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

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Originally published at:
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Abstract

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

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

Running title:
Soft tissue volume augmentation

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**Abstract**

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**Material and Methods:** In 6 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 digitally analyzed. A defined region of interest was measured in all sites and the volume differences between the time-points calculated.

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

**Conclusion:** Within the limits of this animal study, the collagen matrix may serve as a replacement for autogenous connective tissue.
Clinical relevance

Scientific rationale for the study: To date, soft tissue volume augmentation is 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 collagen matrix rendered a soft tissue volume gain on a level non-inferior to the subepithelial connective tissue graft at 28 and 84 days.

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

Introduction


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 has 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 either treated using a FGG or a 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 most often performed at the palate requires an additional surgical site increasing patient morbidity (Farnoush 1978, Griffin et al. 2006). A relative discomfort including pain and numbness is experienced by the patients the days following the surgery.
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 (Soileau & Brannon 2006, Reiser et al. 1996).

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, allogeneic devices were introduced in mucogingival surgery predominantly as replacement for FGGs. Among these allogeneic devices the acellular dermal matrix graft (ADMG; Alloderm™, Life Cell Corporation, The Woodlands, TX) was most frequently used. The ADMG was originally developed for covering full thickness burn wounds (Wainwright 1995). Intraoral applications included procedures to increase the width of keratinized tissue, to cover recession defects, and to deepen the vestibular fornix (Aichelmann-Reidy et al. 2001, Harris 2003, Wei et al., 2000 Andrade et al. 2008). The ADMG has also been used to augment localized alveolar defects (Batista et al. 2001). However, allogeneic 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. Since the vascularization of the acellular dermal grafts appears to be the limiting factor, a folding process could further hamper new vessel formation and provoke 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 fulfill certain demands regarding the mechanical properties. This is based on the observation that shear and compression forces are constantly applied to the grafts in the augmentation sites. For that purpose, collagen-based matrices have been evaluated in preclinical 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) the 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) to 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 1996). With this system a three-dimensional image is obtained after scanning the intraoral anatomy with a camera (Schneider 2003). In a methodological in vitro study, several datasets of three-dimensional objects were captured and volumetric differences 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 a animal study (Fickl et al. 2009a), 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. 2009). 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 leads to volume gain in alveolar ridge defects similar to those obtained by an autogenous subepithelial connective tissue graft.

**Material and Methods**

**Animals**

The present study was designed as a randomized controlled experimental study employing 6 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 anesthesia and sterile conditions in an operating room using Thiopental - NaSolution 4% (Abott Laboratories, N. Chicago, IL, USA), 0.4 ml/kg body weight as a pre-medication. The dogs were placed on a heating pad, intubated, anesthesized 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 premolars (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 enlarged using a round bur (Fig. 1A). Following rinsing with sterile saline, primary wound closure was obtained (GoreTex 5-0®, GoreTex, L. Gore & Associates, Inc., Putzbrunn, Germany). For the first 7 to 14 days after extraction, the dogs were fed a soft diet. After a period of 7 to 10 days, the animals were briefly anesthetized (RAAK @ 1 ml/30 lbs. by IV – Ketamine 50 mg/ml, Xylazine 7.1 mg/ml, Acepromaz 2.1 mg/ml, Atropine 0.1 mg/ml), the sutures removed and the teeth 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, GC Corporation, Tokyo, Japan) and individualized trays 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, Neuss, Germany) of the lower jaws were made using individualized trays (Megatray®, Select Dental Manufacturing Company, Farmingdale, NY, USA). 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 3 treatment modalities were randomly applied to the defects (Table 1):
Group a: a prototype collagen matrix made of porcine collagen (CM) (Geistlich Pharma AG, Wolhusen, Switzerland)

Group b: an autogenous subepithelial connective tissue graft (SCTG)

Group c: sham-operated site (control)

Group a:

The CM (dimensions: width 10mm, length 12mm, thickness 5mm) was soaked in sterile saline for 60 sec (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 10mm, length 12mm, thickness 5mm). Fatty tissue, glandular tissue, and remnants of the epithelium were removed. Any bleeding in the palate was controlled by the use of local anesthetic (Ultracain forte 4%®, Hoechst, Leverkusen, Germany), compression with a sterile gauze and 3 to 4 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, Melsungen, Germany).

