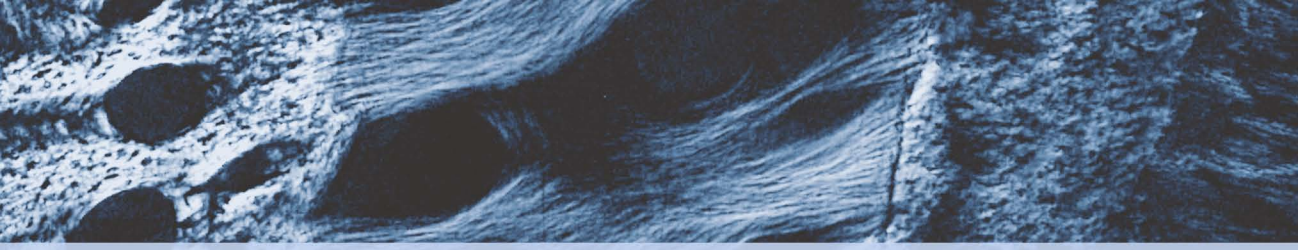
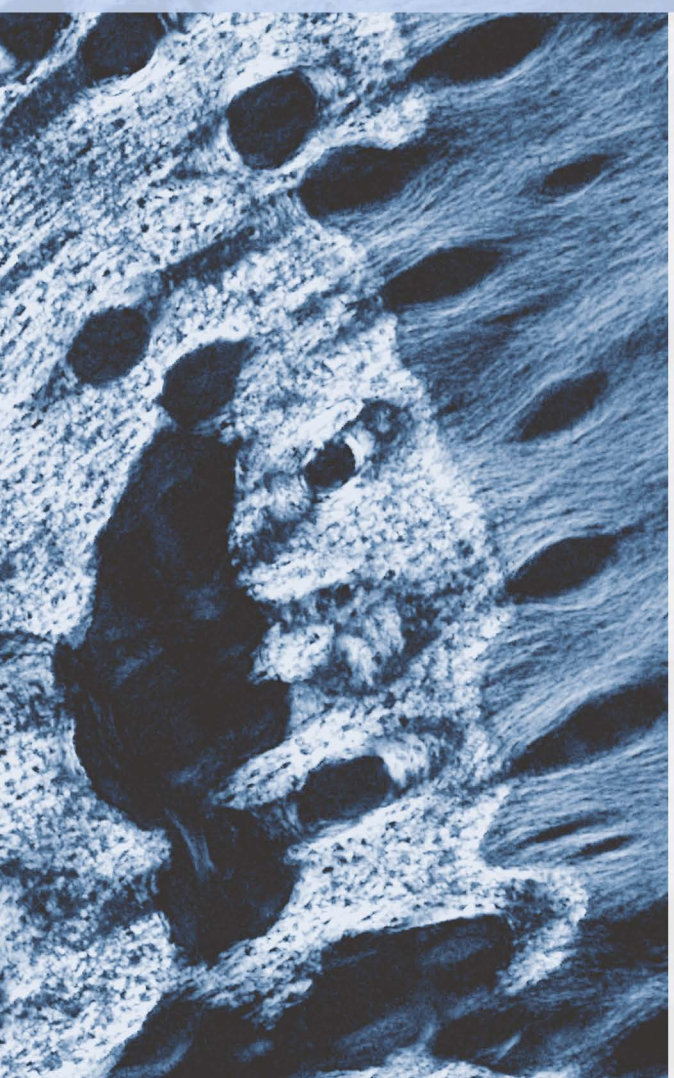


Osteology Guidelines for Oral & Maxillofacial Regeneration

Preclinical Models for Translational Research



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Histologic section illustrating the tooth attachment apparatus. The collagen fibers of the periodontal ligament span between bone and the root surface and insert as Sharpey's fibers into the cementum and into the bundle bone. Undecalcified ground section, unstained and viewed under polarized light. (Courtesy of PD Dr. sc. nat. Dieter D. Bosshardt, Head of the Robert K. Schenk Laboratory for Oral Histology, School of Dental Medicine, University of Bern, Switzerland)

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Vertical Ridge Augmentation

Isabella Rocchietta, David M. Kim, Khalid Al-Hezaimi, and Massimo Simion

11.1 General Overview

The evolution of osseointegrated implants required new regenerative concepts to increase the volume of bone to fulfill the basic tenet of the successful implant residing in bone. New concepts of guided bone regeneration (GBR) needed to be explored in preclinical models prior to human application as it is unethical to apply them without evidence of safety and efficacy. Proof of principle results are extended to small pilot and eventually to multicenter dosing and pivotal randomized controlled trials. Preclinical trials afford the opportunity to perform light microscopic and histomorphometric analyses by harvesting biopsy material (Yukna, 1976; Caton, 1980, 1994).

