

Maximizing Periodontal Defect Creation and Experimental Design in Non-Human Primate Model Study: An Updated Review Article

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Abstract

In relation to periodontal defect, several animals such as rats, hamster, rabbit, dog and non-human primate were used to prove the finding in *in vitro* study and to confirm the efficacy of new material or therapy before settled in human. This review aimed to describe and investigate the use of non-human primate as an animal model in periodontal defect to maximize the experiment design.

Potentially relevant electronic or on-line article from Google Scholar, Science Direct, and PUBMED was screened. Full-text or articles written in English up to November 2017 were collected. Keywords or phrases were used: (Non-human primate OR monkey OR macaca) AND (periodontal defect). Non-human primate animal was reported prone to have systemic infectious disease such as tuberculosis. However, the use of non-human primate in experiment is ethically restricted; it should be performed on the highest beneficial reason.

Combination of acute-chronic defect model in non-human primate is the best way to imitate a periodontitis condition in human. A notch in the base is made as a mark to measure the gain of periodontal ligament, new bone and cementum formations. Due to its limited number of animals used in a study, a good and reliable experimental design is needed.

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Introduction

Periodontal disease is defined as chronic inflammation caused by periodontal bacteria occurred in periodontal apparatus, characterized by increasing of immune-inflammatory mediator in lesion leading to destruction of collagen and alveolar bone.¹ Based on the form, periodontal defect accompanied in periodontitis in proximal area could be divided into horizontal and vertical bone losses. Moreover, there is a periodontal defect in furcation area called furcation involvement. Beside bacterial infection, host susceptibility also investigated as an associated factor in disease.² In human, the definite pathogenesis of periodontal healing and its responses to treatment modalities are remains

unclear. Therefore, both *in vitro* and *in vivo* studies were needed to investigate this fundamental mechanism.

Animal model has been used worthy in periodontology as a linking stage *in vivo* study from basic research to validate a hypothesis before settled in human. *In vivo* experiment also required in periodontology to prove the finding in *in vitro* study and to confirm the efficacy of new material or therapy.³ It been stated that *in vivo* study is aimed to gather new knowledge and testing of compounds, chemicals or devices for safety and its effectiveness.⁴ Reasonable expectation and appropriate experimental design in animal related study will contribute to the improvement of periodontology field. Several animals such as rats, hamster, rabbit, dog and non-human primate are known used to mimicking periodontal disease and its responses to the therapy modalities. Biologically observed, periodontal apparatus and its structures in non-human primate are more parallel to human than others animal models.⁵ Even though, rodents reported massively used in periodontology research due to its age, genetic modification and

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controllable bacteria, but the anatomical structures of periodontal tissue and histopathological features are different from human.⁴ Dog, particularly beagles, has been used to evaluate periodontal disease progress, to observe tissue regeneration, wound healing and osseointegration of dental implant. It stated that the periodontal disease etiology in dog seem identical to human. In mimicking chronic type of periodontal defect in non-human primate (baboon model), it reported that maintenance of wire ligature around the teeth allows the initiation and maturation of periodontal disease.⁶ Using animal, it reported that during periodontitis, pro-inflammatory cytokines (Interleukin-1 β , IL-6 and TNF- α) consistently elevated in gingival crevicular fluid and tissues.⁷

Basavaraju et al. (2015) supported that inflammatory response to periodontal disease gathered in non-human primates is quite similar to human.² Even though it similarities are closely represent of human condition, non-human primate animal were reported prone to infectious disease such as tuberculosis.¹ Trofin et al. (2013) point out that non-human primate study is expensive, difficult to manage and ethically restricted.⁸ Besides highly cost and ethically constricted, considering the possibility of collecting alveolar block biopsy, non-human primate has been considered to be used in periodontal regeneration study. A good, comprehensive and reasonable experimental design by using non-human primate is needed to achieve a valuable outcome. This review is aimed to describe and investigate the use of non-human primate as an animal model in periodontal defect to maximize the experimental design.

Materials and methods

An electronic or on-line article from Google Scholar, Science Direct, and PUBMED was screened (Figure.1). Keywords or phrases were used: (Non-human primate OR monkey OR macaca) AND (periodontal defect). The inclusion criteria for this review analysis are: (1) Non-human primate animal study (2) Periodontal defect creation. Whereas the exclusion criteria are: (1) Human studies; (2) Animal except non-human primate studies; (3) Dental implant study. Information data was compiled, including: (1) First author's name; (2) Publication year; (3) Research topic; (4) Species of non-human

primate used; (5) Tooth or teeth used; (6) Design; (7) Measurement of periodontal defect.

