Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.10.04.Art021

A Reappraisal of Various Animal Models used in Periodontology with Future Perspectives and Alternatives

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Abstract

Periodontitis is an inflammatory infectious disease that occurs when the supporting tissues of the teeth are infected by a mix of gram-positive bacteria (GPB), and anaerobic bacteria. Periodontitis must be treated as soon as possible to avoid more injury and worsening of the condition. Prior to evaluating new treatments, animal studies are used in conjunction with in *vitro studies in periodontitis research. Even though cultured cells can be used to examine physiological processes that occur in periodontitis development, the complex host response that is at the root of the illness is impossible to replicate in vitro. Mice, rats, rabbits, hamsters, nonhuman primates, dogs, and pigs have all been used to imitate human periodontitis, each with its own set of benefits and drawbacks. Animals have played an important part in researching periodontal diseases and developing effective treatments. There is a limitation in the usage of large animals due to their housing problems. Animals for periodontitis are selected based on their resemblance with that human structure and functioning. The usage of these animals will aid in the better and more precise replication of human disease. This will improve the disease's prognosis and treatment outcome. As a result, the medications utilized can provide a better indication of the effect they will have on the human body based on the effects they have on animal models. Therefore, it is critical to use appropriate animals in periodontal research in order to develop improved treatments for these disorders. Thus, animal models play a crucial role in periodontal research.*

Keywords: *Animal models; Bacteria; In vitro; Periodontitis; Treatment.*

Introduction

Periodontitis is a periodontal disease characterized by the deterioration of gingival tissue, the periodontal ligament, and the surrounding supporting alveolar bone [1]. Periodontitis is becoming more common all over the globe. It is also linked to an increased risk of developing serious systemic illnesses such as hypertension, atherosclerosis, rheumatoid arthritis, and diabetes in adults, along with being the leading cause of periodontal attachment loss, bone degradation, and tooth loss [2].

According to the Global Burden of Disease Study, periodontal illness is the 11th most frequent disease worldwide [3]. Periodontal disease affects 20% to 50% of the world's population, according to estimates. Periodontitis is one of the most common forms of tooth decay, and it may have an impact on mastication, attractiveness, self-esteem, and overall quality of life. Periodontal diseases caused 3.5 million years lived with disability

Received: 13.10.2022 ***Accepted: 16.11.2022*** ***Published on: 29.12.2022***
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(YLD) worldwide in 2016. According to the World Health Organization, the worldwide incidence of periodontal disease grew by 57.3 percent between 1990 and 2010. In 2010, it was estimated that severe periodontitis will cost the global economy US$54 billion in lost productivity per year. Due to an older population, the decline in tooth loss in the elderly, and higher natural tooth retention, the global frequency of periodontal disease is expected to rise in the future [3-5].

Periodontitis is caused by bacterial infection along with the host's vulnerability to the harmful consequences of periodontal infections because everyone is not equally sensitive. Individual differences in host responses have a big role in the development of periodontal diseases. Human-derived cell cultures have been proven to be effective models for the replication of components of the disease progression at the cellular level, but knowledge about the complicated host response has been lacking. As a result, animal research into the host response is vital for the development of better treatments [1]. Periodontitis is associated with many systemic disorders (e.g., respiratory disease, cognitive impairment, metabolic syndrome, diabetes, obesity, cardiovascular diseases, rheumatoid arthritis, chronic kidney disease, cancer, etc.) as depicted in Figure 1.

