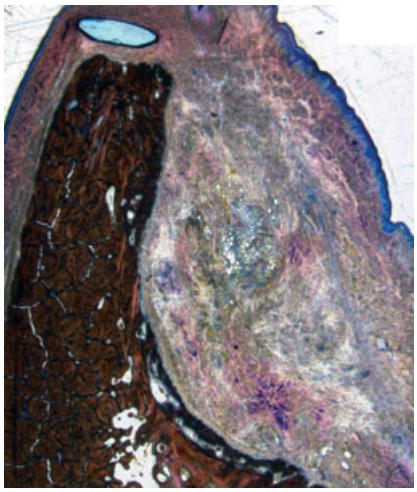

Fig. 4. Histological slide representing SCTG site at 28 days at 25× original magnification. SCTG, autogenous subepithelial connective tissue graft.

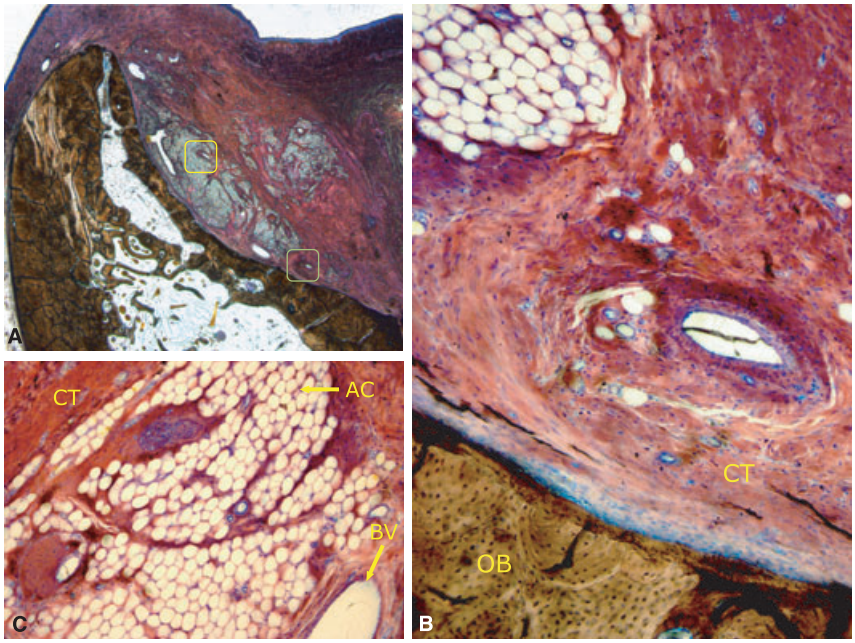

Fig. 5. Histological slide representing 84-day time-point at 25× original magnification (A) and 200× original magnification (B, C). (A) SCTG site with green rectangle indicating position of higher magnification image B and yellow rectangle for image C. (B) High magnification image of border SCTG-bone. (C) High power magnification of SCTG site with large numbers of adipocytes embedded in connective tissue. SCTG, autogenous subepithelial connective tissue graft; CT, connective tissue; BV, blood vessel; AC, adipocytes; OB, old bone.

were noted around a bulk of augmented connective tissue, including a high number of adipocytes. Vascularization was limited within the body of the augmented tissue. Few vessels were noted predominantly at the border towards the bone and towards the covering buccal flap. The border to the underlying bone was distinct and sharply marked. Integration to the covering buccal flap was intimate without a clear distinction between augmented tissue and covering flap (Fig. 4). At 84 days, the augmented SCTG looked very similar to the early time-point (Fig. 5A) with a distinct border between SCTG and the underlying bone (Fig. 5B). The number of vessels appeared to be higher and also covered the middle of the augmented tissue (Fig. 5B). The connective tissue appeared to be denser (Fig. 5C).

