6.1 General Overview

6.1.1 Background
Soft tissue augmentation is a well-established procedure in a variety of disciplines in dentistry. It is indicated in partially and fully edentulous patients to augment areas with a lack of or a reduced width of keratinized tissue, as well as to increase soft tissue volume around dental implants and teeth in conjunction with dental reconstructions. For the latter, a certain amount of keratinized tissue has been considered necessary to maintain periodontal health and to prevent gingival recession (Nabers, 1966; Sullivan and Atkins, 1969). It has also been concluded that for the maintenance of gingival health 2 mm of keratinized gingiva is adequate (Lang and Löe, 1972).

With respect to dental implants, the discussion on the need for a grafting procedure to increase the width of keratinized tissue is somewhat contradictory in the literature. Several studies have suggested associations between an adequate width of keratinized tissue, higher survival rates of dental implants, health of the peri-implant mucosa, and an improved esthetic outcome (Adell et al., 1986; Artzi et al., 1993; Langer, 1996; Schrott et al., 2009).

Soft tissue grafting procedures are also performed to augment soft tissue volume in partially edentulous patients. These procedures have been proposed to surgically correct localized alveolar defects, as preprosthetic site development, as ridge preservation procedures, and to improve the outcome of single-tooth implants (Seibert, 1983a; Studer et al., 2000; Jung et al., 2004; Prato et al., 2004).

6.1.2 Current Treatment Concepts and Limitations
The use of autogenous tissue is associated with a variety of disadvantages including surgical dif-
difficulties, limitations with respect to the quality and quantity of tissue that can be retrieved, lack of color match with the surrounding tissue, prolonged healing time at the donor site and therefore an increased patient morbidity (Farnoush, 1978; McGuire and Nunn, 2005; Griffin et al., 2006; Soileau and Brannon, 2006; McGuire et al., 2008).

To overcome these limitations related to the use of autogenous tissue, various techniques and materials, predominantly of allogenic origin, have been developed. Acellular dermal matrix grafts (ADMGs) were among the first products introduced in dentistry. ADMGs were originally developed for covering full thickness burn wounds (Wainwright, 1995) and were later used in dentistry to increase the width of keratinized tissue, to deepen the vestibular fornix, and to augment localized alveolar defects (Wei et al., 2000; Batista et al., 2001; Harris, 2003). Even though ADMGs are still in use, results from clinical studies are not convincing due to a high shrinkage rate and histologic findings demonstrating a tissue that is substantially different from the oral mucosa (Wei et al., 2000; Wei et al., 2002). Recently, a cellular component has been added to dermal replacement grafts. The results from various studies suggest that these tissue-engineered grafts may be more favorable in terms of clinical outcomes although they may still be associated with difficult clinical handling (McGuire and Nunn, 2005; McGuire et al., 2008; Nevins, 2010).

In contrast to tissue-engineered products derived from human skin, collagen devices of xenogenic origin have been successfully used in dentistry for guided tissue and bone regeneration (GTR and GBR) (Hämmerle and Jung, 2003). These collagen devices are characterized by a hemostatic effect, early wound stabilization, chemotactic properties to attract fibroblasts, and semipermeability (Postlethwaite et al., 1978). A high degree of anastomosis in the vasculature in the regenerated tissue has been reported to be induced by the collagen membrane after subcutaneous implantation in rats (Schwarz et al., 2006).

Recently, a prototype membrane with characteristics similar to the most commonly used resorbable collagen membrane was developed with an additional indication for GTR procedures in periodontal defects. These prototype membranes will allow to further influence the healing cascade, reduce scar retraction, and will serve as a replacement for autogenous tissue to increase the width of keratinized tissue. Results from a randomized controlled clinical trial have demonstrated that this newly developed collagen matrix is as effective and predictable as the gold standard, the connective tissue graft, for attaining a band of keratinized tissue (Sanz et al., 2009).

