

Selection of Animal Models in Dentistry: State of Art, Review Article

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Abstract: Animal experimental models are widely used in medical sciences in general and particularly in dentistry. The reasons for using animal models in dentistry are varied and included satisfying study hypothesis, understanding of progression of pathogenesis of certain diseases including periodontitis. Animal models are also used for other scientific aspects such as testing new medicines prior to their applications in clinical practice. The main challenge is how to select the appropriate animal experimental model. In this review, we reviewed various proposed models to be used in dentistry. Which models to be used and when to be used and what are the conditions that determine the right selection were discussed. Taken together, the use of animal models in dentistry is crucial to establish new findings, prove hypothesis of the study. There are many animal models that have been proposed to be used in dental experiments. Actually, there is no unique animal model for all disease and accordingly, animal model has to be carefully selected.

Key words: Animal models, dentistry, periodontal diseases, implantation, histology, inflammation, microbiology, immunology

INTRODUCTION

Significance of using animal models: The use of animal models is crucial in cases that require developing new dental and orthopedic implants in addition to related biomaterials. Accordingly, animal models must be used prior to application of these materials in humans. It is worth mentioning that although *in vitro* methods including cell and tissue culture may be used at initial stages of testing new materials but these *in vitro* techniques lack the sufficiency to identify biocompatibility of materials, mechanical functioning and safety for being applied in humans (Pearce *et al.*, 2007; Muschler *et al.*, 2010).

WHEN TO USE ANIMAL MODELS

According to ISO 10993-2 (2006), it is required for using experimental animals to test biomedical devices that there is a lack of other sources to obtain data accompanied by the necessity to describe the tested material. An example illustrating the need for using animal models is the need to assess the local effects of an implant after implantation (ISO 10993-6, 2007).

Animals used in dentistry include rodents, rabbits, goats, sheep, dogs and pigs (ISO 10993-6, 2007). These animals have their advantages and disadvantages. Rabbit is considered as an exception due to being its least similarity to humans in respect to histological bone structure and remodeling (Pearce *et al.*, 2007). The reason beyond that is rabbit long bones are generally

made of primary bone tissue compared to the secondary bones in humans (Wang *et al.*, 1998, Martiniakova *et al.*, 2005). Another study indicated that healing of rabbit bones are faster compared to human. As an example, a dental implant fixed in a long bone of rabbit may be osseointegrated within 6 weeks while it takes in humans 3-4 months (Albrektsson *et al.*, 1981). According to this context, it is not an easy task to apply findings from rabbits on humans (Pearce *et al.*, 2007).

EXPERIMENTAL ANIMAL MODELS IN PERIODONTOLOGY

In their study, Struillou *et al.* (2010) have focused on the use of animal models in periodontology. According to the context of their views, animal experiments are required in periodontal studies to complete findings of *in vitro* experiments prior to testing new treatments. Animal models are also used in the purpose of possible validation of hypothesis. Another purpose of using animals in such studies is to provide evidence regarding the safety and efficacy of emerging biomaterials, growth factors or stem cells. According to researchers, several animal models including various species such as rats, hamsters, rabbits, ferrets, canines and primates have been proposed to be used in human periodontal diseases and treatments. It is worth to mention that due to variations in anatomy and physiopathology between humans and animals, it is difficult to make assessment for new therapies. According to this context, experimental models have been

developed to reproduce major periodontal diseases (gingivitis, periodontitis) their pathogenesis and to investigate new surgical techniques.

Tobita *et al.* (2008) pointed to the origin of periodontal diseases to be from infectious origin so that the presence of oral bacterial biofilm in gingival and periodontal tissues which simulates an immune-inflammatory response leading to a possible progressive destruction of the structural components of the periodontium. Accordingly, the clinical signs of periodontitis appear accompanied with the damage of the adjacent tissues which leads to tooth loosening. Various therapeutic approaches have been proposed to arrest the progression of the disease including scaling and root-planning in addition to oral hygiene. Periodontal treatment purposes to regenerate the periodontal tissue through the use of either non surgical or surgical techniques. Other techniques include the introduction of 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.

It is required to have good experimental animal models for testing and validating new regenerative therapies for damaged periodontal tissues. Animal studies can integrate effectively *in vitro* experiments before testing new clinical treatments. Although, several animal species have the potential to be used in for modeling periodontitis and treatments, primates, dogs, rats, rabbits, pigs, hamsters and ferrets are most used (Madden and Caton, 1994; Selvig, 1994).

There are various strategies in periodontology research including the etiology of periodontal diseases and the regeneration of damaged periodontal tissues through surgical creation of bone defects in combination or not with experimental periodontitis (Struillou *et al.*, 2010).

