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Soft tissue volume augmentation by the use of collagen-based matrices: a volumetric analysis

Daniel S. Thoma[1], Ronald E. Jung[1], David Schneider[1], David L. Cochran[2], Andreas Ender[3], Archie A. Jones[2], Christoph Görlach[4], Lorenz Uebersax[4], Ursula Graf-Hausner[5] and Christoph H. F. Hämmerle[1]

[1]Clinic for Fixed and Removable Prosthodontics and Dental Material Science, University of Zurich, Zurich, Switzerland; [2]Department of Periodontics, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; [3]Department of Preventive Dentistry, Cariology, and Periodontology, Zurich, Switzerland; [4]Geistlich Biomaterials, Wolhusen, Switzerland; [5]University of Zurich for Applied Sciences, Wädenswil, Switzerland

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Abstract

Objectives: The aim was to test whether or not soft tissue augmentation with a newly developed collagen matrix (CM) leads to volume 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, sham-operated control). Impressions were taken before augmentation (baseline), at 28, and 84 days. The obtained casts were optically scanned and the images were digitally analysed. A defined region of interest was measured in all sites and the volume differences between the time points were calculated.

Results: The mean volume differences per area between baseline and 28 days amounted to a gain of 1.6 mm (CM; SD ± 0.9), 1.5 mm (SCTG; ± 0.1), and a loss of 0.003 mm (control; ± 0.3). At 84 days, the mean volume differences per area to baseline measured a gain of 1.4 mm (CM; ± 1.1), 1.4 mm (SCTG; ± 0.4), and a loss of 0.3 mm (control; ± 0.3). The differences between CM and SCTG were statistically significant compared with control at 28 and 84 days ($p < 0.001$).

Conclusion: Within the limits of this animal study, the CM may serve as a replacement for autogenous connective tissue.

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

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Soft tissue volume augmentation using autogenous grafts is a widely used procedure in a variety of disciplines in dentistry (Abrams 1980, Coslet et al. 1980, Langer & Calagna 1980, Allen et al. 1985). It is indicated in partially and fully edentulous patients to increase the soft tissue volume around dental implants predominantly for aesthetic reasons (Bichacho 1998, Price & Price 1999, Kinsel & Capoferri 2008). In addition, autogenous soft tissue grafting procedures have also been proposed to surgically correct localized alveolar defects, as pre-prosthetic site development, and as ridge preservation procedures (Seibert 1983, Studer et al. 2000, Jung et al. 2004, Prato et al. 2004, Fickl et al. 2009b).

In a recent systematic review, the dental literature was searched for techniques and materials to augment soft tissue around dental implants and teeth (Thoma et al. 2009). With respect to soft tissue volume augmentation, only a limited number of studies have been identified, rendering a weak level of evidence. It was also shown that the free gingival graft (FGG) and the subepithelial connective tissue graft (SCTG) are most often used to increase soft tissue volume in the oral cavity (Thoma et al. 2009). In a clinical study, localized alveolar ridge defects were treated using either an FGG or an SCTG or left untreated (Studer et al. 2000). The greatest amount of soft tissue volume gain was observed for the SCTG group with significant differences to the control groups (FGG, untreated sites).

Disadvantages of using autogenous tissue are mainly due to the second surgical site. The harvesting procedure

Conflict of interest and source of funding statement

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most often performed at the palate requires an additional surgical site increasing patient's morbidity (Farnoush 1978, Griffin et al. 2006). A relative discomfort including pain and numbness is experienced by the patients the days following the surgery (Del Pizzo et al. 2002, Soileau & Brannon 2006). Furthermore, anatomical and individual limitations exist. The quantity and quality of tissue that can be retrieved varies depending on the shape of the palatal vault, the patient's gender, and age. The location of the palatal vessels and nerves further limits the total amount that is available for obtaining grafts (Reiser et al. 1996, Soileau & Brannon 2006).

In order to overcome difficulties associated with autogenous tissue, research activities have focused on the development of alternative techniques and materials. In the late eighties, allogenic devices were introduced in mucogingival surgery predominantly as replacement for FGGs. Among these allogenic devices, the acellular dermal matrix graft (ADMG; Alloderm™, Life Cell Corporation, The Woodlands, TX, USA) was used most frequently. The ADMG was originally developed for covering full-thickness burn wounds (Wainwright 1995). Intra-oral applications included procedures to increase the width of keratinized tissue, to cover recession defects, and to deepen the vestibular fornix (Wei et al. 2000, Aichelmann-Reidy et al. 2001, Harris 2003, Andrade et al. 2008). The ADMG has also been used to augment localized alveolar defects (Batista et al. 2001). However, allogenic devices are very thin due to the manufacturing process. Volume augmentations normally require larger amounts of tissue. A folding procedure would therefore be necessary to gain greater volume. Because the vascularization of the acellular dermal grafts appears to be the limiting factor, a folding process could further hamper new vessel formation and lead to extensive shrinkage (Batista et al. 2001, Wei et al. 2002).

In contrast to grafts applied to increase the width of keratinized tissue, any device intended to augment soft tissue volume needs to fulfil certain demands regarding the mechanical properties. This is based on the observation that shear and compression forces are applied constantly to the grafts in the augmented sites. For that purpose, collagen-based matrices have been evaluated in pre-clinical and clinical studies in ridge preservation techniques and are currently under investigation for soft tissue volume augmentation (Jung et al. 2004, Heberer et al. 2008, Araujo et al. 2009).