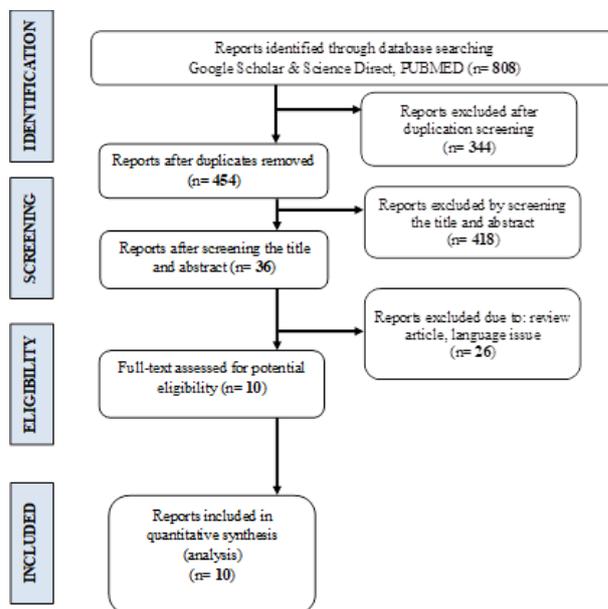


Figure 1. Flowchart for Screening Protocol

Non-Human Primate

The oral and dental structures of non-human primate are phylogenetically similar to human.^{2,9} It also has naturally dental plaque and calculus accumulation, moreover periodontal pathogen bacteria (*Porphyromonas gingivalis*) for periodontal disease.¹ Moreover, it is reported that the composition of dental plaque in cynomolgus monkey (*Macaca fascicularis*) is Gram-positive rods, cocci and anaerobic Gram-negative rod for supragingival and subgingival respectively.² Gaetti-Jardim et al. (2012) revealed that in naturally habituated capuchin monkey (*Cebus apella*), bacterial populations of *C. rectus*, *E. corrodens*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *T. forsythia* were significantly higher in animals with bone loss compared with normal.¹⁰ Rhesus monkey (*Macaca mulatta*), cynomolgus monkey (*Macaca fascicularis*), chimpanzees and baboon (*Papio anubis*) are known susceptible to naturally have periodontal disease amongst other non-human primates.⁵ It reported that the dental anatomy of non-human primate is parallel to human, but smaller. Canines in non-human primate are extended. Premolars in baboons and gorillas are reported have more than one root. Baboons reported have lateral jaw movements that mimic to humans.^{11,12} Higher similarities of dental anatomy, bacterial environment, immune

responses and healing characteristic showed in non-human primate possessed this animal could represent human condition (Table 1).⁹

Naturally Periodontal Defect

Caton et al. (1994) pointed out that there are four types of defect; naturally occurring periodontitis, and three types of experimentally produced.¹³ Interesting study design was performed by Maekawa et al. (2016). Author used pre-existing naturally chronic periodontitis in adult cynomolgus monkey (*Macaca fascicularis*) aged 7-15 years old to investigate the inhibitory effect of peptide Cp40 in periodontitis.¹⁴ However, the natural periodontitis only seen in adult or late in life animal and its patterns are asymmetrical.¹³ Late-adult non-human primate assumed had lower regeneration ability compared to adolescence or early adult.

Acute Periodontal Defect

Basajaravu et al. (2015) explained the type of periodontal defect performed in animal experiment.² Based on the activity, periodontal defect is classified into acute defect, chronic defect and combination of acute-chronic defect. In general comparison, acute defect is defined as surgical approach by removing bone, cementum and periodontal ligament surround the tooth. Chronic periodontal defect was gained by placing a material (e.g. orthodontic elastics, silk suture, wire, metal band) around teeth for 12 to 20 weeks. Whereas, combined acute-chronic model, defects were created surgically, and later materials were positioned in the defect to prevent natural regeneration and to expose it with dental plaque. Coton et al. (1994) pointed out that the disadvantage of acute model is the spontaneous regeneration. Author reported that 50-70% of new bone, cementum and periodontal ligament healed within two months.¹³

Chronic Periodontal Defect

Pellegrini et al. (2009) stated that chronic periodontal lesion is gained by orthodontic elastic ligatures or silk suture placed surround teeth for three until six months.¹⁵ Helieh et al. (2011) stated that plaque accumulating devices (e.g. orthodontic elastic ligature or suture) placed in interproximal area could accelerate periodontitis within 1-2 weeks.¹ The elastic will gradually drifts apically.¹³ Three months after, half of alveolar bone destruction reported by Kostopoulos et al.

(2004).⁹ Additionally an infrabony pocket with six mm loss of attachment exhibited is similar to chronic periodontitis.¹³ Ligature inoculated with *P.gingivalis* reported accelerates disease progression. Bone destruction pattern seen in this model is angular and horizontal, in single and multiple teeth respectively. The advantage of this model is that spontaneous regeneration is not occurred. However, this defect model needs up to six months and costly due to longer animal care expenses.¹³

Acute-Chronic Periodontal Defect

Coton et al. (1994) explained that this combination defect model is created by surgically removing bone, periodontal ligament and cementum. Prior to flap sutured, a foreign object was place in the defect to induce inflammation and prevent natural regeneration.¹³ Pellegrini et al. (2009) pointed out that surgical defect was filled with space-providing mechanical devices (e.g. metal strips, orthodontic wire or bands, cotton floss ligature) for 1-3 months.⁹ This combination model is assumed the best model because defect is produced rapidly, time efficient and bilateral defects pattern expected. Due to foreign material placed, the defect does not spontaneous regenerate naturally.¹³