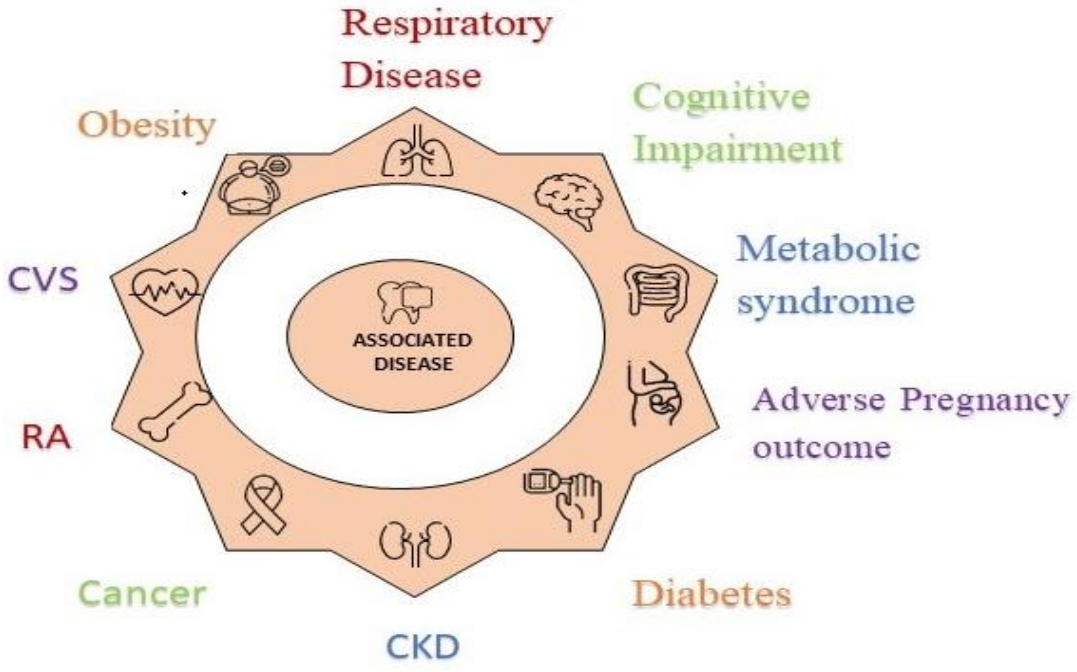

Figure 1. Associated Diseases with Periodontitis

Before conducting clinical trials, it is essential to conduct experimental research in animal models to find out the origin, pathophysiology, and progression of the disease. Thus, animal models are used to investigate the etiopathogenesis, histological, clinical features, and immunologic components of periodontal disease, and to test the success of emerging surgical procedures before their implementation in humans [6]. The animal models can also aid in the creation of more precise diagnostic tools and more successful therapeutic approaches.

Considering the importance of animal models in the research to find out the suitable and safe therapeutic agents for the treatment of periodontitis, the current review has been planned to characterize and discuss the most commonly used animal models for the induction of periodontitis and their advantages and disadvantages.

Periodontitis: Description and Defect Model

Periodontitis is a disease of the periodontium that is characterized by persistent immunoinflammatory symptoms and a high prevalence rate. As the illness progresses, the periodontal ligament, gingival tissue, and surrounding supporting alveolar bone are gradually lost. Periodontal illnesses are caused by bacteria that gather on the surface of the tooth in the form of a biofilm and then harm the periodontal tissue that is next to the tooth. It is caused by anaerobe Gram-negative bacteria (GNB) such as *Aggregatibacterctinomycetemcomitans, Porphyromonasgingivalis,* and *Prevotella intermedia* [1, 7]. The progress of periodontitis is shown in figure 2. Bacteria can trigger periodontal disease directly by releasing endotoxin and indirectly by acting as an immunosuppressive factor. Bacteria and its by-products which spread into the subgingival connective tissue through the junctional epithelium will trigger epithelial cells and fibroblasts to secrete various proinflammatory cytokines. These cytokines can cause connective tissue destruction and alveolar bone resorption in periodontitis [8].

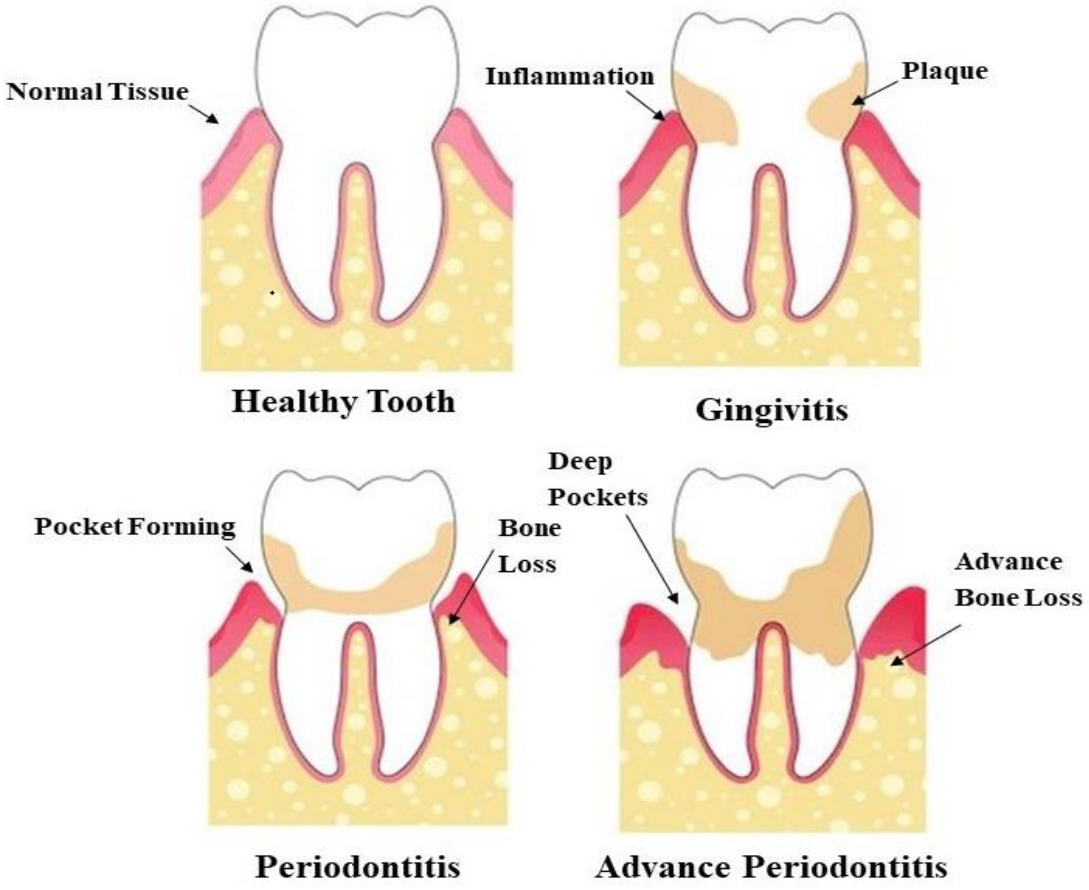

Figure 2. Development of Periodontitis in Healthy Tooth

The induced inflammatory response then activates multiple cells, particularly M1 macrophages, causing them to produce a flood of proinflammatory cytokines such as interleukin 1 beta (IL-1β), IL-4, IL-6, interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α), all of which have a role in modulating the severity and development of periodontal disease [8, 9].