In the systematic review described above (Thoma et al. 2009), a lack of a standardized and reliable technique for the measurement of soft tissue volume changes was observed. This may be one reason for the low number of studies. Methods to measure soft tissue volume changes included time-consuming and complicated procedures like the Moiré method (Studer et al. 2000) as well as simple assessments using a periodontal probe (Batista et al. 2001).

Recently, a new method has been described to measure intra-oral volume changes (Windisch et al. 2007). This technique is based on an optical system derived from operative dentistry, which allows capturing information about the shape of tooth preparations and the adjacent soft tissue contours (Mörmann & Brandestini 1996). With this system, a three-dimensional image is obtained after scanning the intra-oral anatomy with a camera (Schneider 2003). In a methodological in vitro study, several datasets of three-dimensional objects were captured and volumetric differences were measured. The tested optical three-dimensional system showed excellent accuracy and high reproducibility for measuring volume differences between specimens imitating alveolar ridge defects (Windisch et al. 2007). The same method has been used to measure dimensional changes of the ridge contour in an animal study (Fickl et al. 2009a), and to document volumetric soft tissue changes of the interdental papilla (Strebel et al. 2009) and of the buccal mucosal contour at dental implants (Schneider et al. 2010). This non-invasive method is considered to be a technique suitable to measure soft tissue volume changes.

The aim of the present study was to test whether or not soft tissue augmentation with a newly developed collagen matrix (CM) leads to volume gain in alveolar ridge defects similar to those obtained by an autogenous SCTG.

Material and Methods

Animals

The present study was designed as a randomized controlled experimental study employing six male large hound-type dogs. At the beginning, the animals were more than 2 years old, weighed between 60 and 70 kg, and were kept in a purpose-designed room for experimental animals at the University of Texas Health Science Center at San Antonio (UTHSCSA), USA. The protocol was approved by the Institutional Use and Care of Animals Committee at UTHSCSA.

Surgeries

All surgical procedures were performed under general anaesthesia and sterile conditions in an operating room using thiopental – Na solution 4% (Abott Laboratories, North Chicago, IL, USA), 0.4 ml/kg body weight as a premedication. The dogs were placed on a heating pad, intubated, anaesthesized with isoflurane 1.5–2% (Aerane, Ohmeda Carbide Inc., Liberty Corner, NJ, USA), and monitored with an EKG during the surgery.

Surgery 1 (tooth extraction)

Crevicular incisions were made around all mandibular pre-molars (P) and the first molar (M1), and buccal and lingual flaps were reflected. The teeth were sectioned in order to prevent fractures. Following extraction of all mandibular P2, P4, and the distal roots of M1, a rubber dam was placed around the mesial root of each M1 on both sides of the mandible. The pulp tissue of these mesial roots was extirpated and the root canals were filled with gutta-percha. The coronal portion of the pulp was filled using a self-curing composite material (Ketac™ Fil Plus Aplicap™, 3M Espe, Neuss, Germany). Thereafter, the buccal plate of the extraction sites was removed and the defect sites were enlarged using a round bur (Fig. 1A). After rinsing with sterile saline, primary wound closure was obtained (GoreTex 5-0®, GoreTex, L. Gore & Associates Inc., Putzbrunn, Germany). For the first 7–14 days after extraction, the dogs were fed a soft diet. After a period of 7–10 days, the animals were briefly anaesthetized (RAAK at 1 ml/30 lbs by i.v. – ketamine 50 mg/ml, xylazine 7.1 mg/ml, acepromaz 2.1 mg/ml, atropine 0.1 mg/ml), the sutures were removed, and the teeth were cleaned. At this time, polyether impressions of the mandible were taken from every animal. Master casts out of dental stone were obtained (GC Fujirock® type 4,