6.1.3 Animal Models

Based on the different needs, animal models vary for devices/grafts intended to be used for augmentation of keratinized tissue and soft tissue volume. In the two-dimensional augmentation of keratinized tissue, successful integration into the surrounding tissue, degradation and replacement by connective tissue, color match with the surrounding tissue, and stability of the width of the augmented area are desirable. The histologic outcomes should be characterized by the absence of elastic fibers (as this is typical for the alveolar mucosa), an epithelium–connective tissue interface exhibiting rete pegs, and by a distinct keratin layer at the epithelial surface (Lozdan and Squier, 1969; Karring et al., 1971). The understanding of the tissue specificity of transplanted oral soft tissue goes back to the early 1970s. A series of animal studies demonstrated that the specificity of the epithelium is determined by the hereditary mechanism rather than by functional adaptation (Karring et al., 1971). In addition, the connective tissue of a transplanted free gingival graft appears to originate from the periodontal ligament, whereas the epithelium is most likely proliferated from the surrounding tissue (Karring et al., 1975).
The differentiation of the proliferated epithelium is then induced by the periodontal connective tissue (Karring et al., 1975).

Historically, the methods to augment keratinized tissue included: an apically positioned flap (APF) (Friedman, 1962); an APF in combination with autogenous tissue (Edel, 1974); and an APF in combination with allogenic tissue (Yukna and Sullivan, 1978). Therefore, for keratinized tissue positive control groups may include an APF and/or an APF in combination with a free gingival graft (FGG). Sites not undergoing treatment can serve as the negative control group.

For soft tissue volume grafting, three main parameters have to be evaluated clinically and by histologic and histomorphometric analyses when testing an artificial or autogenous graft: (1) successful integration of the device/graft into the surrounding tissue, (2) ability to degrade and being replaced by soft connective tissue, and (3) three-dimensional volume stability over time. Currently, autogenous soft tissue grafts, mainly the subepithelial connective tissue graft (SCTG), are considered as treatment of choice for soft tissue volume augmentation (Seibert, 1983a; Studer et al., 2000). Therefore, positive control groups may include the SCTG. Sham-operated sites and sites without any treatment may serve a negative control groups.

Since soft tissue augmentation surgeries were and are still performed using autogenous soft tissue grafts, available animal models are scarce. Early models used to determine the tissue specificity after auto-transplantation of oral soft tissues have limitations; they were not developed to observe two- and three-dimensional changes of width and thickness of augmented tissues (Karring et al., 1971; Karring et al., 1975). In other models, the palate in dogs served as defect site for the implantation of tissue-engineered mucosal substitutes (Ophof et al., 2004; Ophof et al., 2008). This animal model allows study of tissue integration, re-epithelialization, and revascularization of grafts. However, the palate appears to heal very slowly and no information can be derived with respect to the extent of the regenerated keratinized tissue (Ophof et al., 2008).

Soft tissue regeneration procedures have recently gained attention as new materials have been developed. However, with respect to allogenic devices and preclinical experiments, there is little information as well. This may have at least two reasons. First, allogenic devices have a long tradition in dermatology and were considered as transplants in some countries. This allowed transfer of information from extraoral usage into the oral cavity and probably prevented performance of additional preclinical research. Second, tissue-engineered allogenic devices include living cells from humans. Possible cross-over effects and subsequent auto-immunologic reactions can be expected when these devices are used in animal models.

Very recently, a variety of collagen-based and extracellular matrix-derived matrices of xenogenic origin have been developed for oral soft tissue augmentation and have also been tested in preclinical models and clinical studies (Badylak, 2002; Nevins et al., 2010; Thoma et al., 2010). For this purpose, new animal models specifically designed to evaluate devices for oral soft tissue augmentation (keratinized tissue and soft tissue volume) have been developed.