The inflammatory reactions that cause these disorders are triggered by the bacterial biofilm. To lessen the disease's detrimental impacts, several therapeutic approaches must be discovered. Before being utilized in patients,

the medications, and therapies to be developed must be evaluated for safety. Animal models are used to test new medicines for their safety and effectiveness in humans. Periodontal disease therapy should focus on periodontal tissue regeneration by employing surgical and non-surgical procedures, growth hormones, bone substitutes, and the most recently suggested mesenchymal stem cells [10, 11]. Rats, hamsters, pigs, rabbits, dogs, and ferrets are the most often utilized animals for producing and treating periodontitis in innovative methods. These animals are initially given the illness, and then newer medicines are tested on them to assess how effective they are. However, for ethical considerations, these animal models must be utilized with extreme caution [1, 12]. Animal models have provided a wealth of information on novel biological problems. The utilization of animal models is contingent on their resemblance to the human body. Periodontal research animals are selected based on their close resemblance to human anatomy and physiology.

Periodontal research strives to discover the origins of periodontal illnesses and induce diseases found in the human mouth cavity, such as bone abnormalities, and devise a treatment strategy for them. The animals are either infected with periodontal disease or have the illness produced in them, with different medications utilized to treat it. For periodontal disorders in humans, the result of therapy in terms of medications and treatments is taken into account [12, 13].

Periodontology research employs a variety of approaches, including studying the genesis of periodontal disorders using experimental periodontitis models and regenerating defected periodontal tissues by surgical methods establishing bone defects in conjunction with, or without, experimental periodontitis [12, 13]. Animal studies employing innovative biomaterials and treatments for medical uses other than periodontal illnesses, like correcting bone deficiencies in orthopaedics, are not very useful in the context to periodontal. Periodontal lesions effectively present as open, non-vascularized cavities with continuous tissue inflammation, necessitating the employment of particular experimental models. Nonhuman primates are preferable for etiopathology research, but big series is neither morally nor economically feasible [14]. For periodontal research, small animal models (such as rats or hamsters) have been produced, however, these studies mostly concentrate on bacteriology and immune response [12, 15].

Periodontal diseases may be caused naturally, artificially, or both depending on the species. Large animal models have been used in the context of regenerative medicine employing biomaterials because of the repeatability and surgical accessibility of experimental abnormalities [1, 12]. Dogs have been commonly utilized to mimic the regeneration of periodontal abnormalities using biomaterials, in addition to monkeys, who are the appropriate model in pre-clinical investigations [16]. Rats, minipigs, lambs, rabbits, and cats have all been employed in several research. Various techniques have been offered to ensure repeatable models that may be used for statistical analysis.

The animals were chosen based on their comparable diseases and the simplicity with which surgically induced clinically meaningful abnormalities might be established. The acute defect model, the chronic defect model, and the acute/chronic defect model are the three types of experimental periodontal defects available [12, 17]. All abnormalities are surgically produced in the acute model by surgically removing all periodontal components (bone, periodontal ligament, and cementum). In both the control and experimental sites, reproducible faults are formed. In the chronic model, lesions are formed by wrapping orthodontic elastics, silk sutures, or ligatures around teeth for 3 to 5 months, based on the animal used [18]. The interproximal gaps have deeper flaws than the buccal or lingual surfaces. The lesions are

surgically generated, and ligatures are implanted to assure calculus formation and inhibit spontaneous regeneration of the defects in the combined acute/chronic paradigm. At the alveolar crest of the ramus bone, periodontal and osseous deficiencies form. In order to avoid bias, these investigations focus on critical-sized lesions that would not spontaneously repair throughout the trial [12, 19]. The magnitude of the defect is determined by the species' anatomy and physiology. Monkeys and dogs are the most often utilized periodontal research models.

The ligature model is often used to induce periodontitis because it increases bacterial plaque development and attachment loss [20, 21]. As ligation and oral surgery are easy to perform on dogs, rabbits, and rats, they have been utilized to research periodontitis throughout the last decade [20, 22, 23]. Mouse models are being used to research periodontitis because of the need for gene-editing strains and high-quality immunochemical detection reagents [20, 24]. Mice are cheaper than bigger animals and may be kept in SPF settings. Because the mice's mouth cavity and teeth are too tiny, it's difficult for operators to effectively detect and apply ligatures [20].

Experimental Defect Models

Animals may develop periodontitis spontaneously or artificially. Several standardized experimental defect models according to the morphology are:

1. Periodontal pocket defects: The interdental alveolar bone associated with a mesial or distal root surface, as well as the superficial root covering periodontal ligament (PDL) and cementum layers, are removed to create one, two, or three wall osseous flaws with well-defined dimensions [25, 26].
2. Keyhole defects: In conjunction with superficial root cement and PDL denudation, the alveolar bone filling the furcation between the roots in a molar is removed in a buccolingual direction partially (Class II) or fully (Class III) to generate a 'through and through' defect [25].
3. Dehiscence defects: The alveolar bone that covers the mid buccal section of the roots is removed, as well as the superficial root cement and PDL. Premolars with single roots are usually subjected to this procedure [25].
4. Recession defects: The gingiva on the face is removed, exposing the root surfaces to the oral cavity [25, 27].
5. Irregular-shaped defects: Caused by spontaneous periodontitis, ligature-induced periodontitis, the introduction of virulent bacterial species into the oral microbiota (oral gavage), or the injection of their virulence factors (e.g., LPS) in the periodontal [25].
6. Combined flaws: a mix of the preceding.

