Osteology Guidelines for Oral & Maxillofacial Regeneration

Preclinical Models for Translational Research

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Preclinical Protocols for Periodontal Regeneration

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7.1 General Overview

The periodontium represents the tooth-supporting apparatus and can be described as a dynamic tissue complex, sensitive to a variety of factors, and with an inherent capacity that allows the translation of mechanical stimuli into biochemical signals that govern its homeostasis (Burger *et al.*, 1995; Duncan and Turner, 1995; Marotti, 2000; Marotti and Palumbo, 2007; Bonewald and Johnson, 2008). Its structure and function during remodeling and healing is determined by the orchestration of important bioactive proteins (platelet-derived growth factor [PDGF], vascular endothelial growth factor [VEGF], epidermal growth factor [EGF], fibroblast growth factor [FGF], bone morphogenetic proteins [BMPs], insulin-like growth factor 1 [IGF-1], transforming growth factor β-1 [TGF-β1], etc. (Long *et al.*, 2002; Sato *et al.*, 2002; Tsuji *et al.*, 2004; Yang *et al.*, 2006), thus resulting in an increased adaptive potential that protects and maintains the integrity of its four fundamental components: the alveolar bone, the periodontal ligament (PDL), the cementum, and the gingiva (Fig 7-1).

In humans, the detrimental changes that the tooth-supporting tissues undergo are primarily the result of inflammatory periodontal diseases that undermine and disrupt the functional and structural integrity of the alveolar bone, the PDL, and the cementum. The restoration of the original structure, properties, and function of these tissues represents a therapeutic advantage and the most ideal and desired outcome of periodontal therapy. Unfortunately, altered healing often disrupts the normal restoration of the periodontium and as a result different clinical compromised outcomes can be identified. Today, the available preclinical research models serve as the foundation of successful periodontal regenerative therapies. These *in vivo* animal models represent a valuable approach to elucidate the key factors or devices that promote periodon-
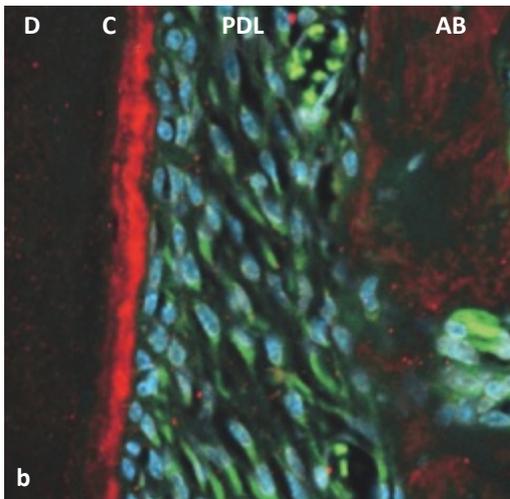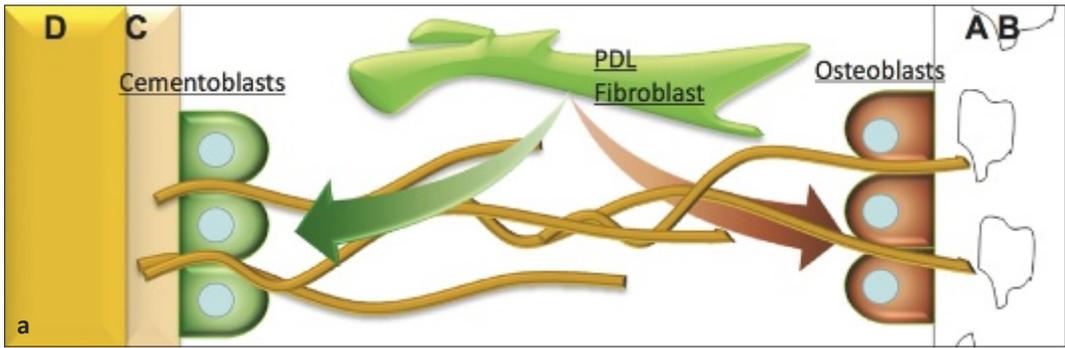
Fig 7-1 (**a**) The mature periodontium contains undifferentiated stem cells that retain the potential to differentiate into osteoblasts, cementoblasts, and fibroblasts. The PDL fibroblasts are important regulators of the periodontal integrity. (**b**) These subpopulations of fibroblasts (green) have the ability to prevent/regulate the invasion of bone (red) and cementum (red) into the PDL space and therefore maintain its structural homeostasis and regulate its remodeling potential. D, dentin; C, cementum; AB, alveolar bone.

tal regeneration and are critical for evaluating the biologic responses before human clinical trial testing.

