Contribution of Animal Models in Periodontal Research

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Abstract

Aim The aim of this review is to define the most pertinent animal models for periodontal research.

Background In periodontal research, animal studies are complementary to in vitro experiments prior to testing new treatments. Animal models and cell cultures have contributed new knowledge in biological sciences, including periodontology. Although cultured cells can be used to study physiological processes that occur during the pathogenesis of periodontitis, the complex host response fundamentally responsible for this disease cannot be reproduced in vitro. Animal models should make possible the validation of hypotheses and prove the safety and efficacy of new regenerating approaches using biomaterials, growth factors or stem cells.

Review results Among the animal kingdom, rodents, rabbits, pigs, dogs, and nonhuman primates have been used to model human periodontitis, each with advantages and disadvantages. Experimental models have been developed in order to reproduce major periodontal diseases (gingivitis, periodontitis), their pathogenesis and to investigate new surgical techniques.

Conclusion A practical and highly reproducible model that truly mimics the natural pathogenesis of human periodontal disease has yet to be developed.

Clinical significance The anatomy, physiology and pathogenicity of experimental models should relate as much as possible to those of patients in order to demonstrate the safety and efficacy of new biomaterials or treatments in periodontal regeneration.

Key words: Biomaterials, experimental animal, periodontal disease.

Background

Periodontitis is an inflammatory disease of the gums, which affects all the dental supporting tissues. This immune-inflammatory disease of the periodontium results in progressive loss of
gingival tissue, the periodontal ligament, and adjacent supporting alveolar bone\textsuperscript{1}. The objective of periodontal treatment should ultimately be to regenerate the periodontal tissue by using non-surgical or surgical techniques, biomaterials for guided tissue regeneration, bone substitutes (e.g. Calcium phosphates or others), growth factors (e.g. enamel matrix derivatives) or, as more recently proposed, mesenchymal stem cells\textsuperscript{2}. Research in periodontology involves different strategies, principally the etiology of periodontal diseases using experimental models of periodontitis, and the regeneration of damaged periodontal tissues by surgically creating bone defects in combination, or not, with experimental periodontitis. Animal models have contributed to the generation of new knowledge in biological sciences, including periodontology\textsuperscript{3}. Periodontal disease can occur naturally or be experimentally induced in animals. Various species have been used to study the pathogenesis of periodontitis and to assess therapeutic modalities against the disease. Appropriate experimental animal models are required for testing and validating new regenerative therapies for damaged periodontal tissues. Animal studies are effectively complementary to \textit{in vitro} experiments prior to testing new clinical treatments where biopsy harvesting for histology is generally not accepted in human. Different animal species could be used for modeling periodontitis and treatments, but primates, dogs, rats, rabbits, pigs, hamsters and ferrets are the most commonly employed. Experimental animal models should be used with care, particularly primates and canines, for ethical reasons\textsuperscript{4,5}. Concerning etiopathologic studies, non-human primates are preferred but large series are not ethically and economically acceptable. Small animal models (e.g. rats or hamsters) have been developed for periodontal research but these studies mainly focus on bacteriology and immune response. Depending on species, periodontal diseases could be induced spontaneously, experimentally or both. In the context of regenerative medicine using biomaterials, large animal models have been preferred due to the reproducibility and surgical accessibility of experimental defects. Apart from monkeys, which are the ideal model in pre-clinical studies, dogs have been widely used for modeling the regeneration of periodontal defects with biomaterials. Some studies have also used rats, mini-pigs, sheep, rabbits and cats. The aim of this review is to summarize the experimental animal models used for periodontal research.

\textbf{Review results}

The selection of an experimental model is determined by research objectives, as well as laboratory constraints such as housing of large or non standard animals. In most cases, small animal models such as rats or hamsters will be sufficient to assess the role of bacteria, diet or other factors in periodontal inflammation at the histological level, providing sufficient statistical significance and pre-clinical relevance. The use of large animals with ethical and social issues such as monkeys and dogs should be reserved for last phase validation of new treatments prior to use in human clinical practice. Experimental studies conducted in monkeys are highly relevant for human clinical practice as they present comparable anatomy and develop similar periodontal diseases with similar clinical symptoms. However, experimental research in monkeys requires a strong ethical justification of their care and use and should take into account the purchasing, transportation and housing expenses of these animals over long periods. The selection of these animals was based on similar pathologies and the ease of surgically created clinically relevant defects. Experimental periodontal defects may be obtained in three different ways\textsuperscript{6}:
(i) The acute defect model 

(ii) The chronic defect model 

(iii) The acute/chronic defect model.

In the acute model, all defects are surgically-induced by removing surgically all the periodontal components (bone, cementum and periodontal ligament). Reproducible defects in experimental and control sites are created. In the chronic model, lesions are obtained by placing orthodontic elastics, silk sutures or ligatures around teeth during 12 to 20 weeks, depending on the type of animal studied. These defects are deeper in the interproximal spaces than in the buccal or lingual surfaces. In the combined acute/chronic model, the defects are surgically-created and ligatures are placed to ensure calculus accumulation and to prevent spontaneous regeneration of the defects. Different animal models used in periodontal research based on pathogenesis of periodontal disease are non-human primates, dogs, minipigs, ferrets and hamsters. They all have showed excellent results during experimental studies. These animal models are also used in periodontal research based on periodontal treatment modalities.

Discussion

Various experimental animal models in periodontal research

1. Non-Human Primates

Nonhuman primates have oral structures and teeth similar to those of humans and have naturally occurring dental plaque, calculus, oral microbial pathogens (e.g., P. gingivalis), and periodontal disease. In particular, rhesus monkeys (Macaca mulatta), cynomolgus monkeys (Macaca fascicularis), and baboons (Papio anubis) are susceptible to naturally occurring periodontal disease. All these species are Diphyodont and have the same dental formula as human: I 2/2, C 1/1, Pm 2/2 and M 3/3. Although periodontitis in primates most closely resembles the human disease, the expense of and special husbandry requirements for these animals limit their use in periodontal studies.

2. Miniature Pigs

Miniature pigs have oral and maxillofacial structures similar to those of humans in terms of anatomy, physiology, and disease development. The Minnesota miniature pig (minipig) was developed about 60 years ago and has been used extensively in biomedical research. After the age of 6 months, minipigs usually develop gingivitis, manifested by inflamed gingival tissue, accumulated plaque and calculus, and bleeding when probed. There is infiltration of inflammatory cells in the gingival tissue that results in progression to severe periodontal inflammation at 16 months of age with identical histopathology to that seen in humans. Periodontitis in minipigs is promoted in about 4–8 weeks using ligatures, and in association with bacterial inoculations of P. gingivalis, S. mutans, and A. actinomycetemcomitans. Minipigs can be suitable for periodontal as well as orofacial investigations. However, minipigs are relatively expensive, with husbandry issues and few studies to support their use.
3. **Dogs**

Many experimental studies on gingival and periodontal diseases have been conducted in dogs. The beagle is one of the most commonly used due to its size and its extremely cooperative temperament. Globally, all periodontal tissues and the size of the teeth are quite similar to those observed in humans. However, some major differences exist between dogs and humans as the lack of lateral movements, no occlusal contacts for all the premolars and presence of open contacts between teeth. The frequent lack of gingival sulci and crevicular fluid, a different composition of periodontal plaque and calculus are other important differences between dogs and humans\(^1\). All dogs are diphyodont with deciduous and permanent dentition. The formula for permanent dentition is I 3/3, C1/1, Pm 4/4, M 2/3.

4. **Mice**

The Baker mouse model of periodontitis has been used to measure alveolar bone resorption caused by oral bacterial inoculums as an outcome for the clinical presentation of periodontitis in humans\(^1\). The dental formula of mice is typical rodent dentition: I 1/1, C 0/0, Pm 0/0, M 3/3.

Mice naturally develop periodontitis starting at about 9 months of age with further increases as a function of age, similar to human periodontitis. This model, however, may not reproduce all aspects of human periodontitis initiation and progression; the bacteria used are one or two of at least 150 microbial types present in any dental plaque biofilm. However mice can be utilized to understand the host-parasite interaction\(^1\).

5. **Rats**

The rat is the most extensively-studied rodent for the pathogenesis of periodontal diseases. Typical rodent dentition is I 1/1, C 0/0, Pm 0/0, M 3/3. The incisor is rootless. The structure of the dental gingival area in rats is quite similar to that observed in humans\(^1\), with a shallow gingival sulcus and attachment of the junctional epithelium to the tooth surface. The most commonly-used strains are Wistar or the Spraque-Dawley. In rats, periodontitis appears to be an infectious process. Inoculations or injections of various periodontal pathogens such as *Prophyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Capnocytophaga*, *Eikenella corrordens*, *Actinomyces viscosus*, and *Streptococcus sobrinus* can induce periodontal lesions\(^1\). Furthermore, rats are easy to handle.

6. **Hamsters**

In hamsters, periodontal disease does not occur spontaneously but may be obtained experimentally. The golden Syrian hamster is the most commonly used. The dentition formula is identical to rodents: I 1/1, C 0/0, Pm 0/0, M 3/3. Histologically, the structure of the periodontal tissue is very similar to that of rats\(^1\) but due to the small size of this kind of animal, the interdental septum is narrower than in rats. Spontaneous periodontal disease was obtained using an appropriate diet containing high concentrations of carbohydrates, particularly sucrose\(^1\). In summary, as in rats, the inflammatory response is very limited in hamsters and is very different from that observed in humans. The mechanisms of alveolar bone resorption in hamsters with diet-
dependent periodontal lesions are quite similar to those observed in rats infected with Gram-positive bacteria.

7. **Ferrets**

Ferrets have a deciduous and permanent dentition. The formula is I 2/2, C 1/1, Pm 4/4, M 2/2. Ferrets (*Mustela putorius*) naturally develop calculus and periodontal disease similar to humans\textsuperscript{18,19}. Unlike rodents, calculus formation in ferrets does not depend on the diet and can be scored in live ferrets. Ferrets are a suitable model to study calculus; however, they can easily escape from standard cages and they need special maintenance.

8. **Minks**

The dentition formula of the adult mink is I 3/3, C 1/1, Pm 3/3 and M 1/2. In minks, spontaneous periodontitis, which is age- and plaque-dependent, is observed. Nevertheless, the extent of this periodontal disease appears to be severe only in very old animals. In minks, neutrophils play a key role in periodontal destruction due to deficiencies in the chemotactic response and massive release of lysosomal enzymes and proteases into periodontal tissue. Minks are therefore interesting experimental models in research on the etiology of periodontal diseases. Nevertheless, housing these animals may be difficult or require specific authorizations that may explain the absence of recent publications in the literature.

9. **Rabbits**

Characterization of the oral microorganisms in rabbits showed numerous pathogenic bacteria, including *F. nucleatum, P. heparinolytica, Prevotella spp., P. micros, S. milleri* group, *A. israelii*, and *A. haemolyticum*, which is somehow consistent with the flora related to periodontal disease in humans\textsuperscript{20}. Rabbits have been used for creation of surgically induced periodontal defect and to study periodontal regeneration, but they have been found less suitable for regeneration of periodontal ligament\textsuperscript{21}.

10. **Other Animals**

Other animal models have been investigated for modeling periodontal diseases.

Horses- Common naturally occurring oral diseases in horses include buccal abrasions, calculus, gingival recession, and periodontal pockets. According a recent equine survey, the prevalence of periodontal pockets and gingival recession is highest in older horses and mostly associated with other dental disorders and tooth loss\textsuperscript{22}. Because of their size and husbandry considerations, horses are not a practical model for basic science studies of periodontitis or for testing of potential therapies.

Sheep- They have also been studied in the context of periodontal diseases. The permanent dentition of sheep consists of 32 teeth with a formula: I 0/3, C 0/1, Pm 3/3, M 3/3.
One publication\textsuperscript{23} used the cat as animal model. In this research, class III furcation defects were surgically created at the level of the premolars in order to study ankylosis at root level during periodontal healing.

Few studies have reported mini-pigs for research, mainly related to dental implant surgery and periodontal regeneration by enamel matrix derivatives\textsuperscript{24,25}, as well as the effects of dental lasers on periodontal healing\textsuperscript{26}.

**Table 1.** Advantages and disadvantages of select animal models for studying periodontal disease development

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>Non Human Primates</td>
<td>Similar dental structure, microflora, and disease to humans’. Natural or experimentally induced periodontitis.</td>
<td>Very expensive, with ethical and husbandry issues</td>
</tr>
<tr>
<td>Dogs</td>
<td>Develop natural or experimental periodontitis similar to humans</td>
<td>Relatively expensive, need special daily care, husbandry issues. Dentition different from humans.</td>
</tr>
<tr>
<td>Minipig</td>
<td>Dental structure and periodontitis have some similarity to humans’. Natural or experimentally induced periodontitis</td>
<td>Relatively expensive, husbandry issues; relatively few studies</td>
</tr>
<tr>
<td>Ferrets</td>
<td>Naturally or experimentally induced disease with similarity to humans</td>
<td>Some husbandry issues</td>
</tr>
<tr>
<td>Rodents</td>
<td>Experimentally induced disease. Similar molar structure to humans’. Inexpensive model</td>
<td>Naturally resistant to periodontitis. Different microbiota from humans’. Small size and therefore amount of tissue for analysis. Large number of animals needed</td>
</tr>
</tbody>
</table>

**Conclusion:** Experimental models for periodontal diseases are essential for understanding the origin and evolution of the pathology in humans. The use of animal models in periodontal research is a necessary step prior to entering into clinical trials with new biomaterials and treatments. Each animal model for periodontal disease has advantages and disadvantages (summarized in Table 1). A more systematic use of these small animal models appears evident for future research, especially from a surgical point of view.

**Clinical significance:** The anatomy, physiology and pathogenicity of experimental models should relate as much as possible to those of patients in order to demonstrate the safety and efficacy of new biomaterials or treatments in periodontal regeneration.

**References**
ANIMAL MODELS IN PERIODONTAL RESEARCH