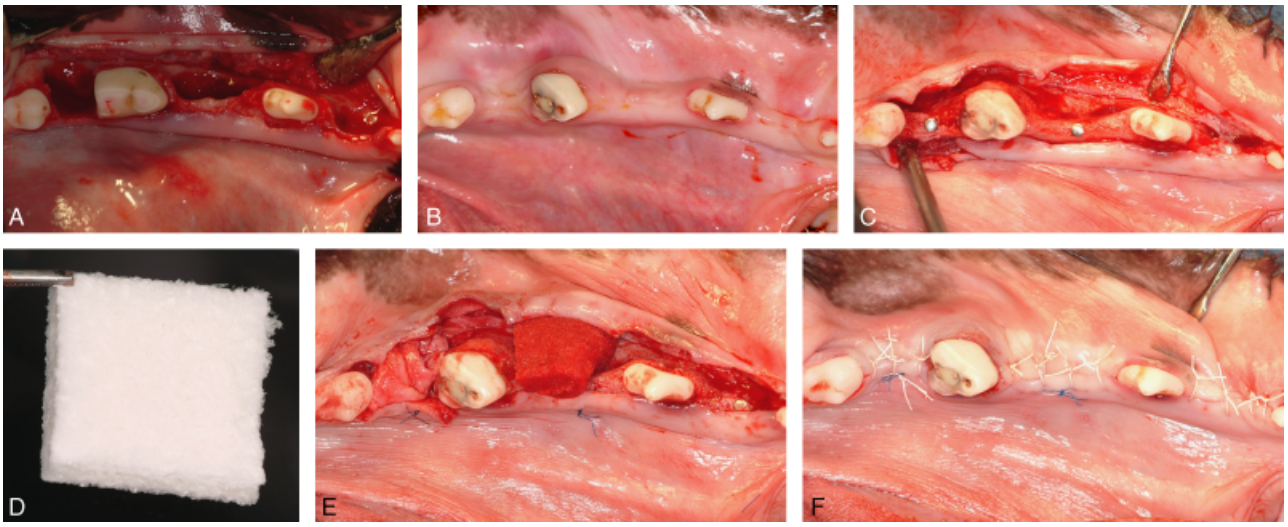

Fig. 1. (A) Lower left mandible after extraction of P2, P4, and the mesial root of M1. The buccal bone plates at all defect sites have been removed and the defects have been enlarged using a round bur. (B) Lower left mandible before starting surgery 2. The volume deficiencies in the three defect sites can be observed. (C) Chronic ridge defects with bone deficiencies can be identified at all three defect sites. One titanium pin has been placed on top of the bone crest in each defect site. (D) Collagen-based matrix before being soaked in sterile saline, folded, and placed in the defect site. (E) Augmented ridge defects before closure of the flaps (from left/posterior to right/anterior: autogenous subepithelial connective tissue graft, collagen-based matrix, sham-operated site). (F) Primary wound closure has been obtained. The result of the augmentation procedures can be identified when comparing with (B).

GC Corporation, Tokyo, Japan) and individualized trays were fabricated using light-curing tray material (Megatray®, Select Dental Manufacturing Company, Farmingdale, NY, USA).

Surgery 2 (soft tissue volume augmentation)

After a healing period of 2 months, surgery 2 was performed on all dogs under the same operating room conditions as surgery 1. Before starting the surgery, the mandibles were inspected (Fig. 1B) and polyether impressions (Impregum F®, 3M Espe) of the lower jaws were made using individualized trays (Megatray®, Select Dental Manufacturing Company). Following midcrestal incisions (between M2 and the mesial root of M1; between the mesial root of M1 and P3; between P3 and P1) and sulcular incisions around the mesial root of M1, P3, and P1, full-thickness mucoperiosteal flaps were elevated over the crest of the ridge. The chronic ridge defects were then inspected and measured in height, depth, and width. A titanium pin (Frios®, Dentsply, Konstanz, Germany) was placed on top of the bone crest in the middle of each chronic defect to simplify the upcoming histological processing (Fig. 1C). Periosteal releasing incisions were made to make room for volume augmentation. The following three treatment modalities were randomly applied to the defects (Table 1):

Group a: a prototype CM made of porcine collagen (Geistlich Pharma AG, Wolhusen, Switzerland),
Group b: an autogenous SCTG,
Group c: sham-operated site (control).

Group a. The CM (dimensions: width 10 mm, length 12 mm, thickness 5 mm) was soaked in sterile saline for 60 s (Fig. 1D), folded once, and then positioned in the pouch under the elevated buccal flap. A horizontal mattress suture was made to immobilize the CM, connecting it to the lingual flap (Dafilon® 5-0, B. Braun Melsungen AG, Melsungen, Germany).

Group b. The autogenous SCTG was harvested from the palatal vault. A u-shaped incision was made in the lateral part of the palatal vault and a mucoperiosteal flap was elevated. A SCTG was then dissected (dimensions: width 10 mm, length 12 mm, thickness 5 mm). Fatty tissue, glandular tissue, and remnants of the epithelium were removed. Any bleeding in the palate was controlled by the use of local anaesthetic (Ultracain forte 4%®, Hoechst, Leverkusen, Germany), compression with a sterile gauze, and three to four single sutures (Ethibond® 4-0, FS-2, Ethicon, Norderstedt, Germany). The SCTG was then folded once and positioned in the pouch under the elevated buccal flap. A horizontal mattress suture was made to immobilize the SCTG, connecting it to the lingual flap (Dafilon® 5-0, B. Braun Melsungen AG).

Group c. No further treatment was applied to sham-operated sites (Fig. 1E).

The buccal flaps in all sites were repositioned without tension to the lingual part. One horizontal mattress suture (Gore Tex 5-0®, W. L. Gore & Associates Inc., Putzbrunn, Germany) was placed over the buccal prominence created by the volume gain through the SCTG, and the CM to stabilize and stretch the grafts towards the vestibular fornix. The flaps were adapted using four to five single sutures (Gore Tex 5-0®, W. L. Gore & Associates Inc.) to ensure primary wound closure (Fig. 1F).

The dogs were maintained on a soft diet for the remainder of the study. The sutures were removed 14 days after surgery 2.

Volumetric analysis to evaluate soft tissue volume changes

Master casts were made out of dental stone (GC Fujirock® type 4, GC Corporation) utilizing the pre-operative (baseline) and follow-up impressions at 28 days (dogs 1–6) and 84 days (dogs 4–6).

For the evaluation of the dimensional changes at the defect sites, the casts were

optically scanned with a 3D camera (Cerec 3 Bluecam®, Sirona Dental Systems GmbH, Bensheim, Germany) (Fickl et al. 2009a, Schneider et al. 2010). Because the accessible area for the optical scanner is limited to a field of 17 × 14 mm at a time, several overlapping optical impressions from the buccal and the bucco-occlusal directions were taken, including the canine, P1, P3, and the mesial root of M1 and M2. The acquired data were then composed into one digital image by a CAD/CAM software (Cerec 3®, Sirona Dental Systems GmbH), encompassing the jaw segments from the canine to the second molar. The obtained digital images of the casts reflecting the different treatment time points (baseline, 28 days, 84 days) were then transferred into another digital imaging software (Match3D, University of Munich, Munich, Germany).