With respect to soft tissue volume augmentation, there is also a lack of suitable techniques for the measurement of volume changes (Thoma et al., 2009). Several methods have been described for noninvasive measurement of volume changes in the oral cavity. The techniques range from simple clinical observation (Allen et al., 1985) and 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 a recent study (Thoma et al., 2010), a three-dimensional optical method has been used to detect volume changes over time. The applied technique showed high reproducibility and excellent accuracy for measuring volume changes in a methodologic study (Windisch et al., 2007). A variety of studies have demonstrated that this method offers great advantages in being easy to apply, noninvasive, and precise (Windisch et al., 2007; Fickl et al., 2009; Strebel et al., 2009; Schneider et al., 2011). A broader use of this technique may be desirable in the future and will allow comparison of volume measurements between different studies.

It is expected that within the coming years the number of available models will continuously increase. The suggested animal models for soft tissue regeneration may potentially undergo changes and further refinements in the future.

6.2 Keratinized Tissue – Pig Model

6.2.1 Aim
The aim of this animal model is to evaluate and compare the tissue integration, the local tolerance, and the clinical performance of experimental devices to increase the width of keratinized tissue by macroscopic and microscopic analysis.

6.2.2 Advantages/Disadvantages
Defect sites that are close to the clinical situation characterize the advantages of this animal model. Sites with a reduced amount of keratinized tissue around teeth are augmented. In addition, a variety of clinical and histologic outcome measures can be evaluated:
• Macroscopically (clinically): width of keratinized tissue over time; thickness of keratinized tissue (by using stents); color match with the surrounding tissue; signs of local inflammation or intolerance; contraction of

defect sites (two-dimensional); and re-epithelialization.
• Microscopically (histologically): histological and histomorphometric measurements.

The disadvantages include possible growth of the animals during the study period resulting in difficulties in performing the clinical measurements and that markings serving as reference points may disappear.

6.2.3 Timing
The experiment is characterized by one surgical procedure. Tooth sites with a reduced amount of keratinized tissue are scheduled for augmentation surgery. On the day of surgery, the width of the keratinized tissue is increased using test and control groups. Follow-up time points are scheduled at 1 and 6 months. This allows studying the healing of the performed augmentation procedures over time with a variety of clinical and histologic parameters that can be analyzed (Fig 6-1).

6.2.4 Surgical Procedures
The surgical procedures performed in this experiment have a long tradition in mucogingival surgery and do not require special training. Surgical procedures to increase the width around dental implants and teeth are indicated for a variety of reasons and have gained further attention in recent years. This is predominantly due to the fact that a minimal amount of keratinized tissue has been considered necessary to maintain periodontal health and to prevent gingival recession around teeth (Nabers, 1966; Sullivan and Atkins, 1969). Surgical procedures to increase the width of keratinized tissue around dental implants have been proposed based on reports of higher survival rates of dental implants, health of the peri-implant mucosa, and an improved esthetic outcome (Adell et al., 1986; Artzi et al., 1993; Langer, 1996; Schrott et al., 2009). The proposed surgical procedures include an apically positioned flap or vestibulo-
plasty procedure (Thoma et al., 2009). These procedures are characterized by a displacement of the mucogingival junction in a further apical direction. The increased distance between the original position of the mucogingival junction and the position after flap positioning is allowed to allow to heal by secondary intention or covered with an artificial or autogenous graft (Jung et al., 2011).

6.2.5 Reparation
On the day of surgery, each animal is anesthetized using atropine for tranquillization, induction with tiletamine-zolazepam and then sodium thiopental, followed by inhalation of an O$_2$–N$_2$O isoflurane (1–4%) mixture. A preoperative injection of a local anesthetic is administered.

Prior to the surgery, the width and thickness of the keratinized tissue are measured using a periodontal probe. In order to reproducibly repeat the measurements (width of keratinized tissue, contraction of sites) at a later stage, four tattoo points are placed coronally, apically, mesially, and distally to each defect site. In addition, impressions are taken from each hemi-mandible using polyether impression material (Fig 6-2a). Individual acrylic stents are then fabricated and used to repeatedly perform measurements of the tissue thickness at five sites per defect area (Figs 6-2b and 6-2c).