At 28 days, the CM appeared to have kept the original shape and demonstrated a dense network of a well-organized residual collagen matrix including large amounts of newly formed connective tissue (Fig. 6A). Vascularization was predominantly apparent at the borders of the CM. The CM body was encapsulated by several layers of newly formed collagen fibres towards the buccal flap (Fig. 6B). In addition, the CM network appeared to be re-modelled specifically at the border towards the bone (Fig. 6C). In contrast to SCTG sites, the border was not smooth, indicating re-modelling processes with initial stages of degradation and simultaneous replacement by bone, and connective tissue at this interface. The number of inflammatory cells was very limited. At 84 days, the integration of the CM into the surrounding hard and soft tissue further increased (Fig. 6D), as did the number of blood vessels, which were also located more in the center of the augmented sites (Fig. 6E). In addition, the turn-over and re-modelling processes led to an increased degradation of the collagen matrix body. Large amounts of connective tissue with a limited number of inflammatory cells were observed within the collagen matrix body demonstrating favourable tissue integration.

Histometric assessment

The calculated differences between the three sites (anterior, middle, posterior) slightly varied within the treatment groups for all parameters at all four levels. However, statistical significance was never reached ($p > 0.10$). The means and standard deviations for all sites, treatment modalities and parameters are presented in Table 1 for the primary end-points at 28 (Table 1A) and 84 days (Table 1B).

The overall mean amount of old bone varied between 6.0 mm (SCTG at 4 weeks) and 6.7 mm (SCTG, CM at 12 weeks). No statistically significant differences were observed between the sites or among the three treatment modalities at any time-point ($p > 0.05$) (data not shown).

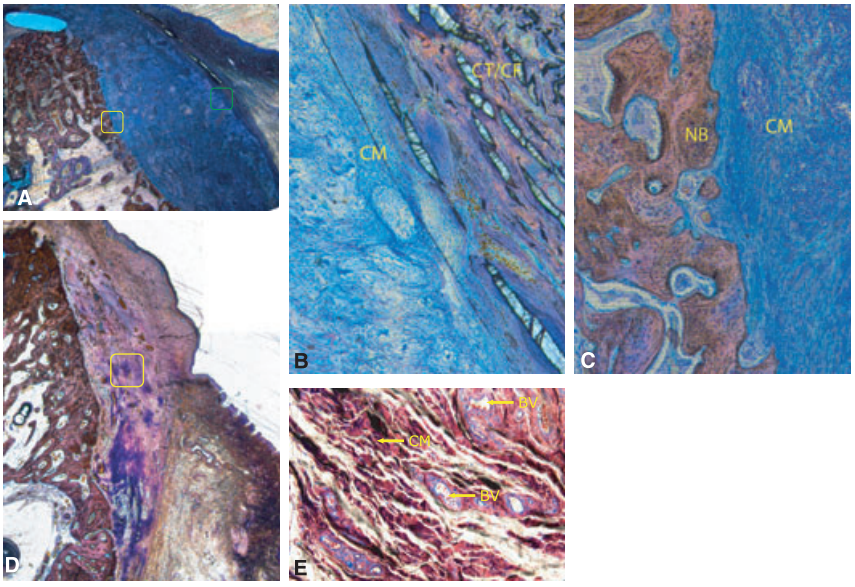

Fig. 6. Histological slides at 25× (A, D) and 200× (B, C, E) original magnification representing CM site at 28 days (A). Green rectangle indicates position of higher magnification images B and yellow rectangle for image C. Interface CM-soft tissue (B) and interface CM-newly formed bone (C). (D) CM site with yellow rectangle indicating position of high magnification image E. (E) CM site demonstrating large number of newly formed blood vessels within collagen network and connective tissue. BV, blood vessel; NB, new bone; CM, collagen matrix; CT/CF, soft tissue (covering flap).

At 28 days, the mean amount of non-mineralized tissue (NMT) increased at all levels (1.5–7.5 mm below the crest) in all groups. The mean overall NMT (mean of all four levels) reached statistically significant differences between CM and Control ($p < 0.05$) and SCTG and Control ($p < 0.01$). The mean overall newly formed bone (NB) values did not reach statistical significance ($p > 0.05$) between the three treatment modalities. The combined calculated overall mean values for NB + NMT demonstrated statistically significant differences between CM and Control ($p < 0.05$) and between SCTG and Control ($p < 0.05$).