The images (Fig. 2A) were then superimposed and matched in one common coordinate system. The buccal surfaces of the remaining teeth were used as reference points for the superposition of the different images. Subsequently, a defined area of interest at each defect site was measured and the volume difference between the time points was calculated (Fig. 2B). Because of an individually variable anatomic situation, the measured area varied between the sites, but was kept constant at one site over time. The region of interest exhibited a trapezoid shape and reached in a bucco-oral dimension from the most coronal aspect of the lingual defect side to roughly 1 cm into the buccal mucosa, and in a mesio-distal dimension from one neighbouring tooth (mesial) to the other neighbouring tooth (distal) at a distance of 1 mm from the neighbouring tooth (Fig. 2C).

Table 1. Randomization of treatment options per dog and mandibular defect

Endpoint (days)	Dog number	Site					
		right anterior	right middle	right posterior	left posterior	left middle	left anterior
28	1	CM	SCTG	Control	CM	SCTG	Control
28	2	SCTG	Control	CM	SCTG	Control	CM
28	3	Control	CM	SCTG	Control	CM	SCTG
84	4	CM	SCTG	Control	CM	SCTG	Control
84	5	SCTG	Control	CM	SCTG	Control	CM
84	6	Control	CM	SCTG	Control	CM	SCTG

CM, collagen matrix; SCTG, subepithelial connective tissue graft; control, sham-operated site.

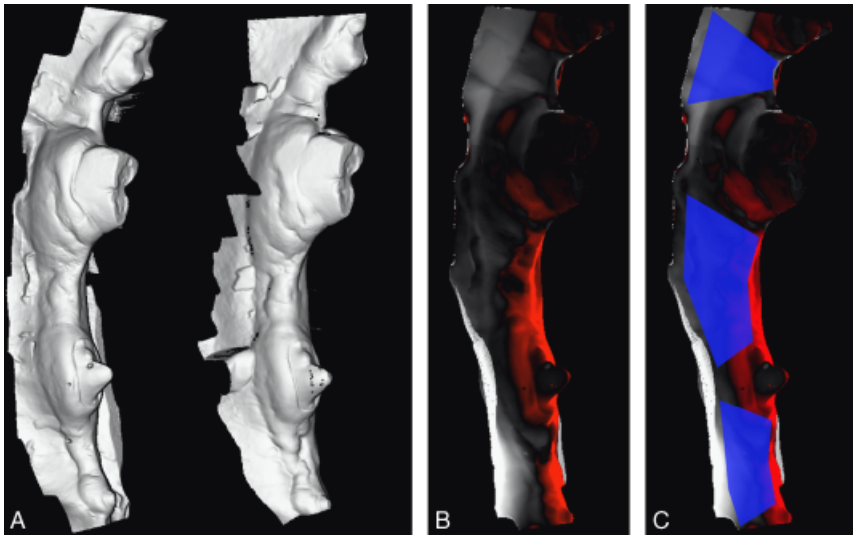

Fig. 2. (A) Optically scanned lower right mandible at baseline (before augmentation; left) and at 84 days (right). (B) Superimposed image [same mandible as in (A)] demonstrating volumetric changes between baseline and 84 days. White colour areas represent a gain in volume; red colour areas represent a loss of volume; black areas show volumetric changes $<50\,\mu$m. (C) Superimposed image [same image as (B)] including the measured areas (region of interest) of all three defects sites in blue colour.

In order to allow a direct comparison of the different sites and the different treatment modalities, the calculated variable Δd was the measured volume difference per measured area (d [mm] = vol [mm^3]/area [mm^2]).

The obtained data were then statistically analysed regarding volume alterations in terms of different treatment modalities and time points.

Statistical analysis

Data analyses were performed using a statistical software (SAS software, SAS Institute, Cary, NC, USA). The volume differences were assessed at 28 and 84 days, relative to the pre-operative dimension of the defect inside 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 and standard deviations. Volume differences were analysed with analysis of variance (ANOVA) with one-factor model in order to describe and compare the three treatment modalities. The paired t-test was used in order to judge the mean changes within treatment groups. The level of significance was set at $p<0.05$.

Results

Clinical findings

The dogs remained healthy and no systemic complications occurred during the entire study period. One site augmented with an SCTG showed some swelling and an incomplete wound closure at the day of suture removal. However, no additional treatment was necessary and further healing was uneventful. No other complications such as local intolerance, inflammation, or wound dehiscences were observed. An optimal integration of the CM and the SCTG into the surrounding soft tissue was detected.

Volumetric measurements

The calculated mean volume differences and standard deviations for all sites and treatment modalities are presented in Table 2 and Fig. 3 for the primary end points at 28 and 84 days.

A tendency for a relationship between site and treatment for the CM and SCTG group was observed with greater absolute

mean values for the CM group in the anterior and posterior sites, and greater mean values for the SCTG group in the middle defects. However, the mean values indicated no statistically significant differences between the three sites ($p > 0.10$). 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.

At 28 days, the CM and the SCTG groups showed a similar and significant increase in soft tissue volume compared with the control group. In detail, the mean difference in soft tissue volume per area compared with baseline amounted to a gain of 1.6 mm (CM; SD ± 0.9), 1.5 mm (SCTG; ± 0.1), and a loss of 0.003 mm (control; ± 0.3) (Table 2A).

A similar soft tissue volume was gained and maintained by the CM and the SCTG group until 84 days. In comparison, the control group lost soft tissue volume over the same observation period. In detail at 84 days, the mean volume differences per area to baseline measured a gain of 1.4 mm (CM; ± 1.1), 1.4 mm (SCTG; ± 0.4), and a loss of 0.3 mm (control; ± 0.3) (Table 2B). No statistically significant differences were observed between the CM and the SCTG group ($p > 0.10$) at 28 and 84 days. The mean values of the control group were statistically significantly lower compared with the CM and the SCTG group at both time points ($p < 0.001$).