Animal Selection

To research the development and genesis of periodontal diseases, the animals should exhibit disease features that are comparable to those present in humans. The experimental investigation should contain indicators like the oral hygiene index, gingival index, and subgingival pocket depth that are used to measure periodontal health in people. Radiographs, bacteria determination, blood immunology tests, and histology analysis are all used in experiments [28]. The study aims, as well as laboratory restrictions such as harbouring big or non-standard animals, influence the choice of an experimental model. Large animals with social and ethical difficulties, such as dogs and monkeys, should be used only for the final phase of validation of innovative therapies before being used in human clinical practice. Small animal models, like hamsters, and rats, are usually enough to analyze the involvement of bacteria, food, or other variables in periodontal inflammation at the histology level, offering enough statistical significance and preclinical relevance [1, 12].

The pathophysiology of periodontitis and therapy methods against the illness has been studied using a variety of animals. While animal models have supplied a wealth of information, determining whether the results are relevant to people may be tricky. Non-human primates have human-like oral structures and teeth, as well as naturally occurring calculus, dental plaque, oral microbial infections (such as *P. gingivalis*), and other periodontal diseases. Naturally occurring periodontal disease affects cynomolgus monkeys (*Macaca fascicularis*), rhesus monkeys (*Macaca mulatta*), and baboons (*Papioanubis*) [7, 12]. In terms of structure, functioning, and disease development, miniature pigs' maxillofacial and oral features are identical to those of humans.

Dogs are an excellent model for studying gingivitis and periodontitis. Similar to human bacteria, the subgingival plaque in dogs is dominated by anaerobic gram-negative cocci and rods, *F. nucleatum*,and *P. Gingivalis* [1, 12, 29]. To supplement primate and human periodontal investigations, rodents offer some unique qualities for evaluating microbial and host responses. The swamp rice rat, also known as the rice rat (Oryzomyspalustris), is a native American species that may be found across the southern United States. Beginning at the age of two weeks, these animals are very vulnerable to periodontal disease. At about 3 months of age, the gingival tissues swell, resulting in pocket development, debris collection, and ulceration [1]. Buccal abrasions, gingival recession, calculus, and periodontal pockets are all frequentlyand naturally occurring oral illnesses in horses [30]. Rabbits' oral microorganisms were characterized and found to include various harmful bacteria, including *F. nucleatum*, Prevotella spp., *P. heparinolytica*, *P. micros*, *S. milleri* group, which are similar to the flora associated with periodontal disease in humans [31]. Unlike rodents, the production of calculus in ferrets (Mustela putorius) is not dependent on the food and may be scored in live ferrets. Hamsters have a similar dental formula to rats, and they use ligatures around their molar teeth to establish experimental periodontitis [12].

Various Animal Models Used for Periodontitis

In periodontal research, we choose animals whose mouth cavities most closely resemble those of humans. This helps us better understand the disease. The periodontal disease is studied using these animal models in order to build a more effective therapy for it and also to determine whether or not the medication is indeed helpful against the illness itself. According to one theory, humans evolved from monkeys, which explains why monkeys and humans have the most similarities. On the other hand, it has been shown that dogs may also spontaneously mimic the periodontal illnesses that are seen in people. Rats have long been used in scientific research and even in the area of periodontics, where they have shown to be of great use. Rodents, dogs, pigs, rabbits, ferrets, horses, and hamsters are among the species that are most often employed in periodontal disease research because of their ability to either mimic the illness or naturally show it [1, 12]. The Pros and Cons of different animal models used for periodontitis are mentioned in Table 1.

Table 1. The Pros and Cons of Different Animal Models Used for Periodontitis

Animal model	Pros	Cons
Non-human primates	Dental structure, microbiota, and diseases comparable to those seen in humans.	Extremely costly, with concerns about both ethics and husbandry.
	Periodontitis that is either naturally occurring or artificially generated in the	

	lab.	
Dogs	Naturally and experimentally generation of periodontitis that is comparable to that of humans.	They are rather costly, need specific daily care, and have husbandry concerns.
		Dentition differs from that of humans.
Ferrets	Periodontitis that occurs naturally or is created deliberately and is analogous to human disease	Certain husbandry-related concerns
Rodents	Disease can be caused by experimentation.	Periodontitis resistance is built in.
	The molar structure is similar to that of humans.	Microbiota differs from that of humans.
	Model is inexpensive	Because of its small size, the quantity of tissue available for examination is limited.
		A large number of animals are required.

Non-human Primates

Since monkeys are so similar to humans, their oral structures, as well as the deposition of plaque, calculus, and bacteria, are almost identical to those found in human mouths. This is due to the fact that monkeys share a common ancestor with humans. Rhesus monkeys, cynomolgus monkeys, and baboons are the species that have been shown to suffer from periodontal disease as a result of natural causes [32, 33]. There are certain non-human ape species that display signs of periodontal disease when they reach adulthood [32, 34]. The ligatures and orthodontic elastics were affixed to the surface of the tooth in order to speed up the progression of periodontitis [32]. This was done in order to increase the amount of plaque that accumulated. Every one to two weeks, the ligatures are switched out based on the forms of the pockets [32].