At 84 days, the mean overall NMT showed statistically significant differences between CM and Control ($p < 0.01$), SCTG and Control ($p < 0.01$) and between CM and SCTG ($p < 0.05$). The overall mean NB values were lowest for SCTG and highest for CM reaching a borderline statistical significance ($p = 0.05$) between CM and SCTG. The overall mean values for NB + NMT showed statistically significant differences between CM and Control ($p < 0.01$) and between SCTG and Control ($p < 0.01$).

All other calculated differences among the three treatment modalities were not statistically significantly different ($p > 0.05$).

Discussion

In the present study, the experimental collagen matrix demonstrated favourable tissue integration towards the covering flap and the underlying bone. Tissue re-modelling increased from 28 to 84 days as observed by an increasing vascularization and connective tissue formation within the matrix body. The gain in hard and soft tissue achieved by the experimental collagen matrix was similar to the gold standard, the autogenous subepithelial connective tissue graft without any statistically significant differences at 28 and 84 days. These outcomes confirm earlier obtained results based on three-dimensional measurements of the volume changes between the two time-points and suggest that the CM could possibly replace the SCTG in the future (Thoma et al. 2010).

From a clinical point of view, tissue integration of both autogenous tissue (SCTG) and the newly developed cross-linked collagen matrix (CM) was favourable, as no clinically relevant complications occurred throughout the entire study period. In the past, cross-linked collagen devices exhibited an increased rate of soft tissue complications (e.g. dehiscences) when used as barrier membranes for guided bone regenerative procedures (Bornstein et al. 2007, Schwarz et al. 2008, Annen et al. 2011). In contrast, numerous preclinical studies demonstrated fast degradation of non cross-linked xenogenic native collagen membranes, by enzymatic activity of immunological cells (Rothamel et al. 2005, Schwarz et al. 2006, 2008). The reason for the uneventful integration of the present collagen matrix might possibly be explained by a modified protocol used to cross-link the matrices. It combines the stability of cross-linked collagen membranes (Brunel et al. 1996) and the favourable tissue integration of non cross-linked collagen membranes (Thoma et al. 2011). The clinical results obtained by the CM were confirmed based on the histological analysis. The CM matrix appeared to serve as a scaffold. At the earlier time-point, vascularization was predominantly eminent in lateral portions of the CM. Over time, new vessels were found throughout the entire collagen network. Similar tissue integration with an early transmembraneous angiogenesis of the collagen matrix body was observed earlier using collagen membranes (Schwarz et al. 2006), whereas an increasing number of vessels from the borders towards the center of the collagen matrix was observed in a murine study using similar prototype collagen matrices, as in the present study (unpublished data). Interestingly, in this study, the interface towards the underlying bone was characterized by re-modelling processes at both time-points. New bone formation concomitantly occurred with connective tissue formation, and the interface was not distinct at all. It is speculated that the collagen network intimately interacted with the underlying bone. This interaction probably resulted in new bone formation, part by enhancement, part by replacement of the collagen matrix. In contrast, SCTG sites appeared to be remodelled quite slowly as demonstrated by high NMT values at both time-

Table 1. Descriptive statistics with mean values (mm) and standard deviations (SD; mm) of ridge width changes (mm) by crest level (1.5 mm, 3.5 mm, 5.5 mm, 7.5 mm; overall mean) and group (CM, SCTG, Control) at 28 days (A) and at 84 days (B).