Preclinical animal models allow the implementation of investigations for determination of the safety and efficacy of regenerative devices and biologics for periodontal repair. In this chapter we provide an overview of the commonly used preclinical animal models for the study of reconstructive procedures to promote bone and soft tissue repair of tooth-supporting periodontal defects. Steps are provided on the animal management for evaluation of outcome measures using descriptive histology, histomorphometry, three-dimensional imaging, and safety assessments. The use of these key measures of periodontal regeneration should aid investigators in the selection of appropriate surrogate endpoints to be utilized in the clinical arena, which are not practical or ethical in humans. These methods will prepare investigators and assist them in identifying endpoints that can then be adapted to human clinical trial planning.

7.2 Small Animal Models for Periodontal Regeneration

Rodents are the most commonly used animal models in biomedical research. Rats are cost-effective, easy to handle, and allow for the

Table 7-1   Advantages and disadvantages of small animal models (mice, rats)

Advantages	Disadvantages
Gives a proof of concept in a short interval	Small size, surgical microscopes required
Relatively low cost	Spontaneous healing
Known age	Narrow healing window
Known genetic background	Different anatomic structures compared to humans
Controllable microflora	Different histopathologic features
Ease of handling and housing	Different host responses compared with humans

standardization of experimental conditions in genetically similar individuals. Rats are suitable to study the effect of systemic diseases and pharmacologic therapies on tissue destruction and regeneration (Graves *et al.*, 2008), and to evaluate physiologic alterations related to aging (Benatti *et al.*, 2006). In addition, tissue destruction and regeneration in an immunodeficient background can be investigated in this model (Klausen, 1991). However, surgery and the evaluation of study endpoints are challenging because of the animals' small size. Moreover, the rodent dentition undergoes continuous tooth eruption, including bone and cementum apposition, which has to be considered in planning a study (Belting *et al.*, 1953) (Table 7-1).

7.2.1 Fenestration or Dehiscence Periodontal Regeneration Model

Rats are not susceptible to developing natural periodontitis. However, chronic inflammation that leads to periodontal destruction can be induced by placing a cotton or silk floss ligature in the sulci around the molars (Jin *et al.*, 2007). Chronic inflammation can also be achieved by repeated intragingival injection of bacterial lipopolysaccharide eliciting the release of proinflammatory cytokines by the host (Park *et al.*, 2007; Rogers *et al.*, 2007). Both models are suitable to evaluate the pathogenesis of periodontitis and therapeutic strategies to modulate disease progression (Graves *et al.*, 2008). Studies on reconstructive therapies, however,

require surgically created periodontal defects, as originally described by Melcher (1970).

The Rat Model
The *Rattus* (Rat) species have being recommended to use for this particular model. The fenestration periodontal wound defect model is widely employed and is accepted as an appropriate one to test periodontal wound healing in small animals. This model provides isolation from the oral environment, due to the extraoral approach, and thus can prevent negative effects such as contamination, and infection by saliva and other microorganisms.

Box 7-1 Aims of the Rat Model
The rat fenestration periodontal wound defect model is primarily adapted to determine the therapeutic efficacy of key factors, material or devices, and provide proof of principle before proceeding to a larger animal model.

7.2.2 Advantages/Disadvantages of the Presented Model

Advantages:
- Proof-of-concept in a short timeframe
- Well-contained defect
- No gingival tissue ingrowth.

Disadvantages:
- Narrow healing time window
- Technically challenging (small size)

- Rapid repair as kinetic healing model
- Not a "natural disease" model.