All primate species other than humans have a wide size range, from 300 to 350g for some marmosets to about the same size as humans for gorillas and chimpanzees. All of these species have two sets of teeth known as diphyodont as that like humans. The teeth of macaques, chimpanzees, and baboons have the same dental formula as that for humans 2123/2123 (I,C,Pm,M) whereas the formula for marmosets is 2132/2132 (I,C,Pm,M). Although the teeth and roots are far smaller, their morphology is quite similar to that of humans. The canines of most non-human primates are prehensile and elongated in comparison to human canines. Premolars of gorillas and baboons have many roots [12].

Several species of non-human primates are plagued with periodontal diseases when they reach adulthood [12]. Additionally, the histological structure of the periodontium is very comparable to that which is seen in human beings. Microbiologically speaking, the makeup of the plaque in Macaca fascicularis (cynomolgus monkeys) is anaerobic Gram-negative rods for subgingival plaque and cocci, and Gram-positive rods for supragingival plaque [12, 35]. The inflammatory reaction that occurs as a result of periodontal disease is fairly comparable to the inflammatory response that occurs in humans. Plasma cells, lymphocytes, and neutrophils all invade connective tissues during an inflammatory response. There is only a very little amount of inflammatory infiltration seen in other species, such as marmosets and squirrel monkeys. In spite of this, the marmoset

is the non-human primate species that have been used in periodontal research the vast majority of the time.

Monkeys have been employed in a variety of experiments including periodontal healing [36], directed tissue regeneration [37], biomaterial filling [38], enamel matrix derivatives [39], and implant surgery [40, 41]. The majority of these surgeries were performed on Macaca fascicularis, as each and every tooth could be utilized, resulting in a more test site with a small number of animals.

Dog

Plaque formation in dogs contains bacteria that are comparable to those found in humans, such as Fusobacterium nucleatum and Porphyromonasgingivalis [42]. Dogs have been used in several experimental research on gingival and periodontal disorders. For the sake of convenience, the most cooperative dogs are used in the study. Due to its small and exceptionally cooperative attitude, the beagle is one of the most widely utilized dogs. However, owing to ethical concerns, the employment of dogs on a broad scale is not viable. All periodontal tissues, as well as the size of the teeth, are reasonably comparable to those seen in humans [12, 43]. Although the human and canine dentitions have many similarities, there are also significant distinctions between the two, such as the absence of lateral motions, the lack of occlusal connections for all premolars, and the existence of open contacts between teeth. Other significant differences between dogs and humans include the absence of crevicular fluid and gingival sulci, as well as a distinct composition of periodontal plaque and calculus [12]. Diphyodont dogs have both deciduous and permanent teeth. 3142/3143 (I, C, Pm, M) is the formula for permanent dentition.

When they reach adulthood, all household dogs have a natural predisposition to develop periodontal illnesses; nevertheless, with proper plaque treatment, it is possible to keep these canines in good condition. Periodontal modifications, such as periodontitis and gingivitis, grow in incidence and severity with age. This rise occurs more rapidly in women than in men, despite the fact that the same variables contribute to the condition. It is possible for gingivitis to become generalized, which is accompanied by bleeding upon probing, the appearance of subgingival and supragingival plaque, significant bone loss, and calculus, which may include osseous abnormalities. At the moment, gingival recessions are seen in the severe kinds of periodontal disorders that may be seen in dogs. Cocci that are Gram-positive make form the vast majority of supragingival plaque. The majority of the subgingival flora consists of Gram-negative rods and anaerobic cocci [1, 12, 42].

The development of gingivitis may be hastened by feeding them a diet consisting of suitably soft foods that have been chopped, which encourages the deposition of supragingival plaque and calculus [12]. The gingival sulcus is almost never seen in dogs who are otherwise in good condition. On the gingival edge, the junctional epithelium and epithelial attachment extend to the highest coronal level. In the early stages of gingivitis, the infiltration of neutrophils and monocytes is confined to the periphery of the gingiva, leaving the majority of the connective tissue unaffected by the disease. Gingivitis may be caused by a number of different bacteria. In the latter stages of the disease, the infiltrate will expand apically subjacent to the junctional epithelium, which will result in gingival pockets being formed.

In dogs, periodontitis develops as a result of pre-existing gingivitis. Periodontal pockets are formed, which are bordered by normal pocket epithelium. Plasma cells and lymphocytes make up the bulk of the dense cellular infiltration in connective tissue. Deep, narrow lesions running vertically around a single root may occur from osteoclastic resorption of alveolar bone, leaving

the interdental space undamaged [12, 44]. The premolar and molar teeth's furcation regions may be affected by bone deficiency. Periodontitis arises sooner and more severely in colony dogs than in household dogs [43]. The interdental gaps are more typically damaged than the bifurcation areas. The initial 2 premolars are the teeth that are most often lost. In addition, the frequency and severity of periodontitis and gingivitis increase with age but differ significantly across breeds. Periodontal disease affects certain canines, whereas it affects others less. Diet has less to do with the changes than the form of the illness or heredity. The magnitude and localizations of periodontal lesions are not uniform in these natural periodontal illnesses [45], which might be regarded as a model limitation.