Group c:

No further treatment was applied to the 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 4 to 5 single sutures (Gore Tex 5-0®, W.L.Gore & Associates, Inc, Putzbrunn, Germany) to ensure primary wound closure (Fig. 1F). Following irrigation with sterile saline, a polyether impression of the jaw was taken.
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 Corp., Tokyo, Japan) utilizing the preoperative (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. 2009). Since the accessible area for the optical scanner is limited to a field of 17 x 14 mm at a time, several overlapping optical impressions from the buccal and the bucco-occlusal direction were taken, including the canine, P2, P4, the mesial root of M1 and M2. The acquired data was then composed into one digital image by a CAD/CAM software (Cerec 3®, Sirona Dental Systems GmbH, Bensheim, Germany), 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 (Figs. 2B). Due to 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 1cm into the buccal mucosa, and in a mesio-distal dimension from one neighboring tooth (mesial) to the other neighboring tooth (distal) at a distance of 1mm from the neighboring tooth (Fig. 2C).

In order to allow a direct comparison of the different sites and the different treatment modalities, the calculated variable $\Delta d$ was the measured volume difference per measured area ($\Delta d [\text{mm}] = \Delta \text{vol} [\text{mm}^3] / \text{area} [\text{mm}^2]$).
The obtained data was then statistically analyzed 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 preoperative 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 analyzed 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 a 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 collagen matrix 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 Figure 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 to the control group. In detail, the mean difference in soft tissue volume per area compared to baseline amounted to a gain of 1.6mm (CM; SD ± 0.9), 1.5mm (SCTG; ± 0.1), and a loss of 0.003mm (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 1.4mm (CM; ± 1.1), 1.4mm (SCTG; ± 0.4), and a loss of 0.3mm (control; ± 0.3) (Table 2B). No statistically significant differences were observed between the CM and SCTG group (p>0.10) at 28 and 84 days. The mean values of the control group were statistically significantly lower compared to the CM and SCTG group at both time-points (p<0.001).

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

Discussion

In the present study, the volume gain achieved with the experimental collagen matrix amounted to 1.6mm at 28 days and 1.4mm 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 three main outcomes: i) the experimental collagen matrix can be used to augment chronic ridge defects in an animal model; ii) no inferiority of the CM group compared to 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. 2002, von Arx et al. 2001). In the present study ridge defects were prepared in order to simulate the clinical situation often encountered at tooth gaps or underneath pontics of fixed
dental prostheses. In these clinical situations pre-prosthetic site development in the form of tissue volume augmentation is frequently conducted to improve function and esthetics.

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 collagen matrix as the test treatment.

Optimal integration of the collagen matrix and the autogenous tissue into the surrounding soft tissue was observed since 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). Due to their chemical properties leading to favorable biological reactions collagen devices were used as barrier membranes in guided bone regeneration (GBR) and guided tissue regeneration (GTR) 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, Jesty et al. 2009, Kimble et al. 2004, Jung et al. 2004, 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 preclinical studies have demonstrated early transmembraneous angiogenesis, but also fast degradation of xenogenic native collagen membranes by enzymatic activity of immunologic cells (Schwarz et al. 2006, Schwarz et al. 2008, Rothamel et al. 2005).

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 preclinical studies have demonstrated hampered tissue integration with subsequent exposure, bacterial colonization and inflammation of the adjacent tissue (Bornstein et al., 2007 Becker et al. 2009, Schwarz et al. 2008). In contrast to the native collagen, the cross-linked type histologically showed a compromised transmembraneous vascularization.

