Animal models for testing biomaterials in periodontal regeneration

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Key Words:
animal model; biomaterials; periodontitis; tissue regeneration

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ABSTRACT

Periodontitis is a prevalent oral disease. It can cause tooth loss and has a significant impact on patients' quality of life. While existing treatments can only slow the progression of periodontitis, they are unable to achieve complete regeneration and functional reconstruction of periodontal tissues. As a result, regenerative therapies based on biomaterials have become a focal point of research in the field of periodontology. Despite numerous studies reporting the superiority of new materials in periodontal regeneration, limited progress has been made in translating these findings into clinical practice. This may be due to the lack of appropriate animal models to simulate the tissue defects caused by human periodontitis. This review aims to provide an overview of established animal models for periodontal regeneration, examine their advantages and limitations, and outline the steps for model construction. The objective is to determine the most relevant animal models for periodontal regeneration based on the hypothesis and expected outcomes.

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Introduction

Periodontitis is a chronic inflammatory disease characterised by progressive destruction of the periodontium, including the gingiva, alveolar bone, periodontal ligament, and cementum. The disease affects about 20–50% of global population, with clinical manifestations such as gingival recession, tooth mobility, and tooth loss.[1] The primary cause of periodontitis is subgingival dental biofilm, which is why traditional treatments aim to control biofilm and reduce inflammation to halt the progression of the disease.[2] However, such treatments are limited in their ability to reverse tissue loss and reconstruct the biomechanical support of the teeth.[2] To address these limitations, regenerative therapies aimed at reconstructing all elements of the periodontium have become a central focus in the field of periodontology. These therapies strive to restore both the structure and function of the periodontium over previously diseased teeth.

Periodontal regeneration is an approach that leverages biomaterials to support tissue growth and repair. By utilizing biomaterials that create a supportive environment and incorporating growth factors and stem cells, periodontal regeneration aims to recreate a functional interface between the various components of the periodontium. Guided tissue regeneration concepts are applied through the use of barrier biomaterials to direct periodontal ligament stem cells and facilitate connective tissue attachment.[3,4] Bone grafting materials, such as autogenous and allograft bone and bone substitutes, are used to fill bone defects and promote bone and cementum regeneration.

Despite promising results from preclinical studies, the clinical outcomes of periodontal regeneration remain limited and unpredictable.[5] To fully restore the mechanical structure of the periodontium, it is necessary to recreate the functional interface between all components of the periodontium. To this end, the regenerative effects of biomaterials must be validated in similar tissue defects prior to their clinical application.

At present, a variety of animal models have been employed to model periodontitis or similar

periodontal defects, including rats, dogs, rabbits, miniature pigs, and non-human primates.[6-8] Large animals are preferred for their similarity in anatomy and physiology to humans, but ethical considerations limit their use. Smaller animals, such as rats and rabbits, are more cost-effective and convenient, and are often used in the early stages of material testing.

To model periodontal defects in animals, various surgical and ligature-induced models have been established, including supra-alveolar defects,[9] dehiscence defects,[10] furcation defects,[11] fenestration defects[12] and intrabony defects.[13] In these models, periodontal ligament and cementum are completely removed to reproduce the characteristics of human periodontal defects.

This review summarises the various animal models used for periodontal regeneration, detailing their advantages and procedures for establishing periodontal defects.

Literature Search

The articles about animal models for testing biomaterials in periodontal regeneration were retrieved by the search terms: ("periodontal regeneration" OR "periodontal tissue engineering") AND ("animal model" OR model OR preclinical) AND (biomaterial OR biomaterials). These searches were performed on Medicine in December, 2022. A total of 185 records were retrieved and carefully screened for eligibility. Finally, 114 articles were included in this review (**Figure 1**).

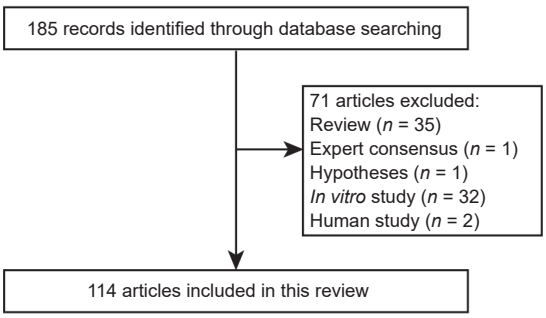

Figure 1. Flowchart of the literature search process.