Dogs are models for implant surgery and directed osseous regeneration. The implant approach uses the ramic angle to produce critical-sized biomaterial-filled defects [46]. Two months of binding dental implants induced peri-implantitis. These osseous anomalies around the implants resembled those in individuals [47]. Dogs are the most extensively used animal model in periodontal research because of repeated critical-size anomalies.

Mice

Although the periodontal disease in mice has been researched, researchers have found that it is quite different from that seen in humans. Mice have the standard rodent dentition, which consists of the following dental formula: 1003/1003 (I, C, Pm, M). With age, the incisors continue to develop, but their molars undergo more complicated physiological changes. At the apical section of each root, there is significant occlusal wear, bucco-occlusal motion, and high levels of hypercementosis [48]. Bone loss is a defining feature of periodontal changes, and it is often more pronounced on the lingual and palatal surfaces of the molars than on the buccal surfaces of the teeth. In addition to this, there are crater-like flaws present in both the interdental and interradicular gaps. On the other hand, periodontal disease does not manifest in mice that are less than one year old. The inflammatory reaction is not all that strong. Mice are not the ideal model for the study of natural or induced periodontal disease because of the substantial physiological changes that occur over time in the location of the molars in the alveolar socket.

Rats

Rats are the most widely studied animal in terms of periodontal disease causation. 1003/1003 (I, C, Pm, M) are the typical rat dentition. The incisor has no roots. With a shallow gingival sulcus and attachment of the junctional epithelium to the tooth surface, the dental gingival region of rats is fairly comparable to that of humans [12, 49]. "However, there are some differences: crevicular epithelium keratinization and gingival and junctional epithelium relationship with the contact of desmosomes between superficial cells of the gingival epithelium and non-keratinized cells present in the junctional epithelium, the first is crevicular epithelium keratinization in rats; the second is gingival and junctional epithelium relationship with desmosomal contact between the most superficial cells of gingival Despite the anatomical differences, the junctional epithelium seems to function as a conduit for foreign chemicals, bacterial endotoxins, and inflammatory cell exudations, similar to what happens in humans". Wistar and Sprague-Dawley are the strains that are used the vast majority of the time. Experimental periodontitis may be induced in periodontal disease-resistant strains of mice, such as germ-free Spraque-Dawley or white Lobund, either by the application of silk ligatures that are wrapped around the molars or through the administration of particular bacterial inoculations [12, 49, 50, 51].

Rats' dental tissues change according to their age, including continual tooth eruption and

permanent cementum and bone apposition in response to wear on all molar occlusion surfaces. In contrast to the occluso-mesial drift found in humans, all of these events produce a gradual change in the location of the molars in 3-D space, culminating in global movement in an occlusal-distal-buccal orientation. The cellular cementum that is deposited on the occlusion surfaces induces anatomical crown attrition, which in turn leads to an increase in root length. The cemento-enamel junction advances faster than bone deposition at the alveolar crest in an occlusal direction throughout time. All periodontal tissues have constantly been remodelled, and the continual occlusal-distal-buccal movement of rat molars seems to be a physiological representation of adaptive alterations necessitated by jaw development and fast occlusal wear [12, 50]. All the age-related and physiological variables have a significant influence on the interpretation of experimental periodontal disease data produced in rats and other rodents.

The rats are exceptionally resistant to periodontal diseases in natural settings, which is significantly different from humans. Nonetheless, certain strains may acquire periodontitis after being inoculated with particular bacteria, or by following a special diet that increases the amount of carbohydrates in the food, which hastens the disease's growth, or by securing ligatures around the teeth. The emergence of marginal gingivitis with edema and ulcerations is the first stage in periodontal changes seen in disease-prone strains. Second, deep pockets are formed, which are filled with food detritus and hair. The lesions impact the inter radicular and interdental gaps in severe cases of periodontitis, resulting in significant alveolar bone resorption and denudation of the molar roots [12].

Periodontitis seems to be an infectious disease in rats. Periodontal infections such as *P.gingivalis*, *A. viscosus*, *A.actinomycetemcomitans*, *F.nucleatum*, and *S.sobrinus* may cause periodontal lesions when inoculated or injected into the mouth [1, 12]. The loss of periodontal tissue begins quickly after infection. GNB inoculation results in a weak inflammatory response that is not comparable to that seen in people. In the gingival tissues, neutrophils predominate, with few lymphocytes and no plasma cells. The kind of bacterial agent used affects tissue damage and host response in distinct ways. Gingivitis does not seem to be a reliable predictor of periodontal disease in rats. Inflammation is limited at the junction epithelium at this point. There is a lot of phagocytic activity in this area, and neutrophils create a defensive wall. The rate of bone resorption is thus unpredictable. Only approximately 10% of rats up to 100 days of age had acute interdental inflammation characterized by an ulcerated junctional epithelium, neutrophil infiltration of all supra-alveolar connective tissue, and significant osteoclastic activity [12]. The osseous flaws that were found were mostly crater-like. On one side, bacteria were in touch with the whole root surface, while on another, the remaining connective tissue was penetrated by macrophages, neutrophils, and lymphocytes. When an infection due to GNB occurs, periodontal lesions appear first in the maxilla, then the mandible. Depending on the kind of bacteria, significant crater-like bone abnormalities might arise six to eight weeks after infection. In the absence of a cell-mediated immune response, the damaging process in reaction to GNB may occur [32, 51].