7.2.3 Timing

Recommended study evaluation time ranges from 2 to 6 weeks to capture early healing events and wound maturation. The early healing process follows the conserved sequence of wound healing that is initiated by blood coagulation and immigration of neutrophils and monocytes for wound debridement and bone resorption. This microenvironment favors the proliferation and migration of mesenchymal progenitors, which can originate from the PDL or the host bone (Lekic *et al*., 1996a,b). After 10 days, a thin cementum layer with a connective tissue attachment can be observed, in particular on the apical side of the teeth where the cementum is thicker compared with the narrow coronal region (King *et al*., 1997). Bone formation starts from the bone margins (Rajshankar *et al*., 1998). In young rats (King *et al*., 1997) periodontal regeneration is complete after 1 month, while geriatric animals at 18 months of age show a delayed healing capacity (Benatti *et al*., 2006). It is therefore crucial to select the appropriate time point to determine the therapeutic efficacy of a candidate molecule, material, or device.

7.2.4 Preparation

Animals require an acclimatization period of approximately 2 to 7 days after arrival in a new housing facility. The surgical area should be conducive to an aseptic surgery, and must not be used for any other purpose during the time of the surgery. Ideally, the surgical area can be located within the housing facilities, therefore limiting stress and potential health hazards to the animals. Disinfectants such as sodium hypochlorite, chlorine dioxide, dimethyl ammonium chloride, or glutaraldehyde-based solutions can be used to clean and disinfect the surgery area, although some may not be as effective at eliminating all contaminants. Animals and instruments must also be prepared in a way to prevent contamination and ensure success of the survival surgery.

Animal preparation for rodent surgery
Following anesthesia, ophthalmic ointment (lubricant) must be applied to the eyes of the animal receiving anesthetic to prevent drying. Incision sites must be cleared of hair if the incision is >1 cm, using clippers, a razor, or a depilatory agent. Hair removal should be performed in a separate location to avoid surgical area contamination. Skin must then be disinfected with three alternating scrubs of povidone-iodine topical antiseptic, and warm, sterile saline, water, or 70% ethanol (ethanol is less desirable) scrubbing in an outward and spiral direction. All instruments should be cleaned and sterilized (e.g., autoclaved) prior to surgery. Disinfection/sterilization of multiple sets of instruments should be carried out for successive surgeries. Following use, instruments should be thoroughly cleaned before sterilization. Hot bead sterilization is a fast, dry method to prevent cross contamination between animals during surgery. Alternative sterilization methods may incorporate the use of glutaraldehyde or chlorine dioxide immersion followed by a sterile water or saline rinse. Aseptic techniques and sterile environments are critical to animal survival and positive experimental results. Effective drug dosage may vary from animal to animal according to body weight, metabolism, and age. There are no exact calculations to relate the effective dose between animal and humans. Dosage can be determined by previous study results, published literature, and veterinary guidelines. Surgeons should also undergo appropriate preparation including, but not limited to, handwashing, wearing sterile gloves, gowns, and masks for each animal's surgical procedures.