The mean shrinkage in soft tissue volume between 28 and 84 days was slightly higher for the CM group (10.0%) compared with the SCTG group (5.7%) without any statistically significant differences between the two treatments ($p > 0.05$).

Table 2. Descriptive statistics with mean values (mm) and standard deviations (SD; mm) of volume changes Δd (mm) by site (posterior; middle; anterior; posterior+middle+anterior) and group (CM, SCTG, control) at (A) 28 days and (B) 84 days

Site	Group					
	CM		SCTG		Control	
	mean value (mm)	SD (mm)	mean value (mm)	SD (mm)	mean value (mm)	SD (mm)
(A)						
Anterior	2.3	1.0	1.9	0.6	−0.5	0.6
Middle	0.8	0.1	1.5	0.2	−0.2	0.0
Posterior	1.7	0.5	1.1	0.6	0.9	0.1
All sites (anterior+middle+posterior)	**1.6**	**0.9**	**1.5**	**0.1**	**0.0**	**0.3**
(B)						
Anterior	1.9	1.6	1.4	0.7	−0.8	0.3
Middle	0.7	0.0	1.7	0.1	−0.1	0.0
Posterior	1.8	1.0	1.2	0.2	0.9	0.6
All sites (anterior+middle+posterior)	**1.4**	**1.1**	**1.4**	**0.4**	**−0.3**	**0.2**

Data in bold signifies p (CM, SCTG versus control) < 0.001 at 28 and 84 days; p (CM versus SCTG) > 0.05 at 28 and 84 days. CM, collagen matrix; SCTG, subepithelial connective tissue graft; control, sham-operated site.

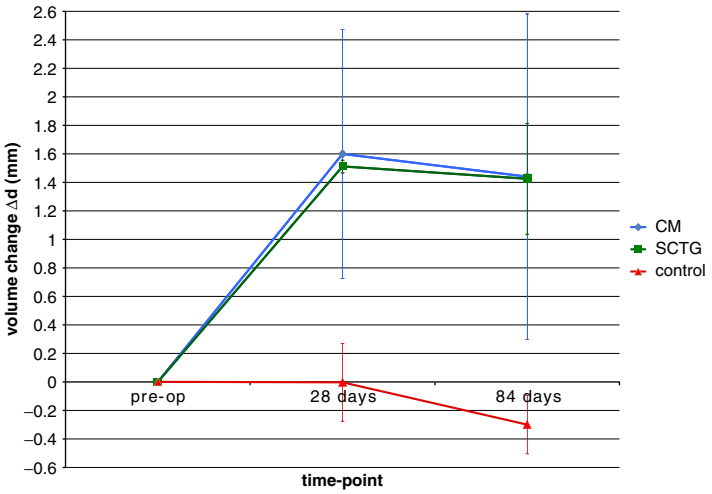

Fig. 3. Measurements of volumetric changes (mm) of all sites between evaluated time points (baseline, 28 days, 84 days). CM, collagen matrix; SCTG, subepithelial connective tissue graft; control, sham-operated site; p (CM,SCTG *versus* control) < 0.001 at 28 and 84 days.

Discussion

In the present study, the volume gain achieved with the experimental CM amounted to 1.6 mm at 28 days and 1.4 mm at 84 days. The differences to the gold standard (SCTG) were not statistically significant. The relative decrease in soft tissue volume between 28 and 84 days was slightly greater (10%) for the CM group than for the SCTG group (5.7%). These results demonstrate two main outcomes: (i) the experimental CM can be used to augment chronic ridge defects in an animal model; (ii) no inferiority of the CM group compared with the SCTG group was observed at 28 and 84 days, which in turn means that the CM could possibly be used as replacement for the SCTG in the future.

The mandible of dogs used in the present study has been validated as a model to study therapeutic interventions for the treatment of chronic ridge defects in earlier studies (von Arx et al. 2001, 2002). In the present study, ridge defects were prepared in order to simulate the clinical situation often encountered at single-tooth gaps or underneath pontics of fixed dental prostheses. In these clinical situations, pre-prosthetic site development in the form of soft tissue volume augmentation is frequently conducted to improve function and aesthetics.

In the present study, the defect sites were augmented using an autogenous soft tissue graft from the palate as the gold standard and a newly developed CM as the test treatment.

Optimal integration of the CM and the autogenous tissue into the surrounding soft tissue was observed because no soft tissue complications occurred throughout the entire study.

Collagen devices from xenogenic origin have been the focus of investigations for years in dentistry (Hammerle & Jung 2003, Jung et al. 2004). Because of their chemical properties leading to favourable biological reactions, collagen devices were used as barrier membranes in guided bone regeneration (GBR) and guided tissue regeneration procedures, for the management of extraction sockets, as haemostatic agents, and recently for the purpose of increasing the width of keratinized tissue (Hammerle & Karring 1998, Jung et al. 2004, Kimble et al. 2004, Jesty et al. 2009, Sanz et al. 2009). The integration of collagen devices into the surrounding soft tissue appears to play a critical role during the wound healing process, which in turn is critical for a successful treatment outcome (Becker et al. 2009). Numerous pre-clinical studies have demonstrated early transmembraneous angiogenesis, but also fast degradation of xenogenic native collagen membranes by enzymatic activity of immunologic cells (Rothamel et al. 2005, Schwarz et al. 2006, 2008).