Parameter	Crest level	CM		SCTG		Control		*p*-value		
		Mean	SD	Mean	SD	Mean	SD	CM *versus* SCTG	CM *versus* Control	SCTG *versus* Control
(A)										
NMT	1.5	3.9	0.8	4.4	1.6	1.8	1.0	0.57	0.02	0.03
	3.5	4.3	1.2	5.4	1.1	2.0	0.8	0.20	0.02	< 0.01
	5.5	5.0	1.8	6.5	0.6	2.3	0.6	0.16	0.03	< 0.01
	7.5	5.3	2.3	7.3	1.0	3.0	0.4	0.16	0.10	< 0.01
NMT overall		4.6	1.5	5.9	0.9	2.2	0.7	0.19	0.03	< 0.01
NB	1.5	1.3	0.5	1.4	0.7	1.1	0.7	0.81	0.58	0.49
	3.5	1.8	0.5	1.5	0.9	1.5	0.8	0.45	0.37	0.92
	5.5	2.0	0.9	1.4	1.1	1.6	0.9	0.24	0.40	0.70
	7.5	2.2	1.0	1.4	1.2	1.2	0.9	0.23	0.09	0.76
NB overall		1.8	0.6	1.4	0.9	1.3	0.8	0.36	0.23	0.88
NMT + NB	1.5	4.9	0.7	5.9	1.7	3.0	1.6	0.35	0.07	0.05
	3.5	6.1	0.6	6.8	1.2	3.6	1.4	0.30	0.02	0.01
	5.5	7.1	1.0	7.7	0.8	4.1	1.2	0.36	0.01	< 0.01
	7.5	7.6	1.6	8.6	1.1	4.3	1.1	0.38	0.02	< 0.01
NMT + NB overall		6.4	0.9	7.2	1.2	3.8	1.2	0.31	0.01	0.01
(B)										
NMT	1.5	3.1	0.6	4.1	0.5	1.5	0.6	0.01	< 0.01	< 0.01
	3.5	4.1	0.8	5.2	0.4	1.7	0.8	0.01	< 0.01	< 0.01
	5.5	4.3	1.3	6.0	0.8	1.7	0.7	0.02	< 0.01	< 0.01
	7.5	4.6	1.5	6.4	1.0	1.6	0.7	0.04	< 0.01	< 0.01
NMT overall		4.0	0.9	5.4	0.5	1.6	0.6	0.01	< 0.01	< 0.01
NB	1.5	1.2	0.9	0.3	0.4	0.7	0.5	0.04	0.27	0.13
	3.5	1.6	1.4	0.4	0.4	0.9	0.4	0.08	0.26	0.09
	5.5	1.7	1.0	0.4	0.5	0.9	0.3	0.02	0.09	0.09
	7.5	1.7	0.9	1.1	1.5	0.7	0.2	0.40	0.03	0.60
NB overall		1.6	1.0	0.5	0.5	0.8	0.3	0.05	0.11	0.27
NMT + NB	1.5	4.3	1.1	4.4	0.6	2.2	1.0	0.90	0.01	< 0.01
	3.5	5.6	1.5	5.6	0.7	2.6	1.1	0.95	< 0.01	< 0.01
	5.5	6.1	1.8	6.4	1.2	2.7	0.9	0.66	< 0.01	< 0.01
	7.5	6.3	2.2	7.5	2.1	2.4	0.6	0.37	< 0.01	< 0.01
NMT + NB overall		5.6	1.5	6.0	2.1	2.4	0.9	0.59	< 0.01	< 0.01

CM, collagen matrix; SCTG, autogenous subepithelial connective tissue graft; Control, sham-operated site; NMT, non-mineralized tissue; NB, new bone.

points. This observation may be explained by the observed encapsulation of the SCTG towards the underlying bone. The connective tissue of the SCTG appeared to be more stable, and probably delayed processes that would lead to new bone formation. This finding, would also explain the differences between SCTG and CM with respect to NB and NMT. SCTG sites exhibited significantly more NMT, but less NB with a borderline statistical difference compared to CM. The SCTG sites were also populated with a large number of adipocytes at both time-points. Adipocytes can frequently be observed in transplanted connective tissue grafts from the palate, which is also reported in human case reports (Majzoub et al. 2001, Roman et al. 2010). In the present study, the amount of fatty tissue varied between the dogs. It depended on the size and shape of the palate, and the amount of tissue that was present, and therefore also depended on the harvesting procedure. However, no additional histomorphometric analysis was performed within the augmented tissue. Nevertheless, the number of adipocytes appeared to have reduced at the later time-point, indicating ongoing re-modelling processes over time within the transplanted autogenous tissue.