7.2.5 Surgical Procedures

For the rat periodontal fenestration defect (King *et al*., 1997; Jin *et al*., 2004; Huang *et al*., 2005), an extraoral buccal approach should be used. After a flap is raised by extraoral access to expose the mandibular alveolus, the distal and buccal roots of the first molar and the mesial root of the second molar are denuded, includ-
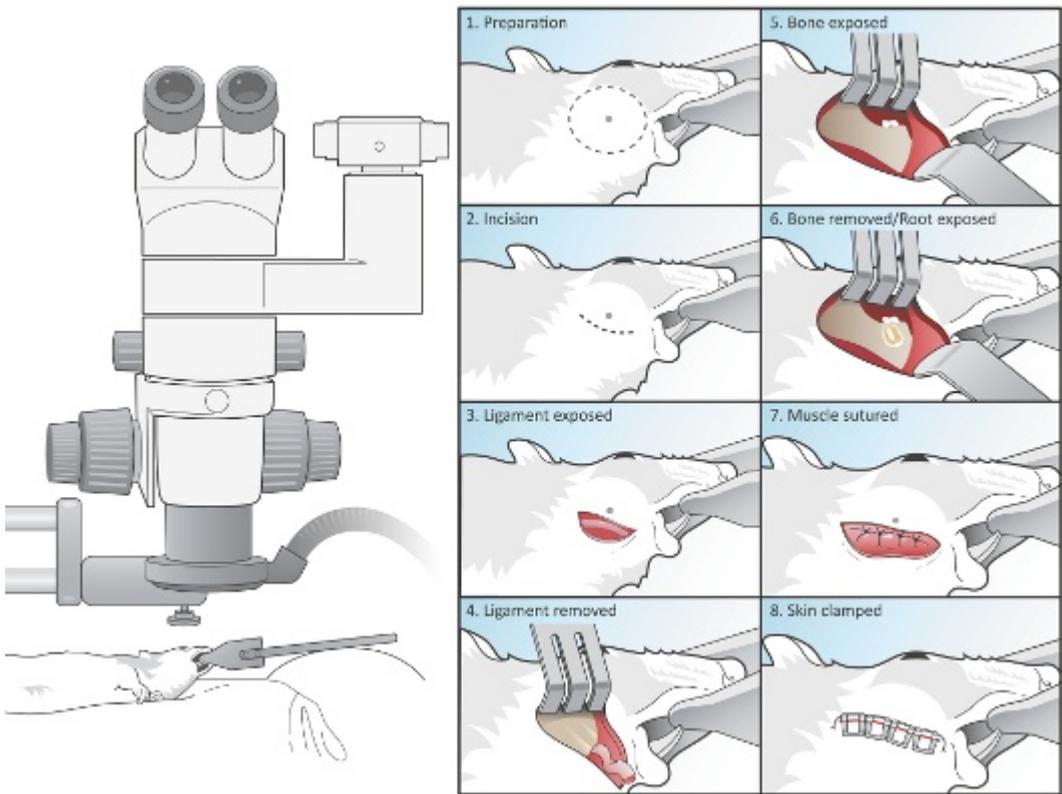
Fig 7-2 Surgical scope. Appropriate magnification with an external light source is essential to overcome the dimensional limitations of the rat fenestration defect model. (**1**) Preparation. Iodine disinfection should be performed together with proper isolation of the surgical site. Soft tissue and hard tissue landmarks should be identified prior to initiating any incision. (**2**) Incision. Incision is designed to expose in an anterior-posterior direction the surface of the masseter muscle. (**3**) Ligamentous line. After the initial cutaneous incision is completed, the operator should identify a ligamentous line that follows the trajectory of the lower border of the mandible. (**4**) Ligament removed. The dissection of the masseter muscle leads to the exposure of an anterior ligament that covers the area of the first molar in a more lateral position. This ligament should be removed to ensure proper access to the buccal plate at the level of the first molar. (**5**) Bone exposure. The periosteum should be elevated in the area of interest with proper flap reflection. (**6**) Defect creation. High-speed burs (no. 4 and ¼) are used to initiate the delineation of the defect and ensure proper cementum removal. (**7**) Muscle sutured. After the regenerative agent or construct is delivered to the defect site, proper would closure is first ensured by single interrupted sutures approximating the masseter muscle to its original position. (**8**) Skin clamped. Surgical staples are utilized to close the cutaneous incision and protect the surgical site. Proper wound stabilization should be maintain for 2 weeks.

ing the superficial dentin. New medical formulations (NMF) can be delivered into the fenestration defects (3 × 2 × 1 mm) and secured by flap repositioning. The surgery should be performed under a magnifying stereoscope (×2 to 10) to allow proper identification of anatomic landmarks and site preparation (Fig 7-2):

1. After preparing the animal for surgery, proper identification of epithelial and hard tissue landmarks should guide the operator for the initial incision.
2. A superficial incision is first used to expose the masseter muscle and gain access to a ligamentous landmark that extends in a pos-