Periodontal defect in rats is induced via surgery creating fenestrations on the radicular surfaces of second mandibular molars. The bone and cement were removed using a burr under saline irrigation after an extraoral incision. Both the depth (1.5 mm) and the breadth (1.5 mm) of the flaws were standardized (3 mm). Huang et al. [50] repeated the surgical model on the rat. The rat has also been utilized to assess the repair of supra and intraosseous defects after the application of enamel matrix derivatives [52]. In these

investigations, standardized flaws in the roots of the first molars were surgically generated. The ramus has also been suggested as a location to test guided osseous regeneration methods [51]. The toothless alveolar crest between the incisor and the first molar, as well as the mandible's edge, are also useful surgical methods for directed osseous regeneration [12,32]. Moreover, rats are often used in scientific experiments. Because the expenses of breeding and housing are inexpensive, investigations with adequate numbers for statistical analysis may be conducted. The constant osseous apposition and occlusal eruption on the dental roots, on the other hand, makes modeling and interpretation of the data challenging and may lead to substantial bias. However, the use of rats as a surgical defect model is a promising animal for periodontitis [12,32].

Hamsters

In hamsters, periodontitis does not develop on its own, but it is possible to induce it via scientific research. The golden Syrian hamster is the one that is utilized almost universally. Dentition in insectivores follows the same pattern as that of rodents: 1003/1003 (I, C, Pm, M). The molars shift throughout time in humans just as they do in rats, following the expansion of the occlusal wear and the jaws. When compared to rats, it seems that the change in the occluso-distal orientation and the ongoing eruption of molars are less noticeable in humans. Anatomically periodontal tissues are extremely like that of rats [12, 52], although because of their smaller size, the interdental septum is narrower compared to rats.

Diet rich in carbohydrates, particularly sucrose is given to these to induce periodontal disease in them [53]. Because of this particular diet, the plaque comprised of formic acid bacteria combined with food debris, and it mostly affected the palatal and lingual surfaces of the teeth rather than the buccal ones. Following the deposition of plaque, a breakdown occurred at the junction epithelium, which was followed by the creation of gingival pockets that resembled craters. The proliferating neutrophils were the primary component of the inflammatory response. The osteoclastic activity was more prevalent on the interdental and palatal sides of the molar. Because of the relatively modest size of the interdental crest, practically all of the bone resorption that occurred was horizontal, and it resulted in a change in the interradicular gaps. Alveolar bone resorption slowed throughout the alveolar walls, and palatal and lingual parts responded more strongly than buccal regions, which looked inert [54]. This was due to the fact that the palatal and lingual regions are closer to the roof of the mouth than the buccal regions are. In conclusion, the inflammatory reaction in hamsters is relatively restricted, similar to the way it is in rats, but it is completely different from the way it is seen in people. In hamsters with diet-dependent periodontal lesions, the processes of alveolar bone resorption are fairly comparable to those described in rats infected with Gram-positive bacteria (GPB) [52].

Minks

The adult mink's dentition formula is 3131/3132(I, C, Pm, M). Minks develop spontaneous periodontitis that is plaque and age dependant. Nonetheless, it seems that the degree of this periodontal disease is chronic only in extremely elderly animals. Chediak-Higashi syndrome (CHS) is a genetically transmitted autosomal characteristic that affects minks bred on ranches [55, 56]. Chediak-Higashi syndrome has caused aggressive periodontitis in young adult minks, as well as chronic bone loss and periodontal lesions. The gingiva of the maxilla and mandible were very distinct in size. The vestibule in the upper jaw was rather large. A broad band of connected gingiva was seen on the incisors and canines. At the level of the premolars and molars, the latter is less essential. The quantity of

connected gingiva in the lower jaw is limited in the incisors and premolars. The attached gingiva around the molars looked to be equivalent to that seen in the upper jaw [12, 55]. Periodontal disease develops quickly in CSH-affected minks, with a significant haemorrhagic inflammatory response in the marginal gingiva. Bone resorption may be varied and linked" to the creation of bone craters and furcation lesions, as well as the location and severity of gingival inflammation.

The inflammatory response seems to be considerably different from that seen in humans, "with an initial exudative and chronic inflammatory response combined with vascular growth. Blood vessels proliferated at the level of the marginal gingiva, and neutrophils infiltrated the whole zone. In comparison to other species, the epithelium's expansion into connective tissue was larger in proportion. The amount of connective tissue in the body shrank drastically. Lymphocytes and plasma cells were uncommon. The number of neutrophils and tiny arteries, the amount and severity of epithelial proliferation, and bone resorption were all significantly higher in CHS-minks than in normal minks. Due to deficits in the chemotactic response and large release of lysosomal enzymes and proteases into periodontal tissue, neutrophils play a crucial role in periodontal deterioration" in minks. Minks are consequently useful experimental animals in the study of periodontal disease pathogenesis [1, 12, 56]. However, keeping these creatures may be challenging or need special permissions, which might explain why there haven't been any recent papers in the literature.