6.2.6 Detailed Methodology
In each animal, two standardized gingival defects (1 cm vertical × 3 cm horizontal distance) are created bilaterally on the buccal side of the mandible. A horizontal incision is made in the keratinized gingiva 2 mm coronal to the mucogingival line (Fig 6-3a). Subsequently, two releasing incisions are made into the vestibular mucosa defining the lateral borders of the defect (Fig 6-3b). To elevate the flap, a split-thickness flap is elevated, creating a standard-
Each animal receives carprofen for analgesia for 3 days. The sutures are removed 14 days following surgery. The animals are fed a soft diet for the remainder of the study.

6.2.8 Endpoint Measurements

Clinical Observations and Measurements

At 1 month, photographs are taken of the defect sites (Figs 6-4a and 6-4b). The thickness and width of the keratinized gingiva are measured using a periodontal probe and individualized stents (Figs 6-2b and 6-2c). The defect sites are checked for signs of inflammation or local intolerance. The re-epithelialization of the defect sites is evaluated on the photographs by digital planimetry using an overlaid grid with 289 intersections and a 0.5 mm distance between them. The re-epithelialization is then
Fig 6-3  Surgical procedure. (a) Horizontal incision 2 mm coronal to mucogingival junction. (b) Two releasing incisions into alveolar mucosa. (c) Split-thickness flap is elevated. (d) Connective tissue is completely removed from defect area. (e) Denuded defect area. (f) Split-thickness flap is sutured at apical border of defect to the underlying periosteum; no further treatment is applied in the control group. (g) Device/graft is sutured in the defect area.
expressed in percentage of the entire grafted area. The contraction of each defect site is evaluated by measuring the gap between the horizontal and vertical tattoo points (height x length in mm). In addition, biopsy samples can be harvested from some or all of the animals.

At 6 months, the same macroscopic observations and measurements are performed as at 1 month (Figs 6-4a and 6-4b). Subsequently, the animals are euthanized. The soft tissues are resected together with the underlying bone and the adjacent intact gingival tissue. The coronal border of each biopsy sample is identified by means of a suture thread.

**Histological Processing and Analysis**

After fixation, each sample is decalcified, dehydrated in alcohol solutions of increasing concentration, cleared in isoparaffin H, and embedded in paraffin. Embedded samples are cut at 5 µm into three blocks (anterior, medial, and posterior according to the corono-apical axis) using a microtome. One section per block is prepared and stained with Masson’s trichrome.

All histologic sections are evaluated using a light microscope for qualitative and semiquantitative histologic analysis.

**Color Match**

For colorimetric analysis, the clinical photographs taken at baseline (prior to surgery) and after surgery at 1 and 6 months are digitized. The images are then assessed and analyzed according to standard colorimetric parameters (Commission Internationale de l’Eclairage [CIE] Lab; L = lightness, a = chroma along red-green axis, and b = chroma along yellow-blue axis). Two areas, one from the defect site and one from the adjacent keratinized tissue, are chosen for comparison (Fig 6-4b). The colorimetric difference between the two areas (ΔE) are calculated according to the following equation: 

\[ \Delta E = \left( (L_{\text{Graft}}-L_{\text{Adjacent tissue}})^2 + (a_{\text{Graft}}-a_{\text{Adjacent tissue}})^2 + (b_{\text{Graft}}-b_{\text{Adjacent tissue}})^2 \right)^{1/2} \]

(Jung et al., 2004).

**6.2.9 Statistical Analysis Plan**

Statistical analyses are performed to compare test devices and control sites at baseline, and at 1 and 6 months. Summary statistics (mean, standard deviation, minimum, and maximum) are used to describe the quantitative parameter. The groups are compared at each time point with analysis of variance (ANOVA). Summary statistics (frequency and percentage) are used to describe the ordinal parameter. The groups are compared at each time point by site with the chi-squared test of association for independent data and with the Cochran Mantel-Haenszel test for dependent data. For all tests a P value lower than 0.05 is considered as statistically significant.
6.2.10 Materials, Consumables, Equipment

Operating room: A purpose-designed room for experimental animals in accordance with the requirements of the FDA “Good Laboratory Practice” (GLP) Regulations.

Animals: Seghers Hybrid pigs, more than 2 years old, weighing between 50 and 61 kg.

Anesthesia:
- Tranquilization by atropine
- Induction by tiletamine-zolazepam, then thiopental sodium followed by inhalation of a O₂–N₂O isoflurane (1–4%) mixture
- Preoperative injection of a local anesthetic is administered.

Surgery:
- Standard surgical instrument kit for mucogingival surgery
- Test devices/graft disinfectant
- Non-resorbable sutures
- Polyether impression material
- Master casts out of dental stone
- Acrylic material for individualized stents
- Ink for tattoo points.

Postoperative care:
- Antibiotic treatment with spiramycin and metronidazole
- Analgesia with carprofen.

Analyses:
- Histology and histomorphometry:
  - Decalcification
  - Dehydration in alcohol solutions of increasing concentration
  - Embedding in paraffin
  - Microtome
  - Staining with Masson’s trichrome
  - A stereoscope with a video camera
  - An automated image analysis system.
- Color measurements:
  - An image analysis program.
- Clinical measurements:
  - Re-epithelialization
  - Using photographs and an overlaid grid with 289 intersections and a 0.5 mm distance between them
  - Thickness
  - Acrylic stents (see above) and a periodontal probe
  - Width of keratinized tissue
  - Periodontal probe or a caliper.

6.3 Soft Tissue Volume – Dog Model

6.3.1 Aim

The aim of this animal model is to histologically and volumetrically evaluate a potential device for soft tissue volume augmentation. For that purpose, single-tooth gaps with chronic ridge defects are created in a dog model.

6.3.2 Advantages/Disadvantages

This animal model is characterized by chronic ridge defects that are close to the clinical situation in single tooth gaps. Three defects sites can be prepared in each hemi-mandible and allow a variety of treatment modalities to be included. The use of chronic ridge defects minimizes bone regeneration as a result of the first surgical intervention (tooth extraction). Any volume changes may predominantly be a result of the second surgery (soft tissue augmentation). The tissue regeneration, which takes part following the preparation of the ridge defects, should be completed at the day of the second surgery.

Possible disadvantages include the need for a variety of interventions that are necessary to create the chronic ridge defects and to obtain volumetric measurements. The defect sites are not standardized on the day of soft tissue augmentation surgery (as it would be when using acute defects). Each defect site has an individual shape and form and therefore individual healing potential. In addition, the differentiation between native bone and regenerated
The surgical technique itself has been described extensively in the literature with a variety of modifications (Langer and Calagna, 1980; Seibert, 1983a, 1983b; Orth, 1996; Studer et al., 2000; Batista et al., 2001). In brief, a full- or split-thickness flap is elevated in sites with chronic ridge defects (osseous and/or soft tissue). Subsequently, either an artificial or autogenous graft is prepared and cut to fit into the recipient site and placed underneath the buccal flap. Periosteal releasing incisions are performed and help to suture the flap back to the lingual side, preventing compression of the augmented site (Thoma et al., 2010).

6.3.3 Timing
The experiment is characterized by two surgical procedures. Initially, single-tooth gaps have to be created during surgery 1. Subsequently, a healing period of 2 months allows the defects to heal and to establish chronic ridge defects. During surgery 2, augmentation procedures are performed using test and control grafts as well as a negative control group. The sacrifice time points are scheduled at 4 and 12 weeks. These two time points allow study of the volumetric and histologic changes over time (Fig 6-5).

6.3.4 Surgical Procedures
The soft tissue volume augmentation surgeries described in the following experiment are routinely performed in single-tooth gaps between dental implants and teeth predominantly to obtain an ideal contour for esthetic reasons. The surgical technique itself has been described extensively in the literature with a variety of modifications (Langer and Calagna, 1980; Seibert, 1983a, 1983b; Orth, 1996; Studer et al., 2000; Batista et al., 2001). In brief, a full- or split-thickness flap is elevated in sites with chronic ridge defects (osseous and/or soft tissue). Subsequently, either an artificial or autogenous graft is prepared and cut to fit into the recipient site and placed underneath the buccal flap. Periosteal releasing incisions are performed and help to suture the flap back to the lingual side, preventing compression of the augmented site (Thoma et al., 2010).

6.3.5 Preparation and Surgical Equipment
All surgical procedures are performed under general anesthesia and sterile conditions in an operating room using thiopental sodium solution 4%, 0.4 mL/kg body weight as a premedication. The dogs are placed on a heating pad, intubated, anesthetized with isoflurane 1.5–2%, and monitored with electrocardiography during the surgery.

Fig 6-5  Timing of surgical phases 1 and 2, and sacrifice time points.
6.3.6 Detailed Methodology

**Surgery 1 (Tooth Extraction)**

Crevicular incisions are made around all mandibular premolars (P) and the first molar (M1) and buccal and lingual flaps are reflected (Figs 6-6a and 6-6b). The teeth are sectioned in order to prevent fractures (Fig 6-6c). Following extraction of all mandibular P2, P4, and the distal roots of M1 (Figs 6-6d and 6-6e), the buccal plate of the extraction sites is removed and the defect sites are enlarged using a round bur (Figs 6-6d and 6-6e). Rubber dam is placed around the mesial root of each M1 on both sides of the mandible. The pulp tissue of the mesial roots is extirpated and the root canals are filled with gutta-percha and a sealer (Figs 6-6f, 6-6g, and 6-6h). The coronal portion of the pulp is filled using a self-curing composite material. Following rinsing with sterile saline, primary wound closure is obtained (Fig 6-6i).

**Surgery 2 (Soft Tissue Volume Augmentation)**

After a healing period of 2 months, surgery 2 is performed on all dogs under the same operating room conditions as surgery 1. Before starting the surgery, the mandibles are inspected and polyether impressions of the mandibles are made using the individualized trays (Figs 6-7a and 6-7b). 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
The following three treatment modalities are then randomly applied to the defects (Fig 6-8 and Table 6-1):

- **Group a:** test device/graft
- **Group b:** an autogenous SCTG (positive control)
- **Group c:** sham-operated site (negative control)

Mucoperiosteal flaps are elevated over the crest of the ridge (Fig 6-7c). The chronic ridge defects are then inspected and their height, depth, and width measured. A titanium pin is placed on top of the bone crest in the middle of each chronic defect to simplify histologic processing (Fig 6-7c). Periosteal releasing incisions are made to make room for volume augmentation.

**Fig 6-6** (continuation) Surgery 1. (e) P2, P4, and the distal root of M1 are removed; the defect sites are enlarged to establish chronic ridge defects. (f–h) Root canal treatment of the mesial root of M1. (f) Preoperative view. (g) Radiograph taken for determining the length. (h) Final radiograph taken after root canal treatment; the occlusal access hole has been closed using composite. (i) Primary wound closure has been obtained.
Fig 6-7  Surgery 2. (a) Preoperative impression using a polyether impression material. (b) Preoperative view after 2 months of healing following surgery 1. (c) Full-thickness flaps are raised; titanium pins are placed on top of the bone crest and in the center of the defects. (d) A connective tissue graft is harvested from the palate. (e) All treatment modalities are placed in the three defect sites (one control site in the mesial defect, the SCTG in the posterior defect, and the test device/graft in the middle defect). (f) Primary wound closure is obtained.

**Group a:** The test device/graft is applied according to the manufacturer’s recommendations. It is positioned in the pouch under the elevated buccal flap. A horizontal mattress suture is placed to immobilize the test device, connecting it to the lingual flap.

**Group b:** The autogenous SCTG is harvested from the palatal vault (Fig 6-7d). A U-shaped incision is made in the lateral part of the palatal vault and a mucoperiosteal flap is elevated. An SCTG is then dissected (dimensions: width 10 mm, length 12 mm, thickness 5 mm). Fatty
tissue, glandular tissue, and remnants of the epithelium are removed. Any bleeding in the palate is controlled by the use of local anesthetic, compression with a sterile gauze, and three to four single sutures. The SCTG is then folded once and positioned in the pouch under the elevated buccal flap. A horizontal mattress suture is made to immobilize the SCTG connecting it to the lingual flap (Fig 6-7e).

Group c: No further treatment is applied to the sham-operated sites (control). The buccal flaps in all sites are repositioned without tension to the lingual part. One horizontal mattress suture is placed over the buccal prominence created by the volume gain through the SCTG, and the test device to stabilize and stretch the grafts toward the vestibular fornix. The flaps are adapted using four to five single sutures to ensure primary wound closure (Fig 6-7f).

6.3.7 Postoperative Care

Surgery 1: For the first 7 to 14 days after extraction, the dogs are fed a soft diet. After a period of 7 to 10 days, the animals are briefly anesthetized with 1 mL/13.6 kg by IV – ketamine 50 mg/mL, xylazine 7.1 mg/mL, acepromazine 2.1 mg/mL, atropine 0.1 mg/mL; the sutures removed and teeth cleaned. At this time, polyether impressions of the mandible are taken from every animal. Master casts out of dental stone are obtained and individualized trays fabricated using light-curing tray material.

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

6.3.8 Endpoint Measurements

Volumetric Analysis to Evaluate Soft Tissue Volume Changes
Master casts are made out of dental stone utilizing the preoperative (baseline) and follow-up impressions at 28 days (dogs 1–6) and 84 days (dogs 4–6).

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Dog number</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>28 days</td>
<td>1</td>
<td>Test</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>SCTG</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Test</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>SCTG</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Control</td>
</tr>
</tbody>
</table>

Table 6-1 Randomization table for six dogs and two time points (28 and 84 days).
6.3 Soft Tissue Volume – Dog Model

For the evaluation of the dimensional changes at the defect sites, the casts are optically scanned with a three-dimensional camera (Fickl et al., 2009; Schneider et al., 2011) (Fig 6-9a). Since 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 direction are taken, including the canine, P1, P3, the mesial root of M1, and M2. The acquired data are then composed into one digital image using CAD/CAM software (Cerec 3D®, Sirona Dental Systems, Bensheim, Germany), encompassing the jaw segments from the canine to the second molar (Fig 6-9b). The obtained digital images of the casts reflecting the different treatment time points (baseline, 28 days, 84 days) are then transferred into another digital imaging software product (Match3D, University of Munich, Munich, Germany) (Figs 6-9c and 6-9d).

Fig 6-9  Volumetric measurements. (a) Master cast is scanned using a digital camera. (b) Digitized image of scanned plaster model; this image is transferred into another software program, capable of superimposing digital images. (c) Three-dimensional image of initial situation prior to augmentation surgery (baseline). (d) Three-dimensional image of clinical situation at sacrifice. (e) Superimposed image demonstrating volumetric changes between baseline and sacrifice; white color areas represent a gain in volume; red color areas represent a loss of volume; black areas show volumetric changes <50 μm. (f) Superimposed image including the measured areas (region of interest) of all three defects sites in blue color.
These images are superimposed and matched in one common coordinate system (Fig 6-9e). The buccal surfaces of the remaining teeth are used as reference points for the superposition of the different images. Subsequently, a defined area of interest at each defect site is measured and the volume difference between the time points is calculated. Due to an individually variable anatomic situation the measured area varies between the sites, but is kept constant at one site over time. The region of interest exhibits a trapezoid shape and reaches 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 mesiodistal dimension from one neighboring tooth (mesial) to the other neighboring tooth (distal) at a distance of 1 mm from the neighboring tooth (Fig 6-9f).

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

The obtained data is statistically analyzed regarding volume alterations in terms of different treatment modalities and time points.

**Histological Preparation**

The non-decalcified specimens are embedded in methyl methacrylate resin. Radiographic recordings are performed for each site in order to accurately determine cutting planes. From each specimen, one central orofacial section through the augmented defect (reference pin), one mesial (at a distance of 2 mm from the reference pin) and one distal (at a distance of 2 mm from the reference pin) section are prepared for histologic assessment. The longitudinal sections of 50 to 60 µm thickness are obtained by a micro-cutting and grinding technique adapted by Donath (Donath and Breuner, 1982). Thereafter, the sections are stained with toluidine blue and basic fuchsin.

**Descriptive Histology**

A qualitative analysis can be performed using a stereoscope, which allows evaluation of the different components according to the standard nomenclature of the International Society for Stereology (Exner, 1987). Digital images of the sections are acquired and descriptive histology is applied evaluating the following parameters: vascularization of the device/graft, remaining device/graft material, tissue integration of the device/graft, newly formed connective tissue, newly formed bone, and inflammatory reaction.

**Histomorphometric Analysis**

Computer-assisted histomorphometric measurements are performed using an automated image analysis system, coupled with a video camera mounted on a light microscope. The following parameters are calculated in all three sections: ridge width at four different levels (1.5 mm, 3.5 mm, 5.5 mm, 7.5 mm below the crest) including measurements of native bone, newly formed bone, and regenerated soft tissue (Fig 6-10).

**6.3.9 Statistical Analysis Plan**

The volume differences are assessed at 28 and 84 days, relative to the preoperative dimension of the defect inside a defined region of interest. The ridge widths are assessed at 24 and 84 days. Based on the two site values by dog and treatment, the mean values are always used in the statistical description and analysis. Measured parameters are summarized in terms of means and standard deviations. Volume differences and ridge width differences are analyzed with analysis of variance (ANOVA) in order to describe and compare the three treatment modalities. The paired t test is used in order to judge the mean changes within the treatment groups. The level of significance is set at $P < 0.05$. 
6.3 Soft Tissue Volume – Dog Model

Surgery 1 (tooth extraction):
- Standard surgical instrument kit for tooth extraction
- Disinfectant
- Non-resorbable sutures
- Standard endo kit for root canal filling of M1 (mesial root)
- Gutta-percha and sealer
- Portable radiographic machine.

Impressions:
- Polyether impression material
- Master casts out of dental stone
- Light-curing tray material for individualized trays.

6.3.10 Materials, Consumables, Equipment

Operating room: a purpose-designed room for experimental animals in accordance with the requirements of the FDA “Good Laboratory Practice” (GLP) Regulations.

Animals: male, large, hound-type dogs, more than 2 years old, weighing between 60 and 70 kg.

Anesthesia:
- Thiopental-sodium solution 4%, 0.4 mL/kg body weight as a premedication
- Isoflurane 1.5–2%.

Fig 6-10 (a) Representative histologic slide (original magnification ×2.5). Central section through center augmented site (middle defect). (b) Same slide including grid used for histomorphometric measurements; measurements are performed at four levels below the bone crest (1.5 mm, 3.5 mm, 5.5 mm, 7.5 mm). (c) Calculated measures shown in colors: yellow = old bone; blue = new bone formation; red = newly formed soft tissue.
Surgery 2 (soft tissue augmentation):
- Standard surgical instrument kit for mucogingival surgery
- Disinfectant
- Test devices
- Titanium pins as reference markers
- Non-resorbable sutures.

Postoperative care:
- Antibiotic treatment with spiramycin and metronidazole
- Analgesia with carprofen.

Analyses:
- Volumetric analyses
- 3D camera (Cerec 3 Bluecam®)
- Digital imaging software (Match3D).
- Histology and histomorphometry
- Poly(methyl methacrylate) resin
- Band-saw
- Microtome
- Staining with basic fuchsin and toluidine
- A stereoscope with a video camera
- An automated image analysis system.

References