The native collagen structure can be changed and cross-linked by chemical or enzymatic means. The resulting cross-linked material has been clinically applied in the form of a barrier membrane for GBR. The results of pre-clinical studies have demonstrated hampered tissue integration with subsequent exposure, bacterial colonization, and inflammation of the adjacent tissue (Bornstein et al. 2007, Schwarz et al. 2008, Becker et al. 2009). In contrast to the native collagen, the cross-linked type histologically showed a compromised transmembraneous vascularization.

In the present study, a CM chemically cross-linked with a modified protocol was applied. In contrast to the above-mentioned application as a GBR membrane, the experimental porous CM served as a scaffold for soft tissue augmentation. An uneventful healing and no clinical complications such as exposure of the CM were encountered, demonstrating excellent soft tissue integration.

Autogenous tissue grafts are still considered the treatment of choice for the correction of localized alveolar ridge defects (Thoma et al. 2009). Commonly, the grafts are harvested from the palate either as full-thickness grafts (FGG) or as SCTG.

In a previous clinical study, single-tooth ridge defects were either treated with an SCTG, an FGG, or were left untreated (Studer et al. 2000). Volumetric measurements were performed at 1 month and 3.5 months. At 1 month, the mean volume gain for the SCTG amounted to 187 mm^3. This value was significantly higher than the untreated control. In the present animal study, similar observations were made including a volume gain of 1.5 mm for the SCTG at 1 month, representing a significant difference to the negative control. In the present study between 1 month (28 days) and 3 months (84 days), a slight decrease of the soft tissue volume of 5.7% was observed for the SCTG group. This decrease was less pronounced than the one in the clinical study reporting 15% of volume loss between 1 and 3.5 months (Studer et al. 2000). Even though the absolute numbers cannot be compared between the two studies due to various factors, the outcomes of the present study indicate this animal model to be suitable for the study of treatment modalities aiming at increasing the ridge volume in single-tooth gaps. It also demonstrated that autogenous tissue might be used as a positive control.

Some limitations exist with respect to the present animal model, and may explain increased standard deviations and variability between the sites: (i) the size of the extracted teeth (distal root of M1, P4, P2) widely differed; (ii) chronic instead of acute, standardized defects were used; and (iii) a relatively small number of animals was treated.

It has been demonstrated earlier that acute bone defects are prone to spontaneous bone regeneration (Schenk et al. 1994, Lundgren et al. 1998). In order to minimize a possible contribution of newly formed bone to the augmentation of the defects, and to evaluate the effect of the augmented soft tissue, chronic ridge defects were chosen. In addition, the chronic defects came close to the clinical situation (single-tooth gap). Variability in defects size was therefore accepted as a limitation of this animal model. Augmentation surgery was then performed according to a standardized procedure. Treatments were applied in a randomized manner, resulting in increased standard deviations, but without any statistically significant differences between the three defect sites (anterior, middle, posterior).

As mentioned earlier, the use of autogenous tissue is associated with disadvantages mainly due to the harvesting procedure, which increases patient morbidity and extends the healing time (Griffin et al. 2006, Wessel & Tatakis 2008). In order to overcome these problems, research has focused on the development of alternative techniques and materials to augment soft tissue volume. Advantages of using materials from the shelf include (i) avoidance of a second surgical site, (ii) unlimited availability, and (iii) possibly more predictable outcomes (Griffin et al. 2006). In a clinical study, a hydroxyapatite implant material has been used as a replacement for autogenous tissue (SCTG) to augment localized alveolar ridge defects (Allen et al. 1985). Less shrinkage was reported using the hydroxyapatite material when compared with the SCTG-augmented sites. In another study, soft tissue ridge deformities were augmented using an acellular dermal matrix (Batista et al. 2001). Even though a horizontal gain of 1.72 mm and a slight gain in a vertical direction were observed at the end of the study, the mean shrinkage over the same observation period exceeded 40%. This important degree of shrinkage limits the clinical applicability. As a consequence, no further studies have reported on the use of this material for augmentation of soft tissue volume (Thoma et al. 2009).

Based on favourable results with respect to soft tissue integration and degradation of currently used xenogenic collagen membranes, a CM was developed for the purpose of soft tissue volume augmentation. Any device intended to be used for soft tissue volume augmentation has to fulfil two main criteria: (i) mechanical stability and (ii) favourable biological behaviour. In vitro testing utilizing a specifically designed bioreactor has been conducted to investigate the biological and mechanical properties of the experimental CM. It has been shown that a prototype CM promoted the growth of primary human fibroblasts. Dynamic loading of seeded CMs resulted in an increased expression of extracellular matrix proteins such as collagen type I and fibronectin (M Mathes, L Wohlwend, L Uebersax, DS Thoma, RE Jung, R von Mentlen, U Graf-Hausner, unpublished data). These results indicated the ability of the CM to serve as a scaffold for fibroblasts and to promote tissue remodelling. In addition, the cultivation of CMs in a biomechanical stimulation apparatus resulted in a stiffening of the biomaterial and an increase in the maximum force needed to compress the CMs (M Mathes, L Wohlwend, L Uebersax, DS Thoma, RE Jung, R von Mentlen, U Graf-Hausner, unpublished data). In a clinical environment, where shear and compression forces constantly occur and the matrix is exposed to wound tissue, this CM may

thus optimally meet the mechanical and biological demands for an artificial soft tissue graft. Still, further research in a variety of animal models, followed by controlled clinical trials, is needed to confirm these promising results using collagen-based matrices for soft tissue volume augmentation.