Histomorphometric analyses confirmed results from the previously published three-dimensional data on the augmented ridge defects. Based on that analysis, no statistically significant differences were found between sites augmented with SCTG or CM (Thoma et al. 2010). The present histomorphometric analyses revealed that although both SCTG and CM presented a gain in ridge width, the mechanism behind was different. The SCTG sites, which generally revealed encapsulation of the augmented sites, predominantly gained volume through re-modelling processes within the augmented soft tissue. Bone formation was limited and did rarely contribute to the gain of the ridge width. Unfortunately, results from histological analyses of transplanted connective tissue grafts with specific focus on the interface between the underlying bone and the

transplanted graft were not found in the literature. In contrast to the SCTG group, sites augmented with CM revealed that the gain in ridge width was obtained by the collagen matrix newly formed connective tissue, and new bone formed at the interface matrix-old bone. Based on highly magnified histological pictures, the collagen matrix appears to have enhanced bone formation, which was documented by the non-existence of a distinct border matrix-bone and the lack of surrounding collagen fibres (i.e. encapsulation) at this specific interface. Favourable integration of collagen devices towards the bony interface has also been reported in a rabbit study (Thoma et al. 2011). In that study, native porcine collagen membranes were placed on top of the rabbit skull. Histological analysis demonstrated excellent tissue integration with the collagen membranes in intimate contact with the underlying bone (Thoma et al. 2011).

In the past, other materials have been evaluated to serve as a replacement for autogenous soft tissue grafts (Batista et al. 2001, McGuire & Nunn 2005). In a clinical case series, an acellular dermal matrix graft (ADMG) was used to augment chronic ridge deformities. However, these grafts are very thin due to the manufacturing process and showed an increased shrinkage rate (Batista et al. 2001). Besides, histological analysis of tissue augmented with ADMG did not resemble native oral soft tissue, but had a more scar-like appearance (Wei et al. 2002). Unlike ADMG, the collagen matrix used in the present study showed favourable characteristics in terms of volume stability and tissue re-modelling. The CM kept the augmented ridge width over an observation period of 3 months. In addition, the collagen matrix served as a favourable scaffold for connective tissue formation as demonstrated by the histological analysis.

Conclusions

The results of this study indicated that the experimental collagen matrix rendered a gain in ridge width on a level non-inferior to the gold standard (subepithelial connective tissue graft) at 28 and 84 days. Although the mechanism behind the gain of ridge width was different for SCTG and CM, the outcome was similar. Collagen matrix generally gained in part through the formation of bone and soft tissue, in part through replacement of the collagen matrix, whereas SCTG gained through soft tissue only. Most interestingly, the CM appeared to enhance bone formation at the border towards the underlying ridge defect. In addition, the main body of the transplanted CM was preserved and demonstrated an increasing vascularization and formation of a connective tissue network within the collagen matrix body. Based on the outcomes of the present study and the limitations associated with this animal model, the experimental collagen matrix fulfiled a number of criteria necessary to replace the autogenous connective tissue graft in the future.

Acknowledgements

The authors acknowledge the help of Ms. Sonja Bustamante (Department of Periodontics at UTHSCSA, TX, USA) for her assistance with the animal work. We thank the Department of Laboratory Animals and Animal Surgery (UTHSCSA, TX, USA). The help of Ms. Brigitte Glaus and Ms. Sonja Hitz (University of Zurich, Switzerland) for the preparation of the histological slides is greatly acknowledged. The investigators thank Dr. Ulrich Kreuter (Consult AG, Bern, Switzerland) for providing expertize and support in the statistical analysis.