Periodontal Defect Model

Mouse

Mice are widely utilised in biomedical research as models for periodontal disease due to their low-cost and convenience. However, the narrow oral cavity of mice poses challenges when performing intricate surgical procedures. This limitation makes it difficult to achieve uniform defect sizes across experimental groups. Hence, the murine periodontal defect was not surgically created, but rather induced through periodontitis. The unique advantage of mice is that stem cells originating from human can be transplanted and evaluated for their regenerative ability in immunodeficient mice. Additionally, mice can be gene-manipulated, enabling researchers to study the impact of specific genes on periodontal regeneration.

In 2018, Dr. Fareeha Batool[14] reported a chronic defect model in mice to test a membrane biomaterial for periodontal regeneration. The model involved inducing periodontitis in the palatal sites of the maxillary 1st molar through the placement of *Porphyromonas gingivlais*-infected silk ligatures. After 40 days, intrabony defects were formed, and the safety and efficacy of the biomaterial were evaluated through a palatal flap technique. The biomaterial was placed over the bony defect, and periodontal regeneration was assessed at 7th and 15th days post-treatment. The assessment included measurements of epithelial attachment, connect tissue attachment and alveolar bone level, analysed through histological imaging, to demonstrate tissue regeneration. This study provides valuable insights into the use of mice as models for evaluating regenerative therapies in periodontal disease.

Rat

The rat has become a commonly used animal model in the study of periodontal disease due to its histological similarities with the human periodontal region. Through inoculating periodontal pathogens, or fixing ligatures around the teeth, rats can develop periodontitis.[15] However, it is important to consider certain differences between rats and humans, including rapid wear of the occlusion surfaces and continuous eruption of molars leading to physiological remodelling of periodontal tissues.[7] Moreover, it is noteworthy that in rats, tooth drift occurs in a distal direction, whereas in humans, it occurs mesially. Cellular cementum progressively deposits to adapt to these physiological changes. This has a significant impact on the interpretation of data from periodontal defect models in rats. In addition, rats are less likely to form intrabony defects in periodontitis model due to the narrow interdental spaces, usually presented with horizontal bone loss.[16]

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Periodontal fenestration defect models have been widely used in research. Male Wistar or Sprague-Dawley rats are the most commonly used strains in these studies, where a critical-size defect is created on the radicular surfaces of the mandibular molars. The surgical procedure involves making an incision along the inferior border of the mandible, dissecting the underlying masseter muscle and periosteum, creating a 2 mm wide, 5 mm long, and 1.2 mm deep defect, and removing residual periodontal ligament and cementum.[17] To assess the regenerative ability of membrane biomaterials, these membranes were placed into the defects. Three weeks after the regenerative surgery, tissue samples are collected for the histological examination. This model can be modified by injecting rats with lipopolysaccharide to establish an inflammatory condition,[18] conducting the procedure in athymic rats to study the cell sheets obtained from human donors,[12, 19] or combining it with ovariectomy to study the effect of biomaterials in patients with both periodontal disease and osteoporosis.[20, 21]

Intrabony defect models can also be created in rats by surgically creating bilateral intrabony defects mesial to the first maxillary molar. After a crestal incision is made on the alveolar ridge, the alveolar bone mesial to the first maxillary molar is eliminated using a piezoelectric device under saline irrigation.[13, 22-29] The final dimensions of the defect are 2 mm by 2 mm by 1.7 mm in length, width, and depth, respectively. The defects are then treated with biomaterials according to the study design, and after 12 weeks, animals are euthanised and dissected for histomorphometry analysis.[24]

Rabbit

Periodontitis can be induced in rabbits.[30] Unlike rats and mice, the use of ligatures alone is not sufficient to induce periodontal disease in rabbits, making them a valuable model for evaluating the specific impact of human origin periodontal pathogens. Surgical creation of periodontal fenestration defects is also possible in rabbits due to their larger size, however, the continuous eruption of their teeth results in shifting of the defects towards the occlusal surface. By 12 weeks post-surgery, the defects have been replaced, rather than regenerated, with no signs of the surgical procedure present. As a result, the rabbit fenestration defect model is not appropriate for the study of periodontal regeneration.[31] Instead, traditional models such as rabbit femoral and calvarial defects have proven to provide valuable insights into bone healing and regeneration, although they may not accurately reflect the specific situation of periodontitis.[32, 33]

Dog

The size and anatomy of teeth and periodontal tissues in dogs are comparable to those observed in humans. Due to the accumulation of dental plaque and calculus, dogs frequently develop periodontitis in adulthood. In dogs, periodontitis is characterised by gingival recession, the formation of deep periodontal pockets, and bone defects.[34] Furcation regions are more commonly affected than interdental spaces, and in the late stages of periodontitis, the first and second premolars are most frequently lost. While naturally occurring periodontal defects in dogs are not uniform in terms of extent and location, this model may not be ideal for studying periodontal regeneration.[35] In contrast, surgical models are often used to test regeneration procedures. Defects can be rapidly and symmetrically created in dogs, allowing for the assessment of scaffolds, membranes, and growth factors for tissue regeneration. However, it is important to note that the care regulations for dogs are more complex and costly compared to rodents. Dogs require weekly periodontal prophylaxis, including ultrasonic cleaning and the use of 0.12% chlorhexidine mouthwash.

Intrabony defects

The production of 1-, 2-, and 3-wall intrabony defects in beagles has been experimentally validated. Among these, 3-wall intrabony defects exhibit greater potential for periodontal tissue regeneration. On the other hand, 1-wall defects are more susceptible to the formation of junctional epithelium, owing to the challenge of preserving the interstitial space between the tooth surface and the soft tissue flap.[36] The outcomes of tissue regeneration in the 2-wall intrabony defect model are influenced by the location of tissue sections, with sections closer to the lateral wall demonstrating greater regeneration than those further away.[35] Thus, the 2-wall intrabony defect may not be the optimal choice for evaluating candidate biomaterials.

The procedures for creating periodontal intrabony defects in dogs have been described in previous literatures.[37-42] Following the extraction of one or two premolars and a 2-month healing period, incisions are made and mucoperiosteal flaps are elevated from the underlying jaw bone. The alveolar bone, cementum, and periodontal ligament of the root surface are then surgically removed to create the intrabony defects, as illustrated in **Figure 2**. In each dog, up to four defects can be produced. An 8-week healing interval post-regenerative surgery is sufficient for the evaluation of periodontal regeneration in tissue samples.[43] Five histometric parameters, as illustrated in **Figure 3**, are useful for the assessment of tissue regeneration in histological images:[36, 44]

1. Defect height: distance from the alveolar crest to the base of the reference notch.
2. Junctional epithelium: distance from the cemento-enamel junction to the apical extension of the junctional epithelium on the root surface.
3. Connective tissue attachment: distance from the apical extension of the junctional epithelium to the coronal extension of newly formed cementum.
4. Cementum regeneration: distance from the base of the reference notch to the coronal extension of newly formed cementum on the root surface.
5. Bone regeneration: distance from the base of the reference notch to the coronal extension of newly formed bone along the root surface.

Supra-alveolar defects

The supra-alveolar defect model in dogs involves the horizontal removal of alveolar bone surrounding teeth. Specifically, critical-size 5-mm supra-alveolar periodontal defects are created around the mandibular premolars after

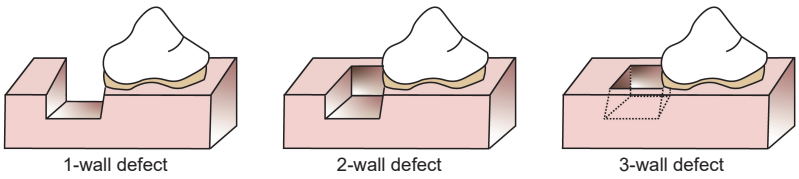

Figure 2. Schematic diagram depicting the surgically created 1-, 2-, and 3-wall intrabony defects.