11.1.1 Advanced Osseous Defects

Horizontal and vertical ridge augmentation procedures are essential for restoring bone deformation following destructive periodontal disease, traumatic tooth extraction, endodontic infection, failing dental implant, or jaw trauma. Horizontal ridge augmentation procedures are routinely performed, but vertical ridge augmentation procedures are considered "elusive treatment" because of potential complications and limited success due to anatomic and technical difficulties. Conventional GBR, autogenous onlay bone grafting, and distraction osteogenesis have all been employed to correct severe bone loss (Nyman, 1991; Simion *et al.*, 1994; Jensen *et al.*, 2002; Levin *et al.*, 2007; Froum *et al.*, 2008). Each of these procedures has potential drawbacks relative to predictability (Simion *et al.*, 2009). Distraction osteogenesis for example may be associated with incomplete bone formation, which requires additional hard and soft tissue grafting and results in compromised esthetic outcome (Jensen *et al.*, 2002; Froum *et al.*, 2008). Autogenous bone grafts offer superior capacity to promote osteogenesis, but the graft harvesting is associated with undesirable

morbidity as well as block graft shrinkage. Thus, extent and configurations of osseous deformity will dictate potential hard tissue augmentation procedures. Predictable treatment modalities that can treat advanced osseous defects require investigation in both preclinical and clinical models.

Recent advances in the areas of biomaterials, and the isolation and application of signaling devices along with technical advancements have enabled clinicians to attempt predictable vertical ridge augmentation in both preclinical and clinical settings (Simion *et al.*, 2006, 2007a–c, 2008, 2009; Wikesjö *et al.*, 2004, 2008). This chapter will introduce appropriate surgical protocols for application of biologics for vertical bone augmentation with and without simultaneous insertion of dental implant. We will suggest a proven defect model for preclinical animal studies that will allow various treatment modalities to succeed.

11.1.2 Construction of the Research Protocol

The eight steps described below should be taken into consideration before embarking on a new preclinical study to test the safety and efficacy of biologics or surgical techniques for vertical ridge augmentation.

Determine What Materials or Techniques are to be Tested

Proper study design and clinical application can minimize or prevent trial and error from occurring in the initial study submission phase. It is important to conduct preclinical trials that have clinical applicability and relevance.

Select Appropriate Model and Method and Become Familiar with Previous Publications

Review of previous publications will give insights regarding proper animal selection as well as limitations of certain animal models. In larger animal models such as nonhuman primates or canines, the oral anatomy and wound healing physiology are similar to humans, and these models might be better suited for reconstructive surgery.

Define Critical-size Defect

Creation of a bony defect that is reproducible and resembles the atrophic ridge in humans is essential. Large bony defects or critical-size defects need to be defined for each animal to prevent spontaneous bone regeneration and to minimize substantial native osteogenic potential. It is also important to realize the limitation of artificially created defects in animals because the results obtained from under-standardized defect conditions do not necessarily imply that the same treatment will produce equivalent results in humans (Selvig, 1994).

Option of Assembling With or Without Implant

Rigid fixation of device or bone grafting material is needed to obtain maximal bone regeneration response and for initial stabilization of the blood clot.

Determine Adequate Sample Size and Length of the Study

Consultation with a biostatistician is recommended to determine an appropriate sample size so that the study will have adequate power. The length of the study should be determined ahead of time to minimize study costs and for ethical considerations regarding use of animals.

Understand Appropriate Analysis of the Results

Potential endpoints of the study can include the rate and amount of new bone formation, resorption of graft particles, and any signs of foreign body reactions. Clinical data collection, radiographic data interpretation, histologic and histomorphometric analyses should all be considered.

