UNIVERSIDAD EAFIT

Vigilada Mineducación

A Novel Injectable Piezoelectric Hydrogel for Periodontal Disease Treatment With Combined Antibacterial And Tissue Regeneration Effects In Vitro and In Vivo Analysis

Article – Based PhD Thesis

Lina María Roldán Lopera

Doctoral adviser

Santiago Correa Ph.D. Bioengineering Research Group (GIB).

Universidad EAFIT, Colombia

ESCUELA DE INGENIERÍAS

DOCTORADO EN INGENIERÍA

MEDELLÍN

2023

INTRODUCTION

Severe periodontal disease impacts approximately 10% of the global population [1], with a similar prevalence of 10.6% in Colombia [2] and affecting nearly half of all adults in the United States [3]. Periodontal disease (PD) is a major oral disease affecting half of adults in the United States PD is caused by pathogenic microflora in the biofilm that forms adjacent to the teeth [4]. The bacterial infection induces an inflammatory response that triggers the progressive destruction of the periodontal tissues and, finally, the loss of teeth [5]. Non-surgical treatment has a limited 39% success rate and faces challenges such as patient compliance, difficulty in treating deep pockets [6,7], limited tissue regeneration, and concerns about antibiotic resistance [8–10]. If non-surgical treatment is ineffective, surgery may be indicated via bone grafting, guided bone tissue regeneration membranes, and scaffolds that deliver bioactive molecules [11–14].

The aim of this work is to develop a piezoelectric hydrogel which could be used as a single therapy with antibacterial and regenerative properties for treating periodontal disease. This claim is substantiated through rigorous scientific inquiry, the details of which are published in two peer-reviewed articles. The first paper, titled **"Smart Dental Materials for Antimicrobial Applications**", [15] serves as a comprehensive review of existing dental materials, categorizing them based on their bioactive and bioresponsive properties. This work laid the foundation for the selection of key components for PiezoGEL, emphasizing its antibacterial efficacy. The second paper, **"A Novel Injectable Piezoelectric Hydrogel for Periodontal Disease Treatment",** [16] delves into the PiezoGEL's formulation and its potential for regenerative treatment in periodontal disease. This article demostrates how PiezoGEL can be easily injected into periodontal pockets and its potential in promoting osteogenic differentiation, thereby aiding in tissue regeneration.

Five primary objectives were identified to steer this research: 1) the selection of ideal PiezoGEL components through an extensive literature review; 2) comprehensive in vitro characterization of PiezoGEL's physicochemical properties; 3) evaluation of its antibacterial efficacy, specifically targeting P. gingivalis; 4) in vitro assessment of cell viability and osteogenic differentiation; and 5) in vivo evaluation focusing on periodontal pocket depth, bone volume, and inflammatory response in mice. These objectives coalesce to form a multi-pronged approach, aimed at delivering a bioactive, bioresponsive, and injectable hydrogel that holds the promise to revolutionize dental healthcare.

In summary, PiezoGEL represents a potential innovative, single-material therapy that addresses both the antibacterial and regenerative challenges in effectively managing PD.

RESEARCH QUESTION

Can be developed a single therapy for treating PD with a single material with antibacterial and regenerative properties?

In this research, such a material, known as PiezoGEL, has been introduced. PiezoGEL is a dual-function material designed to treat PD by both eliminating bacteria and promoting tissue regeneration. Its effectiveness is supported by our two peer-reviewed papers. The first paper reviews existing dental materials and forms the basis for PiezoGEL's antibacterial properties, while the second paper focuses on its regenerative and antibacterial capabilities. Therefore, PiezoGEL offers a promising single-material therapy for comprehensive treatment of PD.

OBJECTIVES

The aim of this work is to develop a piezoelectric hydrogel which could be used as a single therapy with antibacterial and regenerative properties for treating PD.

O.1 *To determine the components, proportions, and mixing procedure of the hydrogel*

The cornerstone of this research began with a thorough literature review that culminated in a peer-reviewed publication titled **"Smart Dental Materials for Antimicrobial Applications"** [15]**.** Through this extensive study, dental materials were stratified based on their 'smartness' levels, ranging from bioinert to autonomous. This review allow to identify Barium Titanate (BTO) and Gelatin Methacryloyl (GelMA) as the optimal components for PiezoGEL. These components were selected based on their bioactive and bioresponsive properties, and their proportions and mixing procedures are outlined in **Section 2.1** in our published paper, **"A Novel Injectable Piezoelectric Hydrogel for Periodontal Disease Treatment".** [16] Bioactive materials elicit particular biological reactions when they come into contact with tissues, cells or bodily fluids. These materials exhibit distinct characteristics, offering deliberate and controlled therapeutic benefits, including antibacterial properties, tissue regeneration support, and precise drug delivery, each with a unique mechanism of action. PiezoGEL is considered bioactive due to its composition and associated effects. To determine suitable concentrations for PiezoGEL, GelMA was subjected to testing at concentrations of 100 mg/mL and 200 mg/mL, while BTO was tested at concentrations of 3 mg/mL, 6 mg/mL, and 9 mg/mL. Notably, the concentration of BTO did not impact degradation rates. GelMA at 100 mg/mL exhibited faster degradation (80%) compared to that at 200 mg/mL after 14 days. Consequently, GelMA at 200 mg/mL was selected for its slower degradation, which is crucial for providing robust support to bone tissue. Furthermore, BTO at 9 mg/mL was chosen due to its ability to generate the highest voltage among the different concentrations. As a result, PiezoGEL, functioning as a bioactive biomaterial, can generate approximately 12 mV/cm2 upon contact with mechanical stimuli. This property contributes to its antibacterial effects against p. gingivalis and its capacity to expedite bone regeneration by stimulating the expression of osteogenic proteins..

O.2. *To characterize the hydrogel properties and its potential to use in periodontal pockets by an in vitro assay.*

This study includes an exhaustive in vitro analysis aimed at fully characterizing PiezoGEL, as described in **Section 2.** [16] This analysis focuses on crucial properties like surface pore size, surface roughness, rheology, injectability, and biodegradation, all of which are pertinent for its potential use in periodontal pockets. The comprehensive characterization results are discussed in **Section 4.1.** [16] The rheological evaluations of GelMA and PiezoGEL with added BTO revealed their suitability for PD treatment, exhibiting shear-thinning behavior for easy application into periodontal pockets. Both hydrogels provided essential mechanical support with a storage modulus of 10^4 Pa and the ability to absorb mechanical forces with a loss modulus of 10^3 Pa. PiezoGEL, slightly more injectable due to BTO, maintained clinical acceptability. Real-time photorheological tests confirmed appropriate curing times, and its capacity to generate ~10 mV/mm^2 under clinical stress conditions highlighted its bioactive potential. Both hydrogels displayed biodegradation profiles conducive to tissue regeneration for PD treatment, with no significant differences between them. Additionally, controlling porosity and surface roughness below ~0.2 μm had no notable impact on their properties, critical for influencing cell attachment and biofilm formation, further enhancing their potential for clinical use in addressing PD.

O.3. *To evaluate antibacterial effects of the hydrogel by an in vitro assay.*

To ensure that PiezoGEL is not just safe but also effective against bacterial pathogens commonly found in periodontal disease, we have developed a specialized in vitro assay methodology. This involves the use of crystal violet (CV) staining, MTT assays, and Colony Forming Unit (CFU) counts to evaluate its efficacy against *P. gingivalis*. Additionally, Subsection **2.3.3, "Molecular**

Biofilm−Biomaterial Evaluations" [16] delves into the RNA extraction process and gene expression assays conducted for a molecular-level evaluation. This multi-pronged approach ensures a comprehensive understanding of the antibacterial efficacy of PiezoGEL. The antibacterial evaluation results are elaborated in **Section 4.2**. [16] PiezoGEL demonstrated substantial reductions in pathogenic biofilm biomass (~41%), metabolic activity (~75%), and the number of viable bacteria (~2-3-log reduction) compared to hydrogels devoid of BTO fillers in an in vitro setting. Molecular analysis unveiled that these antibacterial effects were linked to reduced bacterial adhesion, as indicated by the downregulation of *porP* and *fimA* genes, and an increase in oxidative stress, as evidenced by the upregulation of the *oxyR* gene.

O.4. *To determine cell viability and the promotion of osteogenic differentiation by an in vitro assay.*

With an eye on regenerative capabilities, our methodology in **Section 2.4** [16] involves in vitro assays to assess cell viability and the promotion of osteogenic differentiation. Results related to bone-tissue regeneration are expounded upon in **Section 4.3** [16]. PiezoGEL boosts BMSC viability and osteogenic differentiation via RUNX2, COL1A1, and ALP upregulation. It excels in ECM mineralization. This is due to piezoelectric charges and BTO fillers. BTO generates charges, stimulating BMSCs for tissue regeneration. It pioneers periodontal bone regeneration using BMSCs' potential.

O.5. *To measure the periodontal pocket depths, bone volume and inflammatory response by an in vivo assay*

Finally, this study takes the evaluation a step further by including in vivo tests on mice to measure periodontal pocket depths, bone volume, and inflammatory response, as documented in **Section 2.5.** [16] The findings from these in vivo assays are presented in **Section 4.4.** [16] The in vivo model consistently validated the in vitro results tests, confirming the remarkable potential of PiezoGEL. Notably, it exhibited the most significant reduction in pocket depth compared to all other evaluated groups. Following treatment, the final pocket size for PiezoGEL was an impressive 0.2 mm, starting at 0.6 mm, a level comparable to healthy conditions. Moreover, there was a higher percentage change in the distance between the cementoenamel junction and alveolar bone crest (CEJ - ABC), indicating more substantial regeneration in the PiezoGEL group, with a remarkable 60% increase. Additionally, the bone volume showed a significant difference, with a 75% change compared to the diseased condition, a change significantly higher than in other groups, highlighting the profound regenerative potential of PiezoGEL.

Limitations

Some potential limitations of PiezoGEL include concerns about its residency duration if adhesion proves to be insufficient and the challenge of precisely controlling electrical charges for therapeutic purposes. From a biomaterials perspective, current surgical treatments for periodontal disease rely on methods such as bone grafts, guided tissue regeneration (GTR), and tissue engineering with controlled drug or growth factor delivery. These surgical approaches can be invasive, technically demanding, costly, and are typically recommended after non-surgical options have been exhausted.

Future Studies and Considerations

While the potential of PiezoGEL is indeed promising, further investigations should also consider the translation of this technology to higher animals, such as beagles or dogs, to bridge the gap between preclinical models and clinical use in humans. Evaluating its effectiveness and safety in larger, more complex organisms will provide valuable insights into its real-world applicability. Additionally, the development of PiezoGEL into an industrial product suitable for clinical use should be a priority. This entails rigorous testing, refinement, and regulatory approvals to ensure its reliability, safety, and efficacy for treating periodontal disease in humans. In summary, PiezoGEL represents an exciting advancement, and its journey from research to clinical application should involve comprehensive studies in higher animals and the development of a robust industrial product suitable for human use.

References

[1] C. Serrano, E. Suarez, Prevalence of Severe Periodontitis in a Colombian Adult Population., Journal of the International Academy of Periodontology. 21 (2019) 53–62.

[2] C. Serrano, E. Suarez, Prevalence of Severe Periodontitis in a Colombian Adult Population., J Int Acad Periodontol. 21 (2019) 53–62.

[3] P.I. Eke, G.O. Thornton-Evans, L. Wei, W.S. Borgnakke, B.A. Dye, R.J. Genco, Periodontitis in US adults: National health and nutrition examination survey 2009-2014., Journal of the American Dental Association. 149 (2018) 576–588. https://doi.org/10.1016/j.adaj.2018.04.023.

[4] S.S. Socransky, A.D. Haffajee, M.A. Cugini, C. Smith, R.L. Kent, Microbial complexes in subgingival plaque., J Clin Periodontol. 25 (1998) 134–144. https://doi.org/10.1111/j.1600-051X.1998.tb02419.x.

[5] C. Fi, W. Wo, Periodontal disease and systemic diseases: An overview on recent progresses., J Biol Regul Homeost Agents. 35 (2021) 1–9. https://europepmc.org/article/med/33463138 (accessed May 17, 2023).

[6] N.S. Soysa, H. Waidyarathne, M. Ranaweera, Alles, C.N.R. Alexander, Clinical efficacy of local application of sustained-release metronidazole in periodontal therapy., Dentistry Review. 1 (2021) 100006. https://doi.org/10.1016/j.dentre.2021.100006.

[7] S. Corbella, E. Calciolari, A. Alberti, N. Donos, L. Francetti, Systematic review and meta-analysis on the adjunctive use of host immune modulators in non-surgical periodontal treatment in healthy and systemically compromised patients., Sci Rep. 11 (2021) 1–18. https://doi.org/10.1038/s41598-021-91506-7.

[8] C.-M. Ardila, J.-A. Bedoya-García, D.-E. Arrubla-Escobar, Antibiotic resistance in periodontitis patients: A systematic scoping review of randomized clinical trials., Oral Dis. (2022). https://doi.org/10.1111/odi.14288.

[9] M.E. Davey, G.A. O'toole, Microbial biofilms: From ecology to molecular genetics., Microbiol Mol Biol Rev. 64 (2000) 847–867. https://doi.org/10.1128/MMBR.64.4.847-867.2000.

[10] G. Iviglia, S. Kargozar, F. Baino, Biomaterials, current strategies, and novel nano-technological approaches for periodontal regeneration., J Funct Biomater. 10 (2019) 1–36. https://doi.org/10.3390/jfb10010003.

[11] B. Hernández-Monjaraz, E. Santiago-Osorio, A. Monroy-García, E. Ledesma-Martínez, V.M. Mendoza-Núñez, Mesenchymal stem cells of dental origin for inducing tissue regeneration in periodontitis: A mini-review., Int J Mol Sci. 19 (2018). https://doi.org/10.3390/ijms19040944.

[12] L. Larsson, A.M. Decker, L. Nibali, S.P. Pilipchuk, T. Berglundh, W. V. Giannobile, Regenerative medicine for periodontal and peri-implant diseases., J Dent Res. 95 (2016) 255–266. https://doi.org/10.1177/0022034515618887.

[13] L.KN. Bonito AJ, Lux L, Impact of local adjuncts to scaling and root planing in periodontal disease therapy: A systematic review., J Periodontol. 76 (2005) 1227–1236. https://doi.org/10.1902/jop.2005.76.8.1227.

[14] L. Shue, Z. Yufeng, U. Mony, Biomaterials for periodontal regeneration: A review of ceramics and polymers, Biomatter. 2 (2012) 271–277. https://doi.org/10.4161/biom.22948.

[15] C. Montoya, L. Roldan, M. Yu, S. Valliani, C. Ta, M. Yang, S. Orrego, Smart dental materials for antimicrobial applications., Bioact Mater. 24 (2023) 1–19. https://doi.org/10.1016/j.bioactmat.2022.12.002.

[16] L. Roldan, C. Montoya, V. Solanki, K.Q. Cai, M. Yang, S. Correa, S. Orrego, A Novel Injectable Piezoelectric Hydrogel for Periodontal Disease Treatment, ACS Appl Mater Interfaces. (2023). https://doi.org/10.1021/ACSAMI.3C08336.