It has been recognized that the results of animal studies in which new biomaterials and therapies designed for medical applications other than periodontal diseases were used are not particularly valuable in the specific periodontal context. This is due to considerations that

periodontal lesions look as open and non vascularized cavities accompanied with chronic inflammation in the tissues which requires the use of specific experimental models. In regard to studies that focus in etiopathology, it is preferred to use non-human primates. Here, it is worth to mention that ethical and economical considerations are limiting factors to use large animals and accordingly small animal models including rats or hamsters have been developed for periodontal research with prime concern on bacteriology and immune response (Struillou *et al.*, 2010).

It has been proposed that animal species determines how periodontal disease can be induced spontaneously, experimentally or both. As an example, in case of applying biomaterials in regenerative medicine, it is preferable to use large animal models because of two reasons: the reproducibility of findings and accessibility for experimental defects. Monkeys are considered the ideal model in pre-clinical studies. Dogs have also been used for modeling the regeneration of periodontal defects with biomaterials. Other researchers have used other species including rats, mini-pigs, sheep, rabbits and cats. It is of extreme importance to emphasize that several methodologies have been proposed to ensure that models are reproducible and permits possibility of statistical analysis (Struillou *et al.*, 2010).

HOW TO CHOSE ANIMAL MODELS FOR PERIODONTAL RESEARCH

An experimental model is chosen based on research objectives and laboratory constraints including housing of large or non standard animals. However, it is recommended to delay the use of large animals including monkeys and dogs that are accompanied by ethical and social issues for the last phase validation of new treatments before being used in human clinical practice. It has been shown that small animal models including rats or hamsters are adequate to evaluate the role of bacteria, diet and other factors in periodontal inflammation from a histological point of view to provide a sufficient statistical significance and pre-clinical relevance (Struillou *et al.*, 2010) (Table 1).

Table 1: Decision making of laboratory animal model

Models	Research based on pathogenesis of periodontal disease			Research based on periodontal treatment modalities	
	Disease etiology	Calculus	Microbiology and immunology	Biomaterials	Implants
Non-human primates	Excellent	Medium	Excellent	Excellent	Good
Dog	Good	Good	Good	Excellent	Excellent
Minipig	low	Low	Low	Good	Medium
Rabbit	Low	Low	Low	Medium	Medium
Ferret	Medium	Good	Low	Low	Low
Rat	Low	Medium	Good	Medium	Low
Hamster	Low	Medium	Good	Low	Low

Struillou *et al.* (2010)

EXPERIMENTAL GINGIVITIS AND PERIODONTITIS

Studying periodontal diseases using animal models should be based on having characteristics of disease process that are similar to those in humans (Oz and Puleo, 2011). Attstrom *et al.* (1975) showed that experimental findings derived from monkeys to be highly relevant for human clinical practice because they share comparable anatomy and their periodontal diseases are similar to human clinical symptoms. The researchers also expressed their views in rationality of using dogs since there is a high rate of periodontal diseases in dogs and these diseases are thought to increase with ageing and accordingly their etiopathology follow similar patterns like that in humans.

Several studies have pointed to differences in the inflammatory response and in the bacterial population. As an example, in humans and dogs, calculus deposits induce gingivitis, dogs have some variations in which the sub-connective tissue remains almost normal. It has also been indicated to the possibility of converting gingivitis to periodontitis in dogs by fixing ligatures around the teeth (Lindhe and Ericsson, 1978; Schroeder and Attstrom, 1979; Soames and Davies, 1980).

Other studies pointed to the suitability of rodents and rats from ethical point of view to be used as relevant models for experimental periodontal research. It has been shown that the structure of the dental gingival area is highly similar to that observed in humans (Yamasaki *et al.*, 1979).

It has also been indicated that periodontal diseases in rats are less likely to occur as compared to humans irrespective to the possibility to induce these diseases by inoculating bacteria, giving a carbohydrate rich diet and fixing ligatures around the teeth. Taken together, rats are not valid experimental models for studying the evolution of the disease over long periods using histology because of the continuous growth and migration of the teeth. On the other hand, rats are largely used in microbiological and immunological studies (Peruzzo *et al.*, 2008). Using hamsters in studying the etiopathology of periodontal diseases is similar to that in rats. Periodontitis can be experimentally induced by feeding animals with a carbohydrate-rich diet. Hamsters as models are mainly used in studies of microbiology and immunology (Lallam-Laroye *et al.*, 2006).

ANIMAL MODELS OF CHOICE IN PERIODONTOLOGY

It is worth mentioning that animals involved in periodontal research are not unique in their anatomy,

dentition and structure of periodontal tissues. Moreover, the physiopathology, oral bacteria and inflammation response are varied among species (Struillou *et al.*, 2010). These models include:

NON-HUMAN PRIMATES

Schou *et al.* (1993) reviewed characteristics of non-human primates including monkeys which have the advantage of being very close to humans. Non-human primates have varied sizes from 300-350 g for certain marmosets to large sizes approximate humans including chimpanzees and gorillas. These species have the same dental formula as human: I 2/2, C 1/1, Pm 2/2 and M 3/3. It has also been shown that the anatomy of teeth and roots to be similar to humans with a smaller size.