Endpoint Assessments

Initial consultation with a histologist is recommended to assess the outcome of the study. Sample preparation is as important as surgical preparation, and different tissue processing,

embedding and sectioning techniques can be employed by the histologist to answer the study hypothesis.

Establishing Study Budget
Regulatory requirements as well as the high costs of animal maintenance can exponentially increase the cost of the study. A thoroughly planned out budget is a prerequisite to conduct large animal studies involving canines, pigs, or monkeys. Consultation with a veterinarian as well as animal facility coordinator is recommended before submitting a budget plan.

11.2 Selection of an Appropriate Animal Model

Preclinical research trials provide an estimate of potential osteogenic value of a biomaterial so it does not lead to unexpected complications and negative outcomes for patients. It is important for researchers and clinicians to select an appropriate animal model that has phylogenetically similar oral structures to humans, and develop an experimental defect that is suitable to establish validity of the scientific principle (Habal and Reddi, 1992). The rodent models are frequently used for early experimentation, but extrapolation of clinically relevant data is not optimal due to their high potential for innate bone regeneration (Habal and Reddi, 1992). Their small oral cavity also interferes with surgical access and the delivery of devices to be tested. The vestibular side of the rodents' mandibular ramus is often selected to test vertical bone augmentation in the so-called "capsule model" (Kostopoulous and Karring, 1994). The approach consists of space provision with a Teflon capsule with an internal diameter of 5 mm sutured to the mandibular ramus. According to Kostopoulous and Karring, 70% of the capsule was filled with newly formed bone within the first 4 months, and this would be completed within the first year. This type of bone regeneration is referred to as ectopic beyond the normal bony envelope, and not applicable to the human model.

Larger animal models such as sheep, alpine goats, pigs (normal and minipigs), canines, and nonhuman primates have oral anatomy and wound healing physiology that are similar to humans, and might be better suited for reconstructive surgery. The anatomy and physiology of the cutaneous blood supply and the wound healing characteristics have made the pig a standard model for plastic surgical and wound healing studies (Kerrigan *et al.*, 1986; Mertz *et al.*, 1986). Bone and implant studies in the swine oral cavity are frequently reported; however, long tooth roots and the difficult behavior management does not establish it as a preferred animal model (Ruehe *et al.*, 2009). Sheep and goats are used for long bone defect repair (tibial mid-diaphysis is selected) (Giannoni *et al.*, 2008) or maxillary sinuses to test biologics (Nevins *et al.*, 1996; Gutwald *et al.*, 2010). The majority of the studies in the international literature where vertical bone regeneration is evaluated, suggest the canine model (Foxhounds, mongrel, and Beagles). Some authors used Wistar rats' maxillae to test a tissue engineered block in vertical bone regeneration (Shimazu *et al.*, 2006) while others (Tamimi *et al*., 2009) used New Zealand white rabbits.

11.2.1 Aim for the Use of the Canine Model

Canine or nonhuman primate models have strong applicability in the development of clinical guidelines and endpoints for the initiation of phase II and phase III human clinical trials. Economic considerations, demanding maintenance, as well as regulatory requirements may limit the use of nonhuman primates (Caton *et al.*, 1994; Pellegrini *et al.*, 2009). Hence, the canine emerges as the model of the choice for the vertical ridge augmentation. The investigative advantage of a preclinical model is an opportunity to create paired defects of equal size that may resemble bony defects in human (Selvig, 1994).

Table 11-1 Overview of animal models used in preclinical research on vertical ridge augmentation procedures

