ISSN: 2633-4828 Vol. 5 No.4, December, 2023

International Journal of Applied Engineering & Technology

DEVELOPMENT OF NANOHYDROXYAPETITE AND CURCUMIN BASED INJECTABLE HYDROGEL FOR PERIODONTITIS TREATMENT.

Juvairiya Fathima I[1], Sangeetha S[2*] and [3]Dr. Lavanya Prathap

[1]Department of Anatomy, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077

[2]Assistant Professor and [3]Associate Professor, Department of Anatomy, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077

[1]152001083.sdc@saveetha.com and [2]sangeethas.sdc@saveetha.com

ABSTRACT

Introduction

Periodontitis is a chronic inflammatory disease that affects the supporting structures of the teeth, including the gums and bone.Nanohydroxyapetite is a biocompatible and osteoconductive material that closely resembles the mineral component of natural bone.Curcumin, on the other hand, is a natural polyphenol derived from turmeric with well-documented anti-inflammatory and antimicrobial propertiesHydrogels can provide a three-dimensional network that encapsulates bioactive compounds and allows for controlled release at the site of application. To develop a nanohydroxyapatite and curcumin-based injectable hydrogel for the treatment of periodontitis

Aim:

To develop a nanohydroxyapatite and curcumin-based injectable hydrogel for the treatment of periodontitis

Materials and Methods: *In this study, Simulated body fluid with ion concentrations similar to human blood plasma was used as the nano HAp growth medium. A SBF solution was prepared as previously reported by Leena et al,(2016)[1]. For the synthesis of nHAp at a reduced incubation time, a measured amount of $CaCl_2$ (8.7 M) and Na_2HPO_4 (3.5 M) (3.5 times higher than the reported amount) was added to 1000 ml of SBF in a stepwise manner. The significance of modifying the concentrations of the $CaCl_2$ and Na_2HPO_4 is to maintain the Ca/P ratio at 2.5 and other ionic concentrations (K^+, $Mg2^+$, HCO^{3-}, and $SO4^{2-}$) in SBF as similar to natural HBP thereby, avoiding the process of precipitation of higher resorbable phases of calcium phosphates (CaP).*

Discussion:

Nanohydroxyapetite particles were produced using biomimetic approach. Ca to P ratio 1.67 was confirmed through EDAX spectra. Curcumin and nano HAP loaded injectable hydrogel was formulated and morphology was confirmed by SEM imaging. Uniform distribution of nanoparticles in hydrogel was confirmed. The presence of nHAP and curcumin in the injectable gel was analysed through IR spectra. Mechanical properties was analysed through UTM which explains that after loading of nanoparticle and curcumin the compression strength is reduced.

Conclusion:

The development of a nanohydroxyapatite and curcumin-based injectable hydrogel holds significant promise for the treatment of periodontitis. This innovative approach combines the regenerative properties of nanohydroxyapatite with the anti-inflammatory and antimicrobial effects of curcumin to address the complex nature of periodontal diseases

INTRODUCTION

Periodontitis is characterized by various clinical indicators such as clinical attachment loss (CAL), alveolar bone loss (BL), the formation of periodontal pockets, and inflammation of the gingiva. Additionally, other symptoms may include changes in the size or recession of the gingiva, bleeding of the gingiva under pressure, and heightened tooth mobility, drifting, or exfoliation. Generally, periodontitis presents as a chronic inflammatory condition, which may progress steadily or in intermittent bursts of activity, with some exceptions (1)

They are often produced using physical and chemical methods (2)

Periodontitis is the sixth most prevalent disease, and almost 3.5 billion people are affected globally by dental caries and periodontal diseases (3)

Nanotechnology is one of the most promising technologies which opens a large scope of novel applications in the area of sciences (4)

The primary objective of tissue engineering is to create functional constructs that can repair, maintain, or enhance damaged tissues or organs. Hydrogels made from natural polymers have shown significant promise as artificial scaffolds for tissue repair because they can mimic the properties of the extracellular matrices found in our bodies. Therefore, the goal of this research was to develop nanocomposite hydrogels using chitosan/oxidized-modified quince seed gum/curcumin-loaded within halloysite nanotubes (CS/OX-QSG/CUR-HNTs) for potential applications in tissue engineering.

The synthesized hydrogels underwent various analyses, including assessments of their thermal stability, degradation characteristics, swelling capacity, gelling time, and mechanical properties. The findings indicated that as the content of OX-QSG increased, the thermal stability, swelling capacity, and degradation rate of the hydrogels improved (5)

Injectable hydrogels have garnered increased attention in the field of hard tissue engineering, especially for addressing defects with irregular shapes. Consequently, researchers conducted a study to assess the biocompatibility and immune response of injectable PCL-PEG-PCL-Col/nHA hydrogels in a mouse model.

Histological examination, using H&E staining, was employed to evaluate tissue samples. Immune cell activation was assessed by utilizing antibodies targeting CD68, CD4, and CD8 markers. Gene expression analysis included measuring the levels of CCL-2, BCL-2, IL-10, and CD31 genes. Additionally, serum levels of ALT, ALP, AST, and Urea were measured.

The results of the chemical analysis confirmed the successful integration of collagen and Nano-hydroxyapatite into the PCL-PEG-PCL hydrogels. Histological examination indicated a slower biodegradation rate following the addition of collagen and Nano-hydroxyapatite. Importantly, no significant pro-inflammatory response was observed at the injection site (6)

Injectable hydrogels have become increasingly popular due to their ability to provide controlled release, precise targeting of therapeutic agents, and improved mechanical properties. They hold great promise in a wide range of medical applications, including cardiac regeneration, the treatment of joint diseases, postoperative pain management, and the management of ocular disorders. Hydrogels that incorporate nano-hydroxyapatite are particularly promising for bone regeneration, offering potential solutions for challenges such as bone defects, osteoporosis, and regeneration in tumor-associated conditions.

In the fields of wound management and cancer therapy, these hydrogels enable controlled drug release, expedited wound healing, and the targeted delivery of medications. Injectable hydrogels are also finding applications in addressing conditions like ischemic brain injury, tissue regeneration, cardiovascular diseases, and personalized cancer immunotherapy (7)

In recent years, the application of polysaccharide hydrogel in the field of bone regeneration has shown promising potential. Nano Scaffolding polysaccharide, utilized in the medical process for bone and tissue regeneration, offers the ability to regenerate organs and limbs. These nanoscaffolds are three-dimensional structures composed of minute polysaccharide hydrogel fibers scaled down to the nanometer range (10−9 m). Leveraging their unique chemical and physical properties, such as magnetic and electrical attributes, nanostructured biomaterials have exhibited superior performance compared to their bulk counterparts in enhancing bone regeneration. Additionally, nanostructured biomaterials can be tailored to mirror the composition and nanoarchitecture of bones and emulate crucial features of their biochemical environment at the nanoscale. These attributes facilitate enhanced interaction between the host immune system and progenitor cells at the nanometer scale, resulting in improved outcomes (8)

These hydrogels engage in dynamic interactions with their immediate environment, exhibiting smart and controllable responses to external stimuli. Consequently, hydrogels are attractive options for numerous biomedical applications, including tissue engineering, drug delivery, gene delivery, biosensors, wound healing, mucoadhesives, and bioadhesives, among others (9)

Presently, the primary clinical interventions for addressing bone defects involve autologous and allogeneic bone grafts . However, these conventional treatments are associated with limitations such as immune rejection, foreign body responses, and additional injuries to patients . A variety of materials have been employed in the domain of bone repair, encompassing polymers, bioceramics, hydrogels, metals, and alloys . Notably, the use of metal scaffolds can elicit inflammatory and allergic reactions . Furthermore, bioceramic scaffolds, while non-degradable, are processed under harsh conditions, restricting their applicability in bone tissue engineering and drug delivery(10)

Exploring the microbial composition of peri-implant biofilm holds the potential to unveil targeted treatment approaches, thereby enhancing the effectiveness of peri-implantitis management(11)

Aim of this study is to develop an injectable hydrogel incorporated with curcumin and nano hydroxyapatite for periodontitis treatment. The objective of incorporating curcumin and nanohydroxyapatite into injectable hydrogel are to enhance periodontal tissue regeneration and improve periodontitis treatment outcomes.

MATERIALS AND METHODS:

Preparation of bone Like Hydroxyapatite Nanoparticle (Nhap)

SYNTHESIS OF NANO HYDROXYAPATITE:

In this study, Simulated body fluid with ion concentrations similar to human blood plasma was used as the nano HAp growth medium. A SBF solution was prepared as previously reported by Leena et al,(2016)[1]. For the synthesis of nHAp at a reduced incubation time, a measured amount of $CaCl_2$ (8.7 M) and Na_2HPO_4 (3.5 M) (3.5 times higher than the reported amount) was added to 1000 ml of SBF in a stepwise manner. The significance of modifying the concentrations of the $CaCl_2$ and Na_2HPO_4 is to maintain the Ca/P ratio at 2.5 and other ionic concentrations (K^+, $Mg2^+$, HCO^{3-}, and $SO4^{2-}$) in SBF as similar to natural HBP thereby, avoiding the process of precipitation of higher resorbable phases of calcium phosphates (CaP).

First, 0.4935 g of Na_2HPO_4 (3.5 X 0.141 g) was added to 980 ml of SBF and then 0.9695 g (3.5 X 0.277) of $CaCl_2$ was added to the remaining 20 ml of SBF, separately. Complete mixing for these reagents into the SBF resulted in modifying the ionic concentration of the SBF. Followed by the complete mixing of these reagents in SBF solutions separately, 20 ml of a $CaCl_2$ solution dissolved in SBF was added dropwise at the rate of 0.5 ml/min to 980 ml of SBF containing Na_2HPO_4 under continuous stirring, making the final volume 1000 ml. Addition of these reagents resulted in a pH value decrease to 7.25.

The precipitates after 12 hr incubation time were filtered and washed six times with ultrapure water, followed by drying at 80^{o}C for 24 h. The dried samples were then calcinated at 900^{o}C (as nHAp is stable up to 900^{o}C and undergoes decomposition beyond this temperature) for 2 h in a muffle furnace to study the thermal stability and phase changes in the prepared samples. The final product was crushed using a mortar and pestle to obtain nHAp powder.

Preparation of Curcumin and Nano Hap Injectable Hydrogel

Gelatin solution 10% w/v is prepared in distilled water. Prepared nano HAp was added in to gelatin solution at 10 w/w% and curcumin 5% w/v was added and stirred for 2 hr. Crosslinking agent glutaraldehyde was added at various percentages ranging from 0.5,1,1.5, and 2 % v/v.

Characterization

The developed nanoparticles loaded gel was freezed at -80°C for 12 hr and freeze dried and then was characterized using SEM and XRD.

Morphology and EDAX spectra of nanoparticles and hydrogel was taken using JEOL JSM IT 800 after 30s Platinum coating.

Ftir

FTIR using Bruker Alpha II model – from 500 to 400 cm-1 at 4 cm-1 resolution averaging 32 scans.

Compression Test

Mechanical analysis was completed using a Universal testing machine (Electropuls E3000, Instron) in compression mode using 40 mm sandwich fixtures. Hydrogel samples in disk shape at specific height (8 mm Height X 8 mm Dia) were sliced and analyzed n=2. The hydrogels were loaded into the instrument, ramped to 37°C and held isothermally for 5 minutes. A preload force of 0.01 N was applied, followed by a force ramped up to 3 N at a uniform stress rate of 0.5 N per minute. The compressive modulus was determined from the slope of the initial 20% linear elastic region of the obtained stress–strain curve.

RESULTS

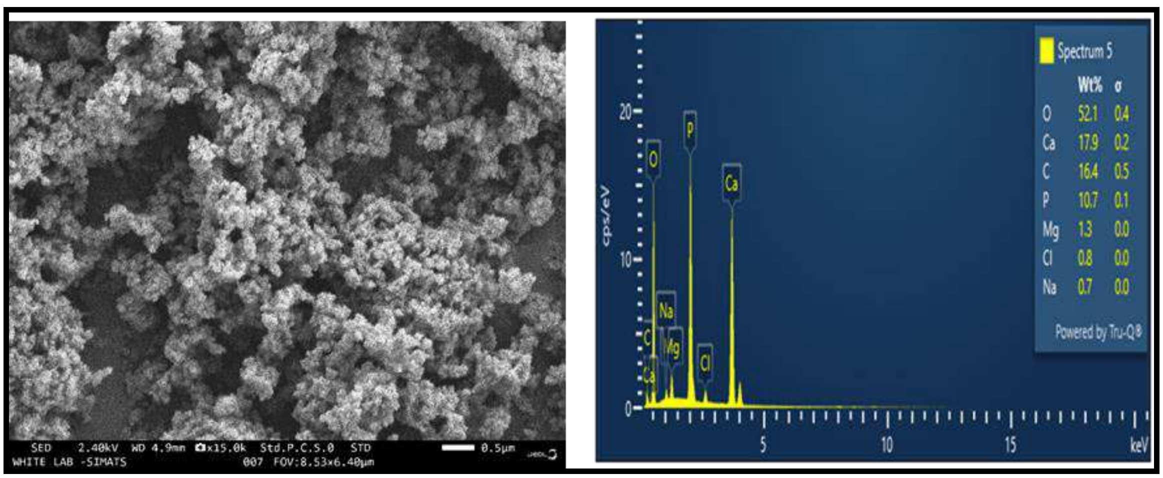

Figure 1: SEM image and EDAX of nanohydroxyapatite

The results of this study revealed several important findings. Firstly, the synthesis of nano-hydroxyapatite (nHAp) using simulated body fluid (SBF) as a growth medium resulted in the successful production of stable nHAp nanoparticles. The modification of CaCl2 and Na2HPO4 concentrations in SBF helped maintain the desired Ca/P ratio, ensuring the formation of nHAp with properties closely resembling natural hydroxyapatite. Characterization using SEM, XRD, and FTIR confirmed the morphology and composition of the synthesized nHAp.

Figure 2: Curcumin and nHAP loaded gel

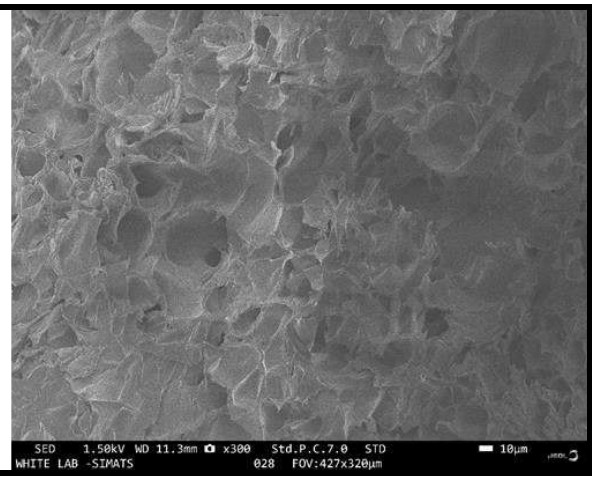

Figure 3: SEM image of Curcumin and nHAP loaded gel

The preparation of the injectable hydrogel incorporated with nHAp and curcumin showed promising results. The addition of nHAp to the gelatin solution enhanced the mechanical properties of the hydrogel, making it suitable for periodontal tissue regeneration applications. The inclusion of curcumin, a known therapeutic agent, further adds potential benefits for periodontitis treatment.

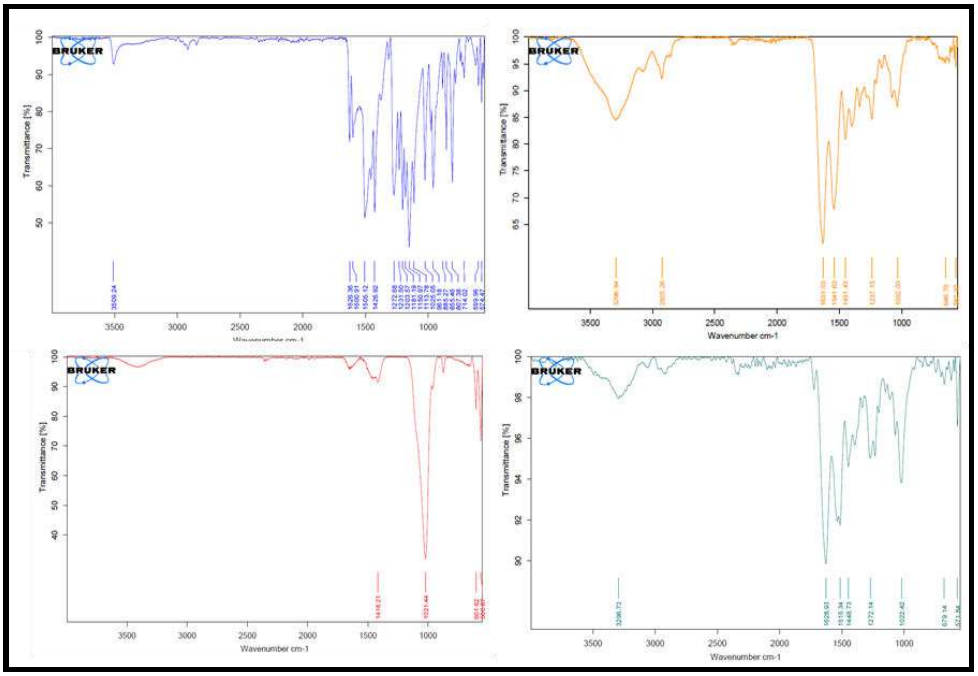

Figure 4: Curcumin (Top Left), Gelatin hydrogel (Top Right), nHAP(Bottom Left), Hydrogel +nHAP+Curcmin (Bottom Right)

Mechanical analysis of the hydrogel demonstrated its suitability for the intended application, with a compressive modulus within an acceptable range. This suggests that the hydrogel can provide the necessary support and stability for periodontal tissue regeneration.

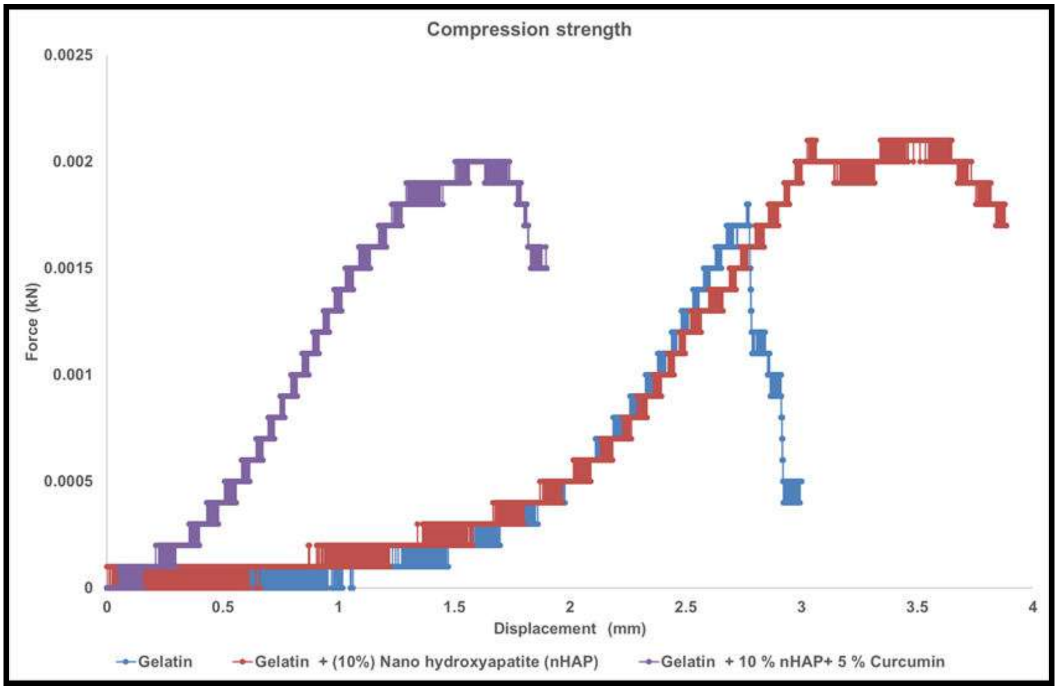

Figure 5:

