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REVIEW ARTICLE

Mouse periodontitis models using whole *Porphyromonas gingivalis* bacteria induction

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Abstract *Introduction:* Due to the increasing prevalence of periodontitis within the general population, it is important to study the progress and stages of periodontal disease and the efficacy of periodontal treatment through *in vitro* and *in vivo* experiments. Mouse periodontitis models are important in many *in vivo* studies. This study presents the findings from a scoping review of the current literature regarding the available method to produce mouse periodontitis models using whole *Porphyromonas gingivalis (P. gingivalis)* bacteria.

Methods: The scoping review was carried out based on the methodology described by Arskey and O'Malley. An electronic literature search was conducted in the PubMed database. Inclusion and exclusion criteria were established. The data were collected on a purpose-made data extraction table for descriptive analysis.

Result: The researchers identified 11 articles that met the inclusion criteria for the review. Factors most considered in the literature relating to this topic are the vehicle to induce periodontitis, the type of strain for mice and *P. gingivalis*, the region of application, sacrifice day and the detection method used to measure the parameters.

Conclusion: The most frequently used vehicle to induce a mouse periodontitis model is the combination of *P. gingivalis* with ligature. Future research on different types of vehicles and bacteria for

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inducing more effective and more time-efficient periodontitis models is needed to guide future researchers on this topic.

Contents

1. Introduction

Periodontitis is considered a worldwide infectious disease that has affected approximately 20–50% of the population around the world (Nazir, 2017). It is also considered the most common dental disease following human tooth decay. Some systemic diseases, such as cardiovascular complications (Desvarieux et al., 2005), rheumatoid arthritis (de Pablo et al., 2009), and adverse pregnancy outcomes (Xiong et al., 2006), are presumed to be related to this disease. Periodontitis in the oral cavity is a chronic inflammatory disease (Alshammari and Amar, 2019; Ruan et al., 2016). It is a polymicrobial, predominant, multifactorial disease that is characterized by the loss of supporting tissues around the teeth, including the alveolar bone and periodontal ligament, which finally leads to tooth loss (Ishida et al., 2017; Rafiei et al., 2017).

Several pathogens play a vital role in periodontitis induction. *Porphyromonas gingivalis* (*P. gingivalis*), an anaerobic gram-negative oral bacterium, is considered a critical pathogen and virulent microbe involved in the onset and development of periodontitis (Ideguchi et al., 2019; Ishida et al., 2017). An investigation on the pathogenesis of *P. gingivalis* has been conducted in various experimental animal models, such as rat, mouse, rabbit, drosophila and cell models, showing the mechanisms between *P. gingivalis* and host response in the development of periodontitis (Rafiei et al., 2017). These models allow researchers to investigate the progress and stages of periodontitis and the efficacy of periodontal treatment (Graves et al., 2008).

There are a variety of animal models that have been employed to separately imitate the various pathogenesis stages of periodontitis and investigate the mechanisms of this disease *in vivo* (Oz and Puleo, 2011). Mouse periodontitis models have been utilized to imitate the different stages of pathogenesis and examine the mechanism of periodontitis *in vivo* (Marchesan et al., 2018). It displays some similarities in anatomic, bacterial, and pathogenic periodontal characteristics to humans, and modifications of their genome allow us to test mechanistic hypotheses (Saadi-Thiers et al., 2013). Mouse periodontitis experimental models are especially informative in examining downstream events related to the host immune reaction. Additionally, there is significant background information on mouse and rat immune systems and a wide range of immunologic and cellular reagents that are accessible for a thorough investigation (Graves et al., 2008).

There have been many variations in the procedures of making a mouse periodontitis model, but the concept of producing mouse periodontitis models using live *P. gingivalis* bacteria is still unclear and hampered by a lack of systematic understanding. Hence, the objective of this article is to present a scoping review that outlines the current literature regarding the available methods to produce mouse periodontitis models using whole *P. gingivalis* bacteria. Furthermore, this scoping review will guide future researchers in studying this topic.

2. Materials and methods

A scoping review was conducted based on the methodology described by Arksey and O'Malley (2005) with some adjustments based on our needs. For this study, the researchers used the five-stage approach, which included identifying the research question, identifying relevant studies and study selection, charting the data, collating, summarizing and reporting results. The researchers choose scoping reviews because it is useful to map, collate and summarize the existing literature on a topic and can assist researchers in classifying the nature and extent of the current research evidence. Scoping reviews can be carried out when little is known about the subject of the research question (Dickson-Swift et al., 2014; Marshall et al., 2016).

2.1. Identifying the research question

This review aims to answer the following question: 'What are the available methods to produce mouse periodontitis models using whole *P. gingivalis* bacteria?'

2.2. Identifying relevant studies: Constructing the search strategy and inclusion and exclusion criteria

The detailed search strategy applied specific MeSH terms and all field keywords to acquire the accuracy and sensitivity of the search to capture the relevant literature. Three independent reviewers performed an electronic literature search on PubMed database articles published between 1991 and 2020. The last period of the literature search was February 2020. Relevant information was sought from peer reviewed publications and grey literature. In this review, key word search terms were established, and a Boolean search string was developed (Table 1). Using truncated words and wild cards (in this case * and "*"), the researchers performed an extensive search that captured all terms with the same root word. There was no limitation on the study design or date of publication. Inclusion and exclusion criteria consistent with this review purpose were created and are outlined in Table 2.

Table 1 Database search terms for PubMed.

Periodont* OR "chronic periodontitis"	Mice OR Rats	Models, animal OR Models, Biological OR Model*	"Porphyromonas gingivalis"

Table 2 Inclusion and exclusion criteria.

Criteria	Inclusion	Exclusion
Language	English	Non-English
Type of article	Original research article published in a peer reviewed journal or grey literature	Any publication that was not original research, peer-reviewed journal article and/or unpublished; for example, PhD theses and reports
Study focus	Using live *P. Gingivalis* bacteria	Using bacteria other than *P. Gingivalis*, using LPS from *P.; Gingivalis*, using only the ligature method

2.3. Study selection

Utilizing the developed search terms, 335 published studies employing various methods to create a mouse periodontitis model using *P. gingivalis* bacteria were identified. After deletion of duplicates, 333 articles remained (Fig. 1). The bibliographic software program Endnote X7 was utilized to import and manage references. The title, abstract and keywords of the articles were scrutinized against the inclusion and exclusion criteria with research team members agreeing and confirming the disposal of irrelevant studies. If there was no abstract available, the original article was utilized. Following abstract screening, the reviewers analysed the full text to choose the final articles. The reviewers also cross-checked the references of the chosen articles to identify any undetected relevant studies. Following their independent full-text screenings, the reviewers compared their choices and discussed each publication individually prior to last article inclusion. Any disparities between the three reviewers regarding the inclusion of articles were resolved through a consensus discussion. Through this process, 11 articles were included in the final review.

2.4. Charting, collating and summarizing the data

Charting tables using an Excel spreadsheet were used to extract the data. To maintain the consistency of the data extraction, this stage was conducted by the three reviewers. The headings of the data extraction spreadsheet were author (s), title of publication, source of publication/journal name, year of publication, type and number of animals used, type and the amount of bacteria, method of application, detection method, and outcome and conclusion.

3. Results

As outlined in Table 3 and Appendix A, the scoping review identified several methods to produce mouse periodontitis models using whole *P. gingivalis* bacteria that have been used since 2000. From the 11 articles reviewed, the most frequently used method was a combination of *P. gingivalis* with ligature (81.8%; n = 9). The remaining articles (n = 2) only used

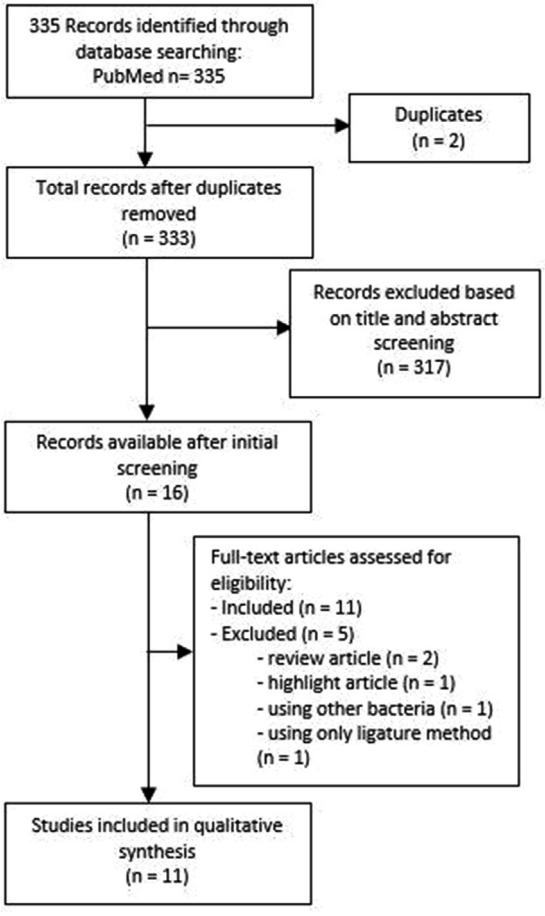

Fig. 1 Flowchart of literature search.

the oral gavage method. Six of the 11 articles used the C57BL/6 mouse strain (54.5%), and two other articles employed the Wistar strain. Each of the remaining three articles used DBA1/BO, BALB/cByJ and C3H/HeN mouse strains. Many types of *P. gingivalis* bacterial strains were used in these 11 articles, but the most widely used type was *P. gingivalis* ATCC 33,277 (54.5%; n = 6), with a bacterial concentration of 1×10^9 CFU. From these 11 articles, the most common area for bacterial application was the region of the first/second maxillary molar (81.8%; n = 9), followed by the mandibular molar and both of the jaws.

For inoculation days, the researchers found that there were very diverse results from studies included in this review. Hence, it was difficult to categorize them. The study of Alshammari et al. applied oral gavage of *P. gingivalis* bacteria every day for 15 days and sacrificed on day 15. Meanwhile, Ideguchi et al. applied *P. gingivalis* inoculated into the ligature on days 0 and 7 and sacrificed on day 14. Duan et al. conducted research on pregnant mice with ligatures soaked in *P. gingivalis* bacteria and applied them on day 8 of pregnancy. The mice were sacrificed 10 days after ligature placement. Another study from Lin et al. applied oral gavage of *P. gingivalis* on day 1 for 4 consecutive days and sacrificed 2 weeks later. Zhang et al. applied oral gavage of *P. gingivalis* four times in two weeks every 3 days and repeated another four inoculations after a 2-week interval. The mice were sacrificed 4 weeks after the second inoculation. Another study using *P. gingivalis* to induce periodontitis was conducted by Jianglin et al. They applied ligatures soaked with *P. gingivalis* bacteria on days 1 and 2 for two weeks and injected anti-RANKL on days 14, 15, 17 and 21. The sacrifice was performed on days 14 and 28. Saadi-thiers et al. used a ligature-only group, ligatures soaked with *P. gingivalis* bacteria and oral gavage of *P. gingivalis*. Ligatures soaked with bacteria were replaced twice a week, while oral gavage was applied twice a week for 3 weeks. The ligature-only group was sacrificed on days 15 and 45, and the ligature and *P. gingivalis* groups were sacrificed on days 15 and 30. The last oral gavage group was sacrificed on day 67. In another study by Meulman et al., the ligature was applied from the first day of the experiment until the day of sacrifice, and *P. gingivalis* bacteria were applied on days 0, 1 and 3. The mice were sacrificed on days 15, 21 and 30 after ligature placement. In the next study by Li and Amar, ligatures soaked with *P. gingivalis* bacteria were applied and replaced every other day. The sacrifice was on days 0, 3, 7 and 10 after ligature placement. Another study by Kitano et al. used ligatures from day 0, and *P. gingivalis* bacteria were inoculated 4 times at 2-hour intervals for 5 consecutive days. The mice were sacrificed 7 weeks after bacterial inoculation. In the last study by Kimura et al., ligatures soaked with *P. gingivalis* bacteria were applied on the first day and left until sacrifice. The mice were sacrificed on weeks 1, 3, 5, 7, 9, 11, 13 and 15.

Many detection methods were used in the 11 articles, and they had one similarity. They performed measurements of alveolar bone loss or bone volume analysis to confirm that periodontitis had already occurred. Some articles even used more than one detection method to ensure alveolar bone loss. Seven of the 11 articles used a histomorphometry approach from histological analysis to measure alveolar bone loss. Three articles used radiology techniques though 2D or 3D micro-CT. Two articles used digital stereomicroscopy, and the other two articles used a morphometry approach. Two of the 11 articles used TRAP staining for osteoclast activity to detect any alveolar bone loss. Bone mineral density was used in one article to confirm alveolar bone loss. One article used the simplest method to confirm alveolar bone loss through direct clinical measurement after defleshing the specimen, and one article did not mention the method used in measuring alveolar bone loss.

Another detection method was used to ensure the presence of *P. gingivalis* bacteria in periodontal tissue, which confirmed that mouse periodontitis models were already established. The methods included bacterial counting analysis, western blot analysis, ELISA, PCR, detection of oral bacterial number using CFU (colony forming units), immunocytochemistry (ICC) and immunohistochemistry (IHC). Each of these methods was used in a different article.

Aside from alveolar bone loss and bacterial load measurements (such as bacterial counting analysis, western blot analysis and colony forming units), seven of the 11 articles used cytokine levels to detect periodontitis in mouse models through cytokine assays, ELISA, PCR, TRAP, ICC and IHC. One article employed cytokine analysis, another three articles used ELISA, four articles used PCR to detect mRNA levels of some pro-inflammatory and anti-inflammatory cytokine, two articles used TRAP to detect osteoclast activity, one article used immunocytochemistry and one article used immunohistochemistry.

4. Discussion

The purpose of this scoping review was to investigate the breadth of evidence related to the available methods for producing mouse periodontitis models using whole *P. gingivalis* bacteria. During the data extraction, it was recognized that this method differs in many factors. The most often considered factors in the literature related to this topic are the vehicle to induce periodontitis, the type of strain for mice and *P. gingivalis*, the region of application, the inoculation day, the sacrifice day and the detection method used to measure the parameters. The main strengths of this review include the scoping review methodology and comprehensive reproducible search strategy. However, the review was limited by one database that was searched and the fact that this scoping review focused only on using *P. gingivalis* bacteria. Further research is also needed with regard to exploring other studies that use different types of bacteria.

In this review, the researchers identified that the most frequently used vehicle to induce a mouse periodontitis model is the combination of *P. gingivalis* with ligature (Ideguchi et al., 2019; Kimura et al., 2000; Kitano et al., 2001; Li and Amar, 2007; Lin et al., 2014, 2017; Meulman et al., 2011; Saadi-Thiers et al., 2013; Xingyu et al., 2019). According to Saadi-Thiers et al. (2013), the *P. gingivalis* soaked ligature creates more active periodontal breakdown and a more excessive systemic response than induction by ligature or oral *P. gingivalis* alone. In their study, *P. gingivalis* induction alone did not result in significant periodontal tissue breakdown compared to the controls, indicating that these bacteria have a role in worsening the inflammatory response to mechanical injury. However, periodontal inflammation and pocket formation are prerequisites. This result is consistent with the study by Li et al., indicating that the *P. gingivalis*-soaked ligature method

provides a simple and straight route to deliver adequate bacteria into the mouse gingival sulcus to create colonization and initiate the pathogenesis of periodontitis (Li and Amar, 2007). However, different results were obtained in the study conducted by Meulman et al. They reported that significant bone loss was observed for both the ligated-only group and the *P. gingivalis*-soaked ligated group compared with the non-ligated group with higher alveolar bone loss observed for the ligated-only group at all experimental time points. This could be explained because, in the ligated group, a more pro-inflammatory and pro-resorptive environment was found. In the *P. gingivalis*-soaked ligated group, the bacteria elicited the host immune response towards the anti-resorptive and anti-inflammatory environment, which may have been responsible for the lower level of alveolar bone loss compared with the ligated only group (Meulman et al., 2011).

Bacterial strain type is considered an important factor in determining whether alveolar bone loss will occur. *P. gingivalis* ATCC 33277, genetically identical to strain 381, was originally isolated from an adult periodontitis patient (Baker et al., 2000). This type of strain is the most commonly used strain in this review because it is easily found in adults who suffer from chronic periodontitis. The study of Wilensky et al. showed that oral infection with *P. gingivalis* 53,977 and *P. gingivalis* 381 (identical to ATCC 33277) strains can induce alveolar bone loss in BALB/c mice with no significant difference between these two groups (Wilensky et al., 2005). However, Baker et al. reported that significant bone loss could be

Table 3 Overview of findings and results.

Findings	Result	% (n)	Source of study
Method of aplication	Combination of P. Gingivalis and ligature	81.8 (9)	(Ideguchi et al., 2019; Kimura et al., 2000; Kitano et al., 2001; Lin et al., 2014; Lin et al., 2017; Li and Amar, 2007; Meulman et al., 2011; Saadi-Thiers et al., 2013; Xingyu et al., 2019)
	Only oral gavage of P. Gingivalis	18.2 (2)	(Alshammari and Amar, 2019; Zhang et al., 2014)
Type of mice used	C57BL/6	54.5 (6)	(Ideguchi et al., 2019; Lin et al., 2014; Lin et al., 2017; Li and Amar, 2007; Saadi-Thiers et al., 2013; Xingyu et al., 2019)
	Wistar rats	18.2 (2)	(Kitano et al., 2001; Meulman et al., 2011)
	DBA1/BO	9.1 (1)	(Alshammari and Amar, 2019)
	C3H/HeN	9.1 (1)	(Kimura et al., 2000)
	BALB/cByJ	9.1 (1)	(Zhang et al., 2014)
P. Gingivalis strain	ATC 33,277	54.5 (6)	(Lin et al., 2014; Lin et al., 2017; Kitano et al., 2001; Saadi-Thiers et al., 2013; Xingyu et al., 2019; Zhang et al., 2014),
	W84	27.3 (3)	(Alshammari and Amar, 2019; Ideguchi et al., 2019; Meulman et al., 2011)
	A7436	9.1 (1)	(Li and Amar, 2007)
	381	9.1 (1)	(Kimura et al., 2000)
Application area	First or second maxillary molar	81.8 (9)	(Alshammari and Amar, 2019; Ideguchi et al., 2019; Lin et al., 2014),(Kitano et al., 2001; Lin et al., 2017; Li and Amar, 2007; Saadi-Thiers et al., 2013; Xingyu et al., 2019; Zhang et al., 2014),
	Mandibular molar	9.1 (1)	(Meulman et al., 2011)
	Both jaws	9.1 (1)	(Kimura et al., 2000)
Detection method	Histomorphometry	21.9 (7)	(Alshammari and Amar, 2019; Ideguchi et al., 2019; Kitano et al., 2001; Li and Amar, 2007; Meulman et al., 2011; Saadi-Thiers et al., 2013; Zhang et al., 2014),
	Radiology technique (2D or 3D micro-CT)	9.3 (3)	(Ideguchi et al., 2019; Li and Amar, 2007; Zhang et al., 2014)
	Digital stereomicroscopy	6.2 (2)	(Lin et al., 2014; Lin et al., 2017)
	Morphometry	6.2 (2)	(Alshammari and Amar, 2019; Li and Amar, 2007)
	BMD (Bone Mineral Density)	3.2 (1)	(Li and Amar, 2007)
	Clinical direct measurement	3.2 (1)	(Kimura et al., 2000)
	Bacterial counting analysis	6.2 (2)	(Alshammari and Amar, 2019; Li and Amar, 2007)
	Western blot analysis	3.2 (1)	(Kimura et al., 2000)
	CFU (Colony Forming Units)	3.2 (1)	(Xingyu et al., 2019)
	Cytokine Assay	3.2 (1)	(Saadi-Thiers et al., 2013)
	ELISA	9.3 (3)	(Ideguchi et al., 2019; Lin et al., 2014; Lin et al., 2017)
	PCR	12.4 (4)	(Lin et al., 2014; Lin et al., 2017; Meulman et al., 2011; Xingyu et al., 2019)
	TRAP	6.2 (2)	(Li and Amar, 2007; Saadi-Thiers et al., 2013)
	ICC (Immunocytochemistry)	3.2 (1)	(Saadi-Thiers et al., 2013)
	IHC (Immunohistochemistry)	3.2 (1)	(Zhang et al., 2014)

obtained with *P. gingivalis* 53,977 but not with *P. gingivalis* 381. He concluded that *P. gingivalis* 381 was not a good inducer of bone loss in mice (Baker et al., 2000). The most suitable explanation for this result is the fact that the alveolar bone loss induced by *P. gingivalis* 381 could not be detected by the morphometric technique, which measures bone loss only in the horizontal direction. According to Evans et al., the alveolar bone loss pattern induced by *P. gingivalis* 53,977 is mostly horizontal in nature, while alveolar bone loss following infection with *P. gingivalis* 381 is mostly vertical in nature (Evans et al., 1992).

The area of bacterial application is an important consideration in this review. Researchers have reported that the most common area for bacterial application is the region of the first or second maxillary molar. For alveolar bone loss assessment, researchers most often examine the bone around the maxillary molars because induction of bone loss in the mandible is slower due to the thicker cortical alveolar bone and wider buccolingual dimensions, whereas incisors are not included in the assessment due to their continuous eruption (Graves et al., 2011, 2008). The study by Kimura et al. also stated that the application of a *P. gingivalis*-adhered ligature into the gingival sulcus around the molar teeth may serve as a notch of bacterial colonization and result in more time-effective and specific periodontal infection (Kimura et al., 2000).

From this review, the researchers could see that there are so many variations in the sacrifice day. The sacrifice day correlates with the duration of bacterial inoculation and duration of illness. It takes some time to produce alveolar bone loss, which becomes a specific marker in periodontitis disease. Saadi-Thiers et al. concluded that the duration of periodontitis induction is one of the main factors for periodontal tissue inflammation and destruction. Their study reported that the combinations of various induction and duration methods of *P. gingivalis* infection showed specific time-dependent patterns of alveolar bone resorption, protease expression, and cytokine blood level variation (Saadi-Thiers et al., 2013). Similarly, Li and Amar also reported that periodontal tissue destruction increased with increasing experimental time and corresponded to obvious inflammatory infiltration. Their histomorphometric results showed that alveolar bone losses significantly increased throughout the experimental period compared to controls (Li and Amar, 2007).

The most commonly used detection method for alveolar bone loss in this scoping review was histological analysis through a histomorphometry approach. This method needs necessary tissue preparation steps and requires substantial effort. However, these types of measurements can simultaneously provide alveolar bone loss quantification and other histology or immunohistochemistry measurements. Therefore, this method could capture both soft and hard tissue information (Li and Amar, 2007). The other method that may have more advantages for detecting alveolar bone loss is digital radiology techniques though 2D or 3D micro-CT. This method is more sensitive in showing interproximal bone loss and providing three-dimensional images. It also provides an adequate possibility to assess bone loss by determining three-dimensional structures of hard tissue using the volumetric method. However, this method is expensive, and it can be time consuming to compose the images and measure alveolar bone loss (Li and Amar, 2007; Wilensky et al., 2005).

5. Conclusion

The most frequently used vehicle to induce a mouse periodontitis model is the combination of *P. gingivalis* with ligature. This scoping review has provided breadth evidence related to the available methods for producing mouse periodontitis models using whole *P. gingivalis* bacteria. Future research to explore different types of vehicles and bacteria to induce more effective and more time-efficient periodontitis models is needed to guide future researchers on this topic.

Ethical statement

All authors declared that this research has follow the ethical procedure.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sdentj.2021.08.001.

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