Animal model	Advantages	Disadvantages	Author/s
Nonhuman primates	Morphologic features similar to humans	Economics	Kleinschmidt and Hollinger, 1992; Pellegrini *et al*., 2009
	Phylogenetically similar oral structures to humans	Regulatory requirements	
	Minimal spontaneous repair	Demanding maintenance	
	Chronic and acute disease models	Small anatomic size of oral cavity hence technically challenging	
Dog	Morphologic features similar to humans	Economics	Rothamel *et al*., 2009; Simion *et al*., 2006, 2009; Wikesjö *et al*., 2004, 2008
	Microbiologic features similar to humans	Regulatory requirements in some countries	
	Oral cavity available to access		
	Favorable anatomic size of oral cavity		
	Easy maintenance		
	Minimal spontaneous repair		
	Reproducibly created		
	Chronic and acute disease models		
Pig	Oral cavity available to access	Demanding maintenance	Cestari *et al*., 2009
	Standard model for plastic surgical and wound healing studies	High self-regenerative bone healing potential	
		Long tooth roots	
Rabbit	Cost effective	Very high self-regenerative bone healing potential	Kon *et al*., 2009; Tamimi *et al*., 2009; Lundgren *et al*., 1995
	Easy maintenance		
	High number of animals per experiment	Oral cavity unable to access	
		Other anatomic structures available differ from human tissues	
		Raised mortality rate when general anesthesia is performed	
Mouse	Cost effective	Very high self-regenerative bone healing potential	Freilich *et al*., 2008; Shimazu *et al*., 2006
	Easy maintenance		
	High number of animals per experiment	Oral cavity difficult to access	
		Other anatomic structures available differ from human tissues	
		Small size of animal	

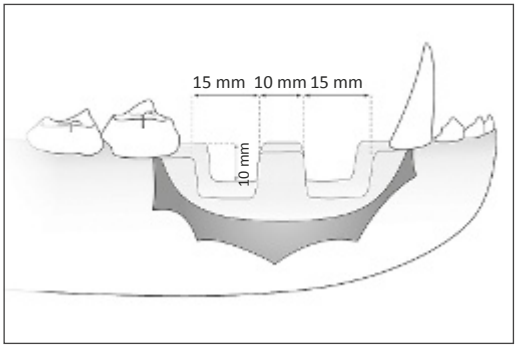

Fig 11-1 Schematic drawing of saddle-like defects in a canine mandible model.

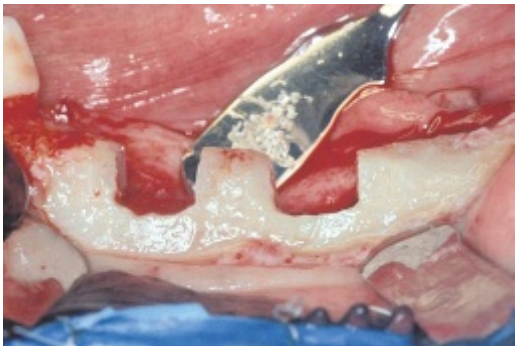

Fig 11-2 Clinical image of saddle-like defects in a canine mandible model.

Canine as a Large Animal Defect Model

A critical-size defect is too large to anticipate spontaneous bone regeneration caused by the native osteogenic potential. The defect size for the bone-regeneration procedure should emulate the atrophic jaw and should explore biologic potential of a surgical procedure. The mandibular jaw is preferred over the maxillary jaw due to proximity of the maxillary sinus around premolars and molars. Vertical bone regeneration may be tested with various research protocols for reconstructive therapy (Table 11-1).

- **Saddle-like mandibular defects**: GBR following cell exclusionary biologic principles of periodontal guided tissue regeneration, i.e., using barrier membranes, has been thoroughly tested using saddle-like mandibular defects in a canine model (Schenk *et al.*, 1994). With the advent of a multitude of barrier membranes to be tested, this model was used by many authors to evaluate their efficacy and potential (Dahlin *et al.*, 1990; Simion *et al.*, 1999). This defect was frequently used to test the barrier membrane. Two defects were created per mandible after extracting all four premolars and eliminating the buccal and lingual cortical plates. Spontaneous bone regeneration could be fostered from the mesial and distal walls as demonstrated by saddle-type defects (10 mm in depth and 15 mm in length, separated by 10 mm in length between two defects) in canine mandibles that achieved spontaneous bone fill of 60% in 3 months (Hunt *et al.*, 2001; Jovanovic *et al.*, 2007) (Fig 11-1 and Fig 11-2).
- **Supra-alveolar peri-implant defect model**: Investigators testing biologics for vertical bone regeneration may do so simultaneously with a dental implant or retention screw (Jensen *et al.*, 1995; Jovanovic *et al.*, 1995; Wikesjö *et al.*, 2006). The Hound Labrador mongrel is selected, and the alveolar bone is removed around the circumference of the mandibular premolar teeth to a level approximately 6 mm from the cemento-enamel junction. Then these teeth are extracted in addition to the first molar. On the same day, three dental implants are placed into the extraction site of the third (distal root) and fourth (mesial and distal roots) premolars. Then 10 mm long implants are placed to a depth of 5 mm (to the level of the reference notch), thus creating 5 mm supra-alveolar peri-implant defects. The maxillary first to fourth premolars are extracted to alleviate potential trauma from the maxillary teeth. The animals are euthanized at 8 weeks post surgery. This experimental model implies the use of a fresh wound with a high