In the present study, a collagen matrix chemically cross-linked with a modified protocol was applied. In contrast to the above-mentioned application as a GBR membrane, the experimental porous collagen matrix served as a scaffold for soft tissue augmentation. An uneventful healing and no clinical complications such as exposure of the collagen matrix 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 (free gingival grafts, FGG) or as subepithelial
connected tissue grafts (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 187mm$^3$. This value was significantly higher than the untreated control. In the
present animal study similar observations were made including a volume gain of 1.5mm 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; 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 the patient morbidity and extends the
healing time (Wessel & Tataakis 2008, Griffin et al. 2006). In order to overcome these
problems, research has focused on the development of alternative techniques and materials to
augment the 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 to 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 1.72mm 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 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 favorable results with respect to soft tissue integration and degradation of currently used xenogenic collagen membranes, a collagen matrix was developed for the purpose of soft tissue volume augmentation. Any device intended to be used for soft tissue volume augmentation has to fulfill two main criteria: i) mechanical stability, ii) favorable biological behavior. In vitro testing utilizing a specifically designed bioreactor has been conducted to investigate the biological and mechanical properties of the experimental collagen matrix. It has been shown that a prototype collagen matrix promoted the growth of primary human fibroblasts. Dynamic loading of seeded collagen matrices resulted in an increased expression of extracellular matrix proteins such as collagen type I and fibronectin (Mathes et al. 2009). These results demonstrated the ability of the collagen matrix to serve as a scaffold for fibroblasts and to promote tissue remodeling. In addition, the cultivation of collagen matrices in a biomechanical stimulation apparatus resulted in a stiffening of the biomaterial and an increase in the maximum force needed to compress the collagen matrices (Mathes et al. 2009). In a clinical environment, where shear and compression forces constantly occur and the matrix is exposed to wound tissue, this collagen matrix 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 collagen matrix to other studies. The weak evidence may not only be due to a lack of suitable devices but first of all 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 demonstrated that this method offers great advantages in being easy to apply, non-invasive and precise (Fickl et al. 2009a, Schneider et al. 2009, Windisch et al. 2007, Strebel et al. 2009). Currently, there is one shortcoming of the technique, since the 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 collagen matrix rendered a soft tissue volume gain on a level non-inferior to the gold standard (subepithelial connective tissue graft) 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 collagen matrix 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.
Acknowledgments

The authors gratefully acknowledge the help of Ms. Sonja Bustamante (Department of Periodontics at The University of Texas Health Science Center at San Antonio, Texas, USA) for her assistance with the animal work. They would also like to thank the Department of Laboratory Animals and Animal Surgery (University of Texas Health Science Center at San Antonio, Texas, USA). The help of Dr. Stephanie Mathes (University of Zurich for Applied Sciences, Wädenswil, Switzerland) is greatly acknowledged for expertise and discussion. The investigators express their thanks to Dr. Ulrich Kreuter (Consult AG, Bern, Switzerland) for providing expertise and support in the statistical analysis.
References


Figure legends

Figure 1A. 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.

Figure 1B. Lower left mandible before starting surgery 2. The volume deficiencies in the 3 defect sites can be observed.

Figure 1C. Chronic ridge defects with bone deficiencies can be identified at all three defect sites. One titanium pins has been placed on top of the bone crest in each defect site.

Figure 1D. Collagen-based matrix before being soaked in sterile saline, folded and placed in the defect site.

Figure 1E. 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).

Figure 1F. Primary wound closure has been obtained. The result of the augmentation procedures can be identified when comparing to Fig. 1B.

Figure 2A. Optically scanned lower right mandible at baseline (before augmentation; left) and at 84 days (right).

Figure 2B. Superimposed image (same mandible as in Figure 2A) demonstrating volumetric changes between baseline and 84 days. White color areas represent a gain in volume; red color areas represent a loss of volume; black areas show volumetric changes <50μm.

Figure 2C. Superimposed image (same image as Fig. 2B) including the measured areas (region of interest) of all three defects sites in blue color.
Figure 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 vs. control)<0.001 at 28 and 84 days.

Table 1. Randomization of treatment options per dog and mandibular defect. CM = collagen matrix; SCTG = subepithelial connective tissue graft; control = sham-operated site.

Table 2A. Descriptive statistics with mean values (mm) and standard deviations (SD; mm) of volume changes $\Delta d$ [mm] by site (posterior; middle; anterior; posterior + middle + anterior) and group (CM, SCTG, control) at 28 days. CM = collagen matrix; SCTG = subepithelial connective tissue graft; control = sham-operated site.

Table 2B. Descriptive statistics with mean values (mm) and standard deviations (SD; mm) of volume changes $\Delta d$ [mm] by site (posterior; middle; anterior; posterior + middle + anterior) and group (CM, SCTG, control) at 84 days. CM = collagen matrix; SCTG = subepithelial connective tissue graft; control = sham-operated site.