The lack of evidence with respect to devices for soft tissue volume augmentation makes it difficult to compare the outcomes of the CM to other studies. The weak evidence may not only be due to a lack of suitable devices but primarily due to a lack of suitable techniques for the measurement of volume changes (Thoma et al. 2009). Several methods have been described to non-invasively measure volume changes in the oral cavity. The techniques ranged from simple clinical observation (Allen et al. 1985), two-dimensional measurements using a periodontal probe (Batista et al. 2001), to complicated volumetric assessments using the Moiré projection method (Studer et al. 2000). The use of different techniques impairs any comparison of volumetric outcomes between studies. In the present study, a three-dimensional optical method has been used to detect volume changes over time. The applied technique showed a high reproducibility and an excellent accuracy for measuring volume changes in a methodological study (Windisch et al. 2007). A variety of studies have shown that this method offers great advantages in being easy to apply, non-invasive, and precise (Windisch et al. 2007, Fickl et al. 2009a, Schneider et al. 2010, Strebel et al. 2009). Currently, there is one shortcoming of the technique, because optical scans were performed on study casts in the present study. The accuracy of the method is highly influenced by the accuracy of the impressions and the casts. For that purpose, every impression was checked in order to ensure that the entire augmented area could be evaluated. Research is ongoing further developing this technique and including optical scans that will be performed directly in the oral cavity. A broader use of this technique may be desirable for the future and will allow comparing volume measurements between different studies.

Conclusions

The results of the present study indicated that the experimental CM rendered a soft tissue volume gain on a level non-inferior to the gold standard (SCTG) at 28 and 84 days. The volume decrease between the two time points 28 and 84 days was similar for the CM and the SCTG group. Within the limits of this animal model, the experimental CM may be a suitable device for soft tissue volume augmentation and may serve as a replacement for autogenous tissue to augment localized alveolar ridge defects.

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References

Abrams, L. (1980) Augmentation of the deformed residual edentulous ridge for fixed prosthesis. *The Compendium on Continuing Education in General Dentistry* **1**, 205–213.

Aichelmann-Reidy, M. E., Yukna, R. A., Evans, G. H., Nasr, H. F. & Mayer, E. T. (2001) Clinical evaluation of acellular allograft dermis for the treatment of human gingival recession. *Journal of Periodontology* **72**, 998–1005.

Allen, E. P., Gainza, C. S., Farthing, G. G. & Newbold, D. A. (1985) Improved technique for localized ridge augmentation. A report of 21 cases. *Journal of Periodontology* **56**, 195–199.

Andrade, P. F., Felipe, M. E., Novaes, A. B. Jr., Souza, S. L., Taba, M. Jr., Palioto, D. B. & Grisi, M. F. (2008) Comparison between two surgical techniques for root coverage with an acellular dermal matrix graft. *Journal of Clinical Periodontology* **35**, 263–269.

Araujo, M., Linder, E. & Lindhe, J. (2009) Effect of a xenograft on early bone formation in extraction sockets: an experimental study in dog. *Clinical Oral Implants Research* **20**, 1–6.

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.

Becker, J., Al-Nawas, B., Klein, M. O., Schliephake, H., Terheyden, H. & Schwarz, F. (2009) Use of a new cross-linked collagen membrane for the treatment of dehiscence-type defects at titanium implants: a prospective, randomized-controlled double-blinded clinical multicenter study. *Clinical Oral Implants Research* **20**, 742–749.

Bichacho, N. (1998) Achieving optimal gingival esthetics around restored natural teeth and implants. Rationale, concepts, and techniques. *Dental Clinics of North America* **42**, 763–780.

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.

Coslet, J. G., Rosenberg, E. S. & Tisot, R. (1980) The free autogenous gingival graft. *Dental Clinics of North America* **24**, 651–682.

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.

Farnoush, A. (1978) Techniques for the protection and coverage of the donor sites in free soft tissue grafts. *Journal of Periodontology* **49**, 403–405.

Fickl, S., Schneider, D., Zuhr, O., Hinze, M., Ender, A., Jung, R. E. & Hurzeler, M. B. (2009a) Dimensional changes of the ridge contour after socket preservation and buccal overbuilding: an animal study. *Journal of Clinical Periodontology* **36**, 442–448.

Fickl, S., Zuhr, O., Wachtel, H., Kebschull, M. & Hurzeler, M. B. (2009b) Hard tissue alterations after socket preservation with additional buccal overbuilding: a study in the beagle dog. *Journal of Clinical Periodontology* **36**, 898–904.

Griffin, T. J., Cheung, W. S., Zavras, A. I. & Damoulis, P. D. (2006) Postoperative complications following gingival augmentation procedures. *Journal of Periodontology* **77**, 2070–2079.

Hammerle, C. H. & Jung, R. E. (2003) Bone augmentation by means of barrier membranes. *Periodontology 2000* **33**, 36–53.

Hammerle, C. H. & Karring, T. (1998) Guided bone regeneration at oral implant sites. *Periodontology 2000* **17**, 151–175.

Harris, R. J. (2003) Soft tissue ridge augmentation with an acellular dermal matrix. *The International Journal of Periodontics and Restorative Dentistry* **23**, 87–92.

Heberer, S., Al-Chawaf, B., Hildebrand, D., Nelson, J. J. & Nelson, K. (2008) Histomorphometric analysis of extraction sockets augmented with Bio-Oss Collagen after a 6-week

healing period: a prospective study. *Clinical Oral Implants Research* **19**, 1219–1225.