Ferrets

Ferrets (Mustela putorius) have a deciduous and permanent dentition. 2142/2142 (I, C, Pm, M) are the components of the dentition formula. Calculus and periodontal disease are both spontaneously occurring in ferrets [57], similar to they are in humans. The ferret's calculus has a physical structure that is comparable to that of hydroxyapatite. Ferret calculus is less calcified than human calculus, which is the primary distinction between the two types of calculus. There was some correlation between the diet and the rate of formation, but not nearly as much as there was in rats. Calculus can be scored on ferrets while the animal is still alive, however, rats cannot have their calculations done while they are still alive [58]. The tissues reacted by inducing typical inflammatory responses, which, in every respect, are indistinguishable from the reactions that are seen in human gingivitis. The accumulation of calculus and plaque, as well as their impingement on the gingival crest, causes a loss of keratin, which in turn leads to the breaking of the junctional epithelium and the creation of pockets. Ferrets can develop ligature-induced periodontitis within a month. At the level of the histological examination, the connective tissue showed an abundance of neutrophils, plasma cells, and lymphocytes. The degree of alveolar bone resorption was considered to be as high as 50% [59].

Other Animals

Research has been done on a variety of other animal models with the purpose of modeling periodontal diseases, such as rabbits, horses, pigs, sheep, etc [1, 12, 32]. Various species such as Arcano bacterium haemolyticum, Prevotella spp., Streptococcus milleri group, and many more were found in the rabbit oral cavity, which is comparable to the flora of periodontal disorders reported in humans [60]. In order to research periodontal regeneration in rabbits, surgically induced periodontal defects are generated, however, periodontal ligament regeneration is shown to be less suited [61]. Local causes such as calculus and plaque induce periodontitis, which leads to bone loss and increasing inflammation [62, 63]. Surgically generated defects may not be identical to the disease's natural form. Rabbits have mostly been employed for biomaterial

testing and peri-implantitis therapy. However, in rabbits, transcortical drilled holes that create tibial or radial critical-sized femoral lesions are commonly utilized models [12]. These lengthy bone deficiencies are unrelated to the particular condition of periodontal disease, but they do seem to be an intriguing model for evaluating bone recovery.

Calculus buildup, the recession of gingiva, periodontal pocket, and buccal abrasion are the four illnesses that are seen in horses the most often. It is not common practice to make use of this animal for experimental research on periodontal disorders and the development of novel treatments because of its big size and the care that it requires [12, 30, 32].

In the context of periodontal disorders, sheep have also been investigated as a research subject. The permanent dentition of a sheep has 32 teeth, with the following formula describing their arrangement: 0033/3033 (I, C, Pm, M). The roots of the incisors are quite short and the teeth themselves are physiologically movable. Periodontitis may damage these front teeth, and when it does, it rather quickly leads to large periodontal pockets and serious bone loss. The wall of the pocket is lined by an example of a pocket epithelium. Plasma cells have made their way into the connective tissue that lies beneath it. At the bottom of the pocket, the junctional epithelium is relatively short, and neutrophils that are migrating to the apical plaque border penetrate the connective tissue, eventually forming an interface layer [12, 64]. It seems as if the irritation of the gingiva is not very severe. Currently, there have been no recent studies published on sheep as an animal model for periodontitis.

Pigs and humans are quite similar when it comes to the progression of illness, anatomy, and physiology [65]. The Minnesota miniature pig has seen a significant amount of usage in a variety of biomedical research settings [66]. Gingivitis will often manifest in the minipigs at the age of 6 months and be characterized by bleeding of the gingiva upon probing, inflammation of the gingiva, and deposition of calculus and plaque. Gingivitis is an early stage of periodontal disease. Periodontitis may be induced in mini pigs in about four to eight weeks with the use of ligature wires and the introduction of bacteria [12, 66].

Alternative to Animal Models

Other than the animal models described above, the mouse calvarial defect model can provide useful information on the effectiveness of several regenerative medicines, allowing researchers to examine the repair of soft and hard tissues as well as the function of different molecules in the control of bone turnover and regeneration. In contrast to the murine models, the rabbit model requires more than just ligature entry into the periodontal fissure to cause periodontal disease. However, by introducing ligatures along with a topical application of a bacterium that causes periodontitis, such as *P. gingivalis*, rabbits can develop the disease [67]. Besides these animal models, cell lines can be used for the study of periodontitis. This will spare the animal's life and ethical consideration is not required.

Future Prospective and Conclusion

Periodontal disease models are useful for studying its pathophysiology in humans. Animal models are employed in periodontal research as the first step to human trials with novel biomaterials and therapies. Experimental models' structure, functioning, and pathogenicity should be as close to that of patients as feasible in order to show the safety and effectiveness of novel therapies in periodontal regeneration. Monkeys are the most human-like models anatomically and physiologically, but their usage is restricted due to ethical and financial concerns. In periodontal research, experimental models in dogs are regularly employed. Dog studies are challenging, similar to monkey studies, and methods should rationalize lesions to that extent so as to generate statistically useable data.

Because anatomically and physiologically they are similar to those of humans, monkeys and dogs should be used only in pre-clinical trials to validate novel therapies. Smaller, simpler to maintain, and less costly species have been suggested in addition to these huge animal models. Experimental periodontal diseases are developed in rats and hamsters. A surgical model for critical-sized periodontal lesions in rats was developed, allowing researchers to test novel biomaterials in conjunction with growth hormones or mesenchymal stem cells. The use of gnobiotic or germ-free rats is a common model in periodontal disease microbiology research. Similarly, the gilded hamster continues to be a fascinating model for immunological study. In periodontal research, new pathways have opened up, allowing for bigger, easier-to-maintain cohorts. For future study, more efficient use of animal models from a surgical standpoint is necessary.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

There are no acknowledgments.

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