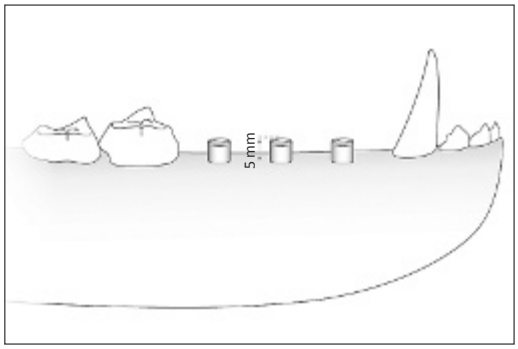

Fig 11-3 Schematic drawing of a supra-alveolar peri-implant defect model in a canine mandible. The implants are left protruding 5 mm from the bone crest.

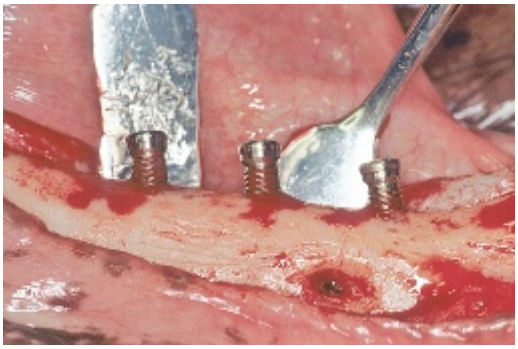

Fig 11-4 Clinical image of the supra-alveolar peri-implant defect model in a canine mandible.

self-regenerative potential of the alveolar bone. However, this condition never occurs in the clinical setting, in that patients present with chronic alveolar defects with no self-regenerative potential at all. The major advantage of this model is costs related to time duration of the experiment. The total duration is only 8 weeks (Fig 11-3 and Fig 11-4).

- **Chronic defect model**: A chronic defect model has been validated for the canine mandibular ridge defects to establish the appropriate biologics for vertical ridge augmentation (Simion *et al.*, 2006, 2007a, 2009). It is proven to be reproducible and reliable for assessment of the potential of a three-dimensional scaffold in conjunction with dental implant placement (Simion *et al.*, 2007b,c, 2008). A critical-size alveolar bone defect in foxhounds (weighing at least 25 kg) is created by bilateral removal of all four mandibular premolars (P1–P4) and surgical reduction of the ridge height and width with a carbide bur (size # 8) and a high-speed handpiece, or an acrylic bur and slow-speed handpiece. Adequate irrigation is required to avoid overheating the bone. The critical defect size dimensions of 20 to 25 mm mesiodistally, 7 to 8 mm apicocoronally and 10 mm buccolingually are created to mimic a flat atrophic ridge. A healing period of 3 months is allowed to create a chronic bony defect (Fig 11-5 and Fig 11-6).

11.2.2 Selection of the Species

In general, female foxhounds (aged between 8 to 24 months and weighing between 25 and 30 kg) that have been bred for research are recommended. Younger animals heal more completely and at a faster rate than their adult counterparts, thus false high expectations of the biologics or devices can occur (Kleinschmidt and Hollinger, 1992). Smaller animals usually present with small jaws and teeth, and larger animals are difficult to manage postoperatively (Wikesjö *et al.*, 1994).

11.2.3 Timing

Selection of appropriate time points to determine the therapeutic efficacy window of a candidate therapeutic is important (Pellegrini *et al.*, 2009). The limitation of the canine model described above is that this is not a naturally occurring bony defect, but an experimentally induced bony defect. Thus, it is very important to delay vertical ridge augmentation procedure up to 3 months post defect creation to diminish spontaneous bone regeneration potential and to confirm the chronicity of the created defects.