Jesty, J., Wieland, M. & Niemiec, J. (2009) Assessment in vitro of the active hemostatic properties of wound dressings. *Journal of Biomedical Materials Research. Part B, Applied Biomaterials* **89B**, 536–542.

Jung, R. E., Siegenthaler, D. W. & Hammerle, C. H. (2004) Postextraction tissue management: a soft tissue punch technique. *The International Journal of Periodontics and Restorative Dentistry* **24**, 545–553.

Kimble, K. M., Eber, R. M., Soehren, S., Shyr, Y. & Wang, H. L. (2004) Treatment of gingival recession using a collagen membrane with or without the use of demineralized freeze-dried bone allograft for space maintenance. *Journal of Periodontology* **75**, 210–220.

Kinsel, R. P. & Capoferri, D. (2008) A simplified method to develop optimal gingival contours for the single implant-supported, metal-ceramic crown in the aesthetic zone. *Practical Procedures and Aesthetic Dentistry* **20**, 231–236; quiz 237.

Langer, B. & Calagna, L. (1980) The subepithelial connective tissue graft. *The Journal of Prosthetic Dentistry* **44**, 363–367.

Lundgren, A. K., Sennerby, L. & Lundgren, D. (1998) Guided jaw-bone regeneration using an experimental rabbit model. *International Journal of Oral and Maxillofacial Surgery* **27**, 135–140.

Mörmann, W. H. & Brandestini, M. (1996) The fundamental inventive principles of Cerec CAD/CIM and other CAD/CAM methods. In: Mörmann, W. H. (ed). *CAD/CIM in Aesthetic Dentistry. Cerec 10 Year Anniversary Symposium*, pp. 81–110. Chicago: Quintessence.

Prato, G. P., Cairo, F., Tinti, C., Cortellini, P., Muzzi, L. & Mancini, E. A. (2004) Prevention of alveolar ridge deformities and reconstruction of lost anatomy: a review of surgical approaches. *The International Journal of Periodontics and Restorative Dentistry* **24**, 434–445.

Price, R. B. & Price, D. E. (1999) Esthetic restoration of a single-tooth dental implant using a subepithelial connective tissue graft: a case report with 3-year follow-up. *The International Journal of Periodontics and Restorative Dentistry* **19**, 92–101.

Reiser, G. M., Bruno, J. F., Mahan, P. E. & Larkin, L. H. (1996) The subepithelial connective tissue graft palatal donor site: anatomic considerations for surgeons. *The International Journal of Periodontics and Restorative Dentistry* **16**, 130–137.

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.

Schenk, R. K., Buser, D., Hardwick, W. R. & Dahlin, C. (1994) Healing pattern of bone regeneration in membrane-protected defects: a histologic study in the canine mandible. *The International Journal of Oral and Maxillofacial Implants* **9**, 13–29.

Schneider, D., Grunder, U., Ender, A., Hämmerle, C.H.F. & Jung, R. E. (2010) 3D volumetric measurements of the long-term stability of periimplant tissue volume following bone and soft tissue augmentation – 1-year results from a prospective cohort study (in press).

Schneider, W. (2003) Cerec 3D – a new dimension in treatment. *International Journal of Computerized Dentistry* **6**, 57–66.

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. *The Compendium on Continuing Education in General 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.

Strebel, J., Ender, A., Paqué, F., Krähenmann, M., Attin, T. & Schmidlin, P. R. (2009) In vivo validation of a three-dimensional optical method to document volumetric soft tissue changes of the interdental papilla. *Journal of Periodontology* **80**, 56–61.

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

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

von Arx, T., Cochran, D. L., Hermann, J. S., Schenk, R. K. & Buser, D. (2001) Lateral ridge augmentation using different bone fillers and barrier membrane application. A histologic and histomorphometric pilot study in the canine mandible. *Clinical Oral Implants Research* **12**, 260–269.

von Arx, T., Cochran, D. L., Schenk, R. K. & Buser, D. (2002) Evaluation of a prototype trilayer membrane (PTLM) for lateral ridge augmentation: an experimental study in the canine mandible. *The International Journal of Oral and Maxillofacial Surgery* **31**, 190–199.

Wainwright, D. J. (1995) Use of an acellular allograft dermal matrix (AlloDerm) in the management of full-thickness burns. *Burns* **21**, 243–248.

Wei, P. C., Laurell, L., Geivelis, M., Lingen, M. W. & Maddalozzo, D. (2000) Acellular dermal matrix allografts to achieve increased attached gingiva. Part 1. A clinical study. *Journal of Periodontology* **71**, 1297–1305.

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.

Wessel, J. R. & Tatakis, D. N. (2008) Patient outcomes following subepithelial connective tissue graft and free gingival graft procedures. *Journal of Periodontology* **79**, 425–430.

Windisch, S. I., Jung, R. E., Sailer, I., Studer, S. P., Ender, A. & Hammerle, C. H. (2007) A new optical method to evaluate three-dimensional volume changes of alveolar contours: a methodological in vitro study. *Clinical Oral Implants Research* **18**, 545–551.

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

Clinical Relevance

Scientific rationale for the study: To date, soft tissue volume augmentation has been performed using autogenous tissue from the palate. This procedure is associated with an increased patient morbidity due to the second surgical site. Furthermore, anatomical and individual variations exist with respect to the quantity and quality of tissue that can be retrieved.

Principal findings: The experimental CM rendered a soft tissue volume gain on a level non-inferior to the SCTG at 28 and 84 days.

Practical implications: The CM is unique as an artificial device for soft tissue augmentation. Further research is necessary to confirm the results of this animal experiment.