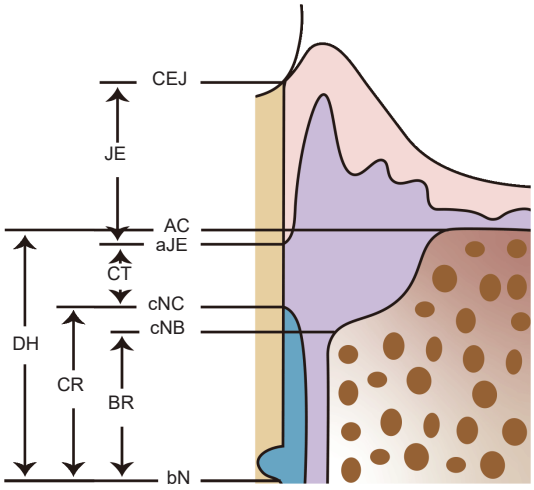

Figure 3. Schematic drawing representing measurement points for histometric parameters. AC: alveolar crest; aJE: apical extension of the junctional epithelium; bN: base of the reference notch; BR: bone regeneration; CEJ: cemento-enamel junction; cNB: coronal extension of newly formed bone; cNC: coronal extension of newly formed cementum; CR: cementum regeneration; CT: connective tissue attachment; DH: defect height; JE: junctional epithelial attachment.

elevating the mucoperiosteal flaps.[9, 45] The alveolar bone is removed using chisels and a hand-piece with fissure burs under water irrigation. The residual periodontal ligament and cementum are then removed from the roots through planing. As previously described, sterile ligatures are placed around the affected teeth for 8 weeks to induce an inflammatory condition in the periodontium.[46] This model is used to evaluate the efficacy of new biomaterials in promoting periodontal regeneration.

Furcation defects

The anatomy of the upper and lower premolars and the progression of periodontitis at these sites in beagles and mongrel dogs are highly similar to those in humans, making these dogs a valuable model for studying periodontal disease. The furcation defect model is often established around premolars, with class II furcation defects exhibiting the greatest potential for periodontal tissue regeneration. Conversely, class III furcation defects are associated with limited and unpredictable tissue regeneration due to the limited number of periodontal stem cells present on the defect surface.[47]

To create a class II furcation defect, the buccal mucoperiosteal flap is elevated to reveal the underlying alveolar bone, which is then removed to expose the buccal roots. The root surfaces are scaled to remove debris, periodontal ligament, and cementum. The defects are created with dimensions of 5 mm apicocoronally, 5 mm mesiodistally, and 3 mm buccolingually.[48, 49] The surgical procedure for creating class III furcation defects is similar to that of class II, with the difference being that more alveolar bone is removed to form a buccolingually penetrating defect on the root.[50] After surgery, the defects are filled with polyether impression material for 21 days to induce an inflammatory response and suppress natural bone repair.

Recession-type defects

The creation of artificial recession-type defects on the buccal surfaces of maxillary canine teeth in dogs is a common surgical procedure. This model is established by making a semicircular incision and two vertical incisions on the buccal gingiva, followed by elevation of a full-thickness flap. The resultant buccal dehiscence defects are 6 mm apicocoronally and 5 mm mesiodistally in dimensions.[51]

Interproximal periodontal defects

The Interproximal periodontal defect model was first established on dogs by Jung et al.[44] in 2011 as a means of studying a common clinical defect. The surgical procedure involves the creation of a vertical incision in the area surrounding the maxillary second premolar and the elevation of a full-thickness flap to expose the underlying alveolar bone between the second and third premolars. The removal of the alveolar bone and remaining periodontal tissues from the root surfaces results in the creation of a 5 mm vertically oriented and 3 mm horizontally oriented pocket defect that lacks any supporting bony walls.

Miniature pig

The miniature pig has proven to be a valuable animal model in periodontal research due to its similarity to humans in periodontal anatomy and the presence of naturally occurring periodontitis.[52] Additionally, its economical advantage over larger animals has further facilitated its use in various experiments. The induction of experimental periodontitis through ligature placement and pathogen inoculation in these animals takes approximately 4 weeks to develop, with the resultant destruction not fully restored even upon removal of the ligatures. Over the years, miniature pigs have been utilised to assess the regenerative capabilities of various biomaterials and technologies, with dehiscence-type defects created in the buccal aspect of mandibular premolars as one such example.[10]

Nonhuman primate

The anatomy of teeth and periodontium in nonhuman primates, particularly Macaca fascicularis, closely resembles that of humans and is characterised by the presence of dental plaque, calculus, and periodontal disease in adulthood.[53] Thus, nonhuman primates are considered an excellent animal model for evaluating regenerative procedures in periodontics. However, the use of nonhuman primates in experimental research raises ethical concerns and is expensive due to the requirement of specialised facilities and high costs associated with procurement and maintenance. As a result, the evaluation of new biomaterials in lower order phylogenetic species is recommended prior to testing in nonhuman primates.

There are three methods for inducing periodontal defects in nonhuman primates: the acute defect model, the chronic defect model, and the acute/chronic defect model. In the acute defect model, standard surgical procedures are followed, and spontaneous regeneration occurs in approximately 50–70% of cases, making it difficult to determine the extent to which regenerative outcomes are due to the biomaterials.[54] In the chronic defect model, orthodontic elastics are placed around the teeth, leading to accelerated plaque accumulation, inflammation, and destruction of periodontal tissues over a 3–6-month period. This defect model is usually conducted in single root teeth. If the interproximal space between teeth is large, then an angular intrabony defect will be obtained. If the interproximal space is narrow or there is an elastic on the adjacent tooth, suppra-alveolar defects will be achieved.[55] This model accurately mimics human periodontitis, but is time consuming and costly. The acute/chronic defect model combines surgical removal of periodontal tissues with the placement of ligatures or alginate to induce chronic inflammation and inhibit spontaneous regeneration.[11, 56-58]

Other Defect Models

The mandibular angle defect model is a widely accepted method for evaluating the efficacy of biomaterials in promoting bone regeneration. This model involves creating round, through-and-through osseous defects measuring 5 mm in diameter on both sides of the mandible in Sprague-Dawley rats. It has been successfully used to assess the performance of membrane materials and the results are obtained by fixing the membranes to the bone defects for 6 weeks and monitoring the progress of bone regeneration.[59]

The calvarial defect model, on the other hand, is a powerful tool for evaluating the potential of biomaterials for craniofacial skeleton regeneration. This model is also useful for periodontal research, as the physiological remodelling of calvaria is similar to that of the human mandibular bone.[60] This model is conducted in rats and rabbits and involves exposing the calvarial bone through a sagittal incision, elevating a full-thickness flap, and creating a round transosseous defect of 8 mm in diameter in the centre of the calvarium of rats[61-67] or four bony defects of the same diameter in rabbits.[33, 68, 69] The created bony defect is regarded as a critical-sized defect, which will not heal spontaneously during the lifetime of the animal.[70] To evaluate the effectiveness of the biomaterials, histological evaluation and micro-computed tomography analysis are performed at 2- and 8-week intervals post-surgery. Micro-computed tomography analysis calculates parameters such as total augmented volume, new bone volume, bone volume fraction, and bone mineral density, which are analysed for statistical significance.[63] Meanwhile, histomorphometric analysis measures the areas and percentages of new bone, residual material, and defect closure, providing additional information on the efficacy of the biomaterials.[64, 71]

Heterotopic Regeneration Model

The heterotopic subcutaneous transplantation model has been extensively utilised in stem cell-based regeneration research. This well-established model eliminates the influence of microenvironmental factors on tissue regeneration, making it a valuable tool for evaluating stem cell-based therapies. The model involves the assembly of stem cell sheets, specifically human periodontal ligament stem cells, onto scaffold materials[72-75] or bone grafts,[76-80] which are then subcutaneously implanted into the dorsal area of athymic mice or rats. Some studies have incorporated dentin slices and bone substitutes to simulate the periodontal space.[72-74] Following a 6–8-week healing interval, tissue samples are collected and analysed for the formation of cementum, bone, and the periodontal ligament. These evaluations provide insight into the efficacy of stem cell-based therapies for periodontal tissue regeneration.

Conclusions & Perspectives

The testing of periodontal regenerative procedures in animal models is crucial prior to the initiation of clinical trials. By creating periodontal defects similar to those found in human patients, the safety and effectiveness of new biomaterials and treatments can be assessed. The availability of sufficient tissue samples allows for a comprehensive evaluation of the quality and extent of regenerated periodontal support tissues.

Rats, due to their ease of feeding and maintenance, have become the standard animal model for testing new biomaterials. However, it is important to note that the physiology of the periodontium in rats differs from that of humans. Dogs and non-human primates, on the other hand, provide the closest approximation of human periodontium, making them ideal models for the verification of regenerative procedures. However, the use of these animals must be justified by ethical considerations and the limitations imposed by the number of animals used must be carefully considered during the

experimental design. In conclusion, a stepwise approach is recommended for evaluating new biomaterials, starting with testing in small animals, followed by validation in dogs and non-human primates.

While acute defects, namely surgically created defects, are predominantly employed in these included articles due to their ability to ensure uniform defect sizes across experimental groups and reduce the duration of animal experiments, it's worth highlighting that acute defects do not faithfully replicate the pathological characteristics of defects caused by periodontitis. Discrepancies exist in the pathophysiological conditions and the processes involved in tissue healing and regeneration between acute defects and those caused by periodontitis. Consequently, experimental findings derived from acute defect models may not offer precise predictability for clinical applications. As such, we advocate the adoption of chronic periodontal defect models, with the aim of furnishing more clinically pertinent insights for translational purposes. Furthermore, periodontitis-induced tooth mobility and tooth loss represent the most immediate factors impacting patients' quality of life. In the context of these animal experiments, there is limited documentation regarding the effectiveness of biomaterials in ameliorating tooth mobility. We contend that these functional outcome assessments should be incorporated into the evaluation of animal models, in addition to histomorphological measurements, to provide a more comprehensive understanding.

This review provides a comprehensive overview of frequently employed animal models as reported in the literature (**Table 1**). The rationale for the selection of these animal models in the

Table 1. Animal models for periodontal regeneration

Animal species	Characteristic	Type of model	Site of defect	Size of defect	Longest period for evaluation
Mouse	Low-cost; Immunodeficient mice and gene-manipulated mice are available; Regenerative procedures are difficult to conduct due to the narrow oral cavity.	Chronic periodontal defect model			15 days
		Heterotopic transplantation model			8 weeks
Rat	Well established as a model for periodontitis; Physiological remodelling of periodontal tissues due to continuous eruption of molars; More likely to form horizontal bone loss due to the narrow interdental spaces.	Acute periodontal defect model	Fenestration defect	2 × 5 × 1.2 mm^3	3 weeks
		Other defect models	Intrabony defect	2 × 2 × 1.7 mm^3	12 weeks
			Mandibular angle defect	5 mm in diameter	6 weeks
			Calvarial defect	8 mm in diameter	12 months
Rabbit	Periodontitis can be induced; Fenestration defects may be replaced rather than regenerated due to the continuous eruption of their teeth.	Other defect models	Calvarial defect	8 mm in diameter	8 weeks
Dog	Size and anatomy of periodontal tissues are comparable to humans; Naturally developed periodontitis; The daily care and postoperative maintenance are more complex than rodents.	Acute periodontal defect model	Intrabony defect	5 × 5 × 5 mm^3 or 5 × 5 × 4 mm^3 or 4 × 4 × 4 mm^3	24 weeks
			Supra-alveolar defect	5 mm	8 weeks
			Furcation defect	5 × 5 × 3 mm^3	120 days
			Recession-type defect	6 × 5 mm^2	8 weeks
			Interproximal periodontal defect	5 × 3 mm^2	8 weeks
Miniature pig	Similarities to humans in periodontal anatomy and the presence of naturally occurring periodontitis.	Acute periodontal defect model	Recession-type defect	5 × 4 mm^2	3 months
Nonhuman primate	The anatomy of teeth and periodontium in nonhuman primates closely resembles that of humans; An excellent animal model for evaluating regenerative procedures; Ethical concerns, requirements of specialised facilities and high costs.	Acute/chronic periodontal defect model		7–9 × 2–3 mm^2 or 3 × 4 × 5 mm^3	5 months

cited articles was typically based on references to previously published research. Consequently, our review lacks an in-depth exploration of the physiological characteristics of periodontal tissue in various animal species but centres on the methodologies for establishing these models. Additionally, it should be noted that only a limited number of the included articles provided data on the incidence of adverse events following regenerative procedures, thus this critical aspect is not addressed in our review.

Author contributions

QS and HH conceptualised and designed the review; QS drafted the manuscript; YL, PL and HH checked and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

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Conflicts of interest statement

The authors declare no conflict of interest.

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