Measurement of the Periodontal Defect

Several methods of measurement the defects were described varied. Vilacca et al. (2005) reported by using a notch in the base apical level to measure the gain of junctional epithelium, new cementum and also new bone formation.¹⁶ Studies by Sculean et al (2000), Takayama et al. (2001), Shirakata et al. (2016) make a mark by using a small diameter of round-bur.¹⁷⁻¹⁹

Hurzeler et al. (1987) pointed out the measurement by planing the root and perform histology examination.²⁰ Emerton et al. (2011) measured the intrabony defects (7- to 9-mm depth by 2- to 3-mm width) using clinical measurement after debridement of granulation tissue.¹² Where as other authors use radiograph and or computed tomography (CT) scans approach to measure it.^{14,21}

Conclusions

Regarding to higher similarities of dental anatomy, bacterial environment, immune responses and healing characteristic, non-human primate assumed represent human condition. The use of non-human primate in experiment is ethically restricted and should be performed on a good reason. Combination of acute-chronic

defect model in non-human primate is the best way to imitate a periodontitis condition in human. A notch in base is made as a mark to measure the gain of periodontal ligament, new bone and cementum formatted. Due to the limitation of number of animals used for an experiment, a good and reliable experimental design is suggested.

Author (year)	Research Topic	Non-Human Primate	Tooth	Design	Measurement of Periodontal Defect
Hurzeler et al. (1987). ²⁰	Periodontal regeneration	<i>Macaca mulatta</i>	Central Incisor	Periodontal pockets were produced using orthodontic elastics for five months.	Root surfaces were then planed to the apical part of the defect as a histology marker
Sculean et al. (2000). ¹⁷	Periodontal regeneration	<i>Macaca fascicularis</i>	First-Premolar Central Incisor	Intrabony periodontal defects with a depth of approximately 6– 8 mm measured from the CEJ were produced using a slowly rotating cylindrical. Metal strip was placed to prevent spontaneous healing and enhance plaque accumulation.	Reference notches indicating the bottom of the defect were prepared on the root surfaces using small diameter of round bur.
Takayama et al. (2001). ¹⁸	Periodontal regeneration	<i>Macaca fascicularis</i>	First-Molar	Furcation class II bone defect were surgically made by elevating mucoperiosteal flap. Furcation defect was created (4 mm inferior x 3 mm horizontal) using steel burs. Impression material was placed to promote inflammatory response.	Horizontal groove was made using a small ½ round bur on each root to indicate the base of the defect
Villaca et al. (2005). ¹⁶	Periodontal healing	<i>Cebus apella</i> (monkeys)	Premolar	2-Wall intrabony defects (3x2 mm, depth x width) were made with manual and rotary instruments. Gutta-percha was placed in defects to prevent spontaneous regeneration.	A notch was made on the root surface at the apical border of the bone defect with a ½ round carbide bur.
Emerton et al. (2011). ¹²	Periodontal regeneration	<i>Papio hamadryas</i> (baboons)	First-Premolar, First-Molar	One-wall intrabony defects created by remove the teeth, create mesial lesions and create plaque accumulation via wire ligatures.	Clinical measurement (7- to 9-mm depth by 2- to 3-mm width) after debridement of granulation tissue
Jimbo et al. (2014). ²¹	Periodontal regeneration	<i>Macaca fascicularis</i>	First-Molar, Second-Molar	Furcation class II bone defect made surgically (5 x 3 mm, height x depth) using 2 mm diameter cylindrical bur. Alginate impression material was placed in the defects to induce experimental inflammation.	Computed tomography (CT) scans were performed to analyze bone, cementum and periodontal ligament percentage filled.
Shirakata et al. (2016). ¹⁹	Periodontal regeneration	<i>Macaca fascicularis</i>	First-Molar, Second-Molar	Class III furcation defects were surgically created using bone chisels and slowly rotating diamond burs (5 mm wide x 5 mm high). Defects were filled with impression materials.	Reference notches were made using a #1 round bur on the root surface at the base of the defects for histometric analysis.
Bachtiar et al. (2016). ²²	Scaffold degradation (bio-material)	<i>Macaca nemestrina</i>	First-Molar, Second-Premolar	The defect size was approximately 10 × 20 mm surgically made by bur.	NA
Maekawa et al. (2016). ¹⁴	Periodontal pathogenesis (mechanism)	<i>Macaca fascicularis</i>	Maxillary teeth	Animal selected for the study were have pre-existing natural periodontitis	Presence of at least 30% of sites with probing pocket depth, clinical attachment level ≥4 mm, associated with bleeding on probing, and radiographic evidence of bone loss
Kajikawa et al. (2017). ²³	Periodontal inflammation	<i>Macaca fascicularis</i>	Third molars (maxilla and mandible)	Natural model of periodontitis in non-human primates were included	Gingival Index and plaque index were represented periodontal inflammation; clinical attachment loss and periodontal probing depth were considered as a tissue destruction.

Table 1. Summary of Periodontal Defect Experimental Studies using Non-Human Primate

Declaration of Interest

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