References

Annen, B. A., Ramel, C. F., Hämmerle, C. H. F. & Jung, R. E. (2011) Use of a new cross-linked collagen membrane for the treatment of peri-implant dehiscence defects: a randomised-controlled double-blinded clinical trial. *European Journal of Oral Implantology* **4**, 87–100.

Araujo, M. G., Sukekava, F., Wennstrom, J. L. & Lindhe, J. (2005) Ridge alterations following implant placement in fresh extraction sockets: an experimental study in the dog. *Journal of Clinical Periodontology* **32**, 645–652.

Batista, E. L., Jr., Batista, F. C. & Novaes, A. B., Jr. (2001) Management of soft tissue ridge deformities with acellular dermal matrix. Clinical approach and outcome after 6 months of treatment. *Journal of Periodontology* **72**, 265–273.

Bornstein, M. M., Bosshardt, D. & Buser, D. (2007) Effect of two different bioabsorbable collagen membranes on guided bone regeneration: a comparative histomorphometric study in the dog mandible. *Journal of Periodontology* **78**, 1943–1953.

Brunel, G., Piantoni, P., Elharar, F., Benque, E., Marin, P. & Zahedi, S. (1996) Regeneration of rat calvarial defects using a bioabsorbable membrane technique: influence of collagen cross-linking. *Journal of Periodontology* **67**, 1342–1348.

Cardaropoli, G., Araujo, M. & Lindhe, J. (2003) Dynamics of bone tissue formation in tooth extraction sites. An experimental study in dogs. *Journal of Clinical Periodontology* **30**, 809–818.

Del Pizzo, M., Modica, F., Bethaz, N., Priotto, P. & Romagnoli, R. (2002) The connective tissue graft: a comparative clinical evaluation of wound healing at the palatal donor site. A preliminary study. *Journal of Clinical Periodontology* **29**, 848–854.

Donath, K. & Breuner, G. (1982) A method for the study of undecalcified bones and teeth with attached soft tissues. The Sage-Schliff (sawing and grinding) technique. *Journal of Oral Pathology & Medicine* **11**, 318–326.

Exner, H. E. (1987) A model stereological nomenclature. *Acta Stereologica* **6**, 179–184.

Jung, R. E., Hurzeler, M. B., Thoma, D. S., Khraisat, A. & Hammerle, C. H. (2011) Local tolerance and efficiency of two prototype collagen matrices to increase the width of keratinized tissue. *Journal of Clinical Periodontology* **38**, 173–179.

Majzoub, Z., Landi, L., Grusovin, M. G. & Cordioli, G. (2001) Histology of connective tissue graft. A case report. *Journal of Periodontology* **72**, 1607–1615.

Mathes, S. H., Wohlwend, L., Uebersax, L., von Mentlen, R., Thoma, D. S., Jung, R. E., Gorlach, C. & Graf-Hausner, U. (2010) A bioreactor test system to mimic the biological and mechanical environment of oral soft tissues and to evaluate substitutes for connective tissue grafts. *Biotechnology and Bioengineering* **107**, 1033–1043.

McGuire, M. K. & Nunn, M. E. (2005) Evaluation of the safety and efficacy of periodontal applications of a living tissue-engineered human fibroblast-derived dermal substitute. I. Comparison to the gingival autograft: a randomized controlled pilot study. *Journal of Periodontology* **76**, 867–880.

Roman, A., Campian, R., Domsa, I., Soanca, A. & Gocan, H. (2010) Subepithelial connective tissue graft for root coverage: clinical case reports and histologic evaluation. *Romanian Journal of Morphology and Embryology* **51**, 793–797.

Rossmann, J. A. & Rees, T. D. (1999) A comparative evaluation of hemostatic agents in the management of soft tissue graft donor site bleeding. *Journal of Periodontology* **70**, 1369–1375.

Rothamel, D., Schwarz, F., Sager, M., Herten, M., Sculean, A. & Becker, J. (2005) Biodegradation of differently cross-linked collagen membranes: an experimental study in the rat. *Clinical Oral Implants Research* **16**, 369–378.

Sanz, M., Lorenzo, R., Aranda, J. J., Martin, C. & Orsini, M. (2009) Clinical evaluation of a new collagen matrix (Mucograft prototype) to enhance the width of keratinized tissue in patients with fixed prosthetic restorations: a randomized prospective clinical trial. *Journal of Clinical Periodontology* **36**, 868–876.

Schropp, L., Wenzel, A., Kostopoulos, L. & Karring, T. (2003) Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. *International Journal of Periodontics & Restorative Dentistry* **23**, 313–323.

Schwarz, F., Rothamel, D., Herten, M., Sager, M. & Becker, J. (2006) Angiogenesis pattern of native and cross-linked collagen membranes: an immunohistochemical study in the rat. *Clinical Oral Implants Research* **17**, 403–409.

Schwarz, F., Rothamel, D., Herten, M., Wustefeld, M., Sager, M., Ferrari, D. & Becker, J. (2008) Immunohistochemical characterization of guided bone regeneration at a dehiscence-type defect using different barrier membranes: an experimental study in dogs. *Clinical Oral Implants Research* **19**, 402–415.

Seibert, J. S. (1983) Reconstruction of deformed, partially edentulous ridges, using full thickness onlay grafts. Part I. Technique and wound healing. *Compendium of Continuing Education in Dentistry* **4**, 437–453.

Soileau, K. M. & Brannon, R. B. (2006) A histologic evaluation of various stages of palatal healing following subepithelial connective tissue grafting procedures: a comparison of eight cases. *Journal of Periodontology* **77**, 1267–1273.

Studer, S. P., Lehner, C., Bucher, A. & Scharer, P. (2000) Soft tissue correction of a single-tooth pontic space: a comparative quantitative volume assessment. *Journal of Prosthetic Dentistry* **83**, 402–411.

Thoma, D. S., Benic, G. I., Zwahlen, M., Hammerle, C. H. & Jung, R. E. (2009) A systematic review assessing soft tissue augmentation techniques. *Clinical Oral Implants Research* **20**(Suppl 4), 146–165.

Thoma, D. S., Jung, R. E., Schneider, D., Cochran, D. L., Ender, A., Jones, A. A., Gorlach, C., Uebersax, L., Graf-Hausner, U. & Hammerle, C. H. (2010) Soft tissue volume augmentation by the use of collagen-based matrices: a volumetric analysis. *Journal of Clinical Periodontology* **37**, 659–666.

Thoma, D. S., Subramani, K., Weber, F. E., Luder, H. U., Hämmerle, C. H. & Jung, R. E. (2011) Biodegradation, soft and hard tissue integration of various polyethylene glycol hydrogels: A histomorphometric study in rabbits. *Clinical Oral Implants Research*; doi: 10.1111/j.1600-0501.2010.02075.x.

Wei, P. C., Laurell, L., Lingen, M. W. & Geivelis, M. (2002) Acellular dermal matrix allografts to achieve increased attached gingiva. Part 2. A histological comparative study. *Journal of Periodontology* **73**, 257–265.

Address:
Daniel S. Thoma
Clinic of Fixed and Removable Prosthodontics and Dental Material Science Center of Dental Medicine, University of Zurich
Plattenstrasse 11
CH-8032 Zurich, Switzerland
E-mail: daniel.thoma@zzm.uzh.ch

Clinical relevance

Scientific rationale for the study: To date, soft tissue volume augmentation is performed using the patient's own tissue, mostly harvested from the palate. These procedures are associated with limitations mainly due to an increased morbidity at the second surgical site, and currently unknown long-term stability.

Principal findings: The experimental collagen matrix rendered a similar gain in newly formed hard and soft tissue as the gold standard, the autogenous-subepithelial connective tissue graft at 28 days and 84 days.

Practical implications: Based on recently published volumetric and the present histomorphometric outcomes, this collagen matrix may be used as an alternative to autogenous soft tissue in the future.