Histological features regarding the structure of the periodontium have been found to be similar between these species and humans. From a microbiological point of view, monkeys have a plaque composed of Gram positive rods and cocci in supragingival plaque and anaerobic Gram negative rods in subgingival plaque (Socransky and Haffajee, 1992; Giannobile *et al.*, 1994).

In a study (Page and Schroeder, 1982), the pathological characteristics of periodontal disease in non-human primates have been found to be similar to humans. Connective tissues are infiltrated by plasma cells, lymphocytes and neutrophils. Other species including squirrel monkeys and marmosets exhibited very limited inflammatory infiltrate. According to this context of having these large differences from pathological picture of humans they are inappropriate models for studying the pathogenesis of periodontitis.

Reviewing the literature revealed that monkeys have been widely used in many studies relating to periodontal healing (Blomlof *et al.*, 1989; Ling *et al.*, 1994; Sculean *et al.*, 1997), filling with biomaterials (Drury and Yukna, 1991; Karatzas *et al.*, 1999), guided tissue regeneration (Kostopoulos and Karring, 2004), enamel matrix derivatives (Sculean *et al.*, 2000a, b; Donos *et al.*, 2003) or implant surgery (Schou *et al.*, 2003a, b; Trejo *et al.*, 2006).

Dogs: Dogs have been reported to be used in many experimental studies on gingival and periodontal diseases. Studies showed the use of beagle to predominate over other species because of its size and its extremely cooperative nature. Periodontal tissues and the size of the teeth are very similar to that of humans. Some differences have been reported to exist between dogs and humans including the lack of lateral movements, no occlusal contacts for all the premolars and presence of open contacts between teeth (Sorensen *et al.*, 1980).

Table 2: Advantages and disadvantages of select animal models for studying periodontal disease development

Animal models	Advantages	Disadvantages
Non-human primates	Similar dental structure, microflora and disease to human's Natural or experimentally induced periodontitis	Very expensive with ethical and husbandry issues
Dogs	Develop natural or experimental periodontitis similar to humans	Relatively expensive, need special daily care, husbandry issues. Dentition different from humans
Minipig	Dental structure and periodontitis have some similarity to human's. Natural or experimentally induced periodontitis	Relatively expensive, husbandry issues; relatively few studies
Ferrets	Naturally or experimentally induced disease with similarity to humans	Some husbandry issues
Rodents	Experimentally induced disease. Similar molar structure to human's. Inexpensive model	Naturally resistant to periodontitis. Different microbiota from human's. Small size and therefore amount of tissue for analysis. Large number of animals needed

Bhardwaj and Bhardwaj (2012)

Rats: The rat is considered the most extensively-studied rodent for the pathogenesis of periodontal diseases. The incisor is rootless. The structure of the dental gingival area in rats is quite similar to that observed in humans (Yamasaki *et al.*, 1979). Some differences exist between rats and humans relating to the keratinisation of the crevicular epithelium in rats and the relationship between the gingival and junctional epithelium with desmosomal contact between the most superficial cells of the gingival epithelium and the non keratinized cells of the junctional epithelium (Listgarten, 1975).

Hamsters: Hamsters do not develop periodontal disease spontaneously but it is rather induced experimentally. The golden Syrian hamster is mostly used. The dentition structure is identical as in rodents. Its molars move following the growth of the jaws and occlusal wear (Struillou *et al.*, 2010). From a histological point of view, the structure of periodontal tissue in hamster and rats are very similar (Eggert *et al.*, 1980).

Spontaneous periodontal disease has been reported to occur using an appropriate diet with high concentrations of carbohydrates, particularly sucrose (Lallam-Laroye *et al.*, 2006). It has been shown that there is a limited inflammatory response in hamsters and is very different from that observed in humans. Furthermore, 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 (Struillou *et al.*, 2010).

Ferrets: Ferrets have been shown to have a deciduous and permanent dentition. Ligature-induced periodontitis can be obtained within 4 weeks (Harper *et al.*, 1990; Mann *et al.*, 1990). It has been shown that there is a similarity of evolution in the periodontal lesions to that observed in humans (Weinberg and Bral, 1999). Taken together, there is no unique animal model to be used for all purposes and each model has its own advantages and disadvantages (Table 2).

CONCLUSION

The use of animal models in dentistry is crucial to establish new findings prove hypothesis of the study. There are many animal models that have been proposed to be used in dental experiments. Actually, there is no unique animal model for all disease and accordingly, animal model has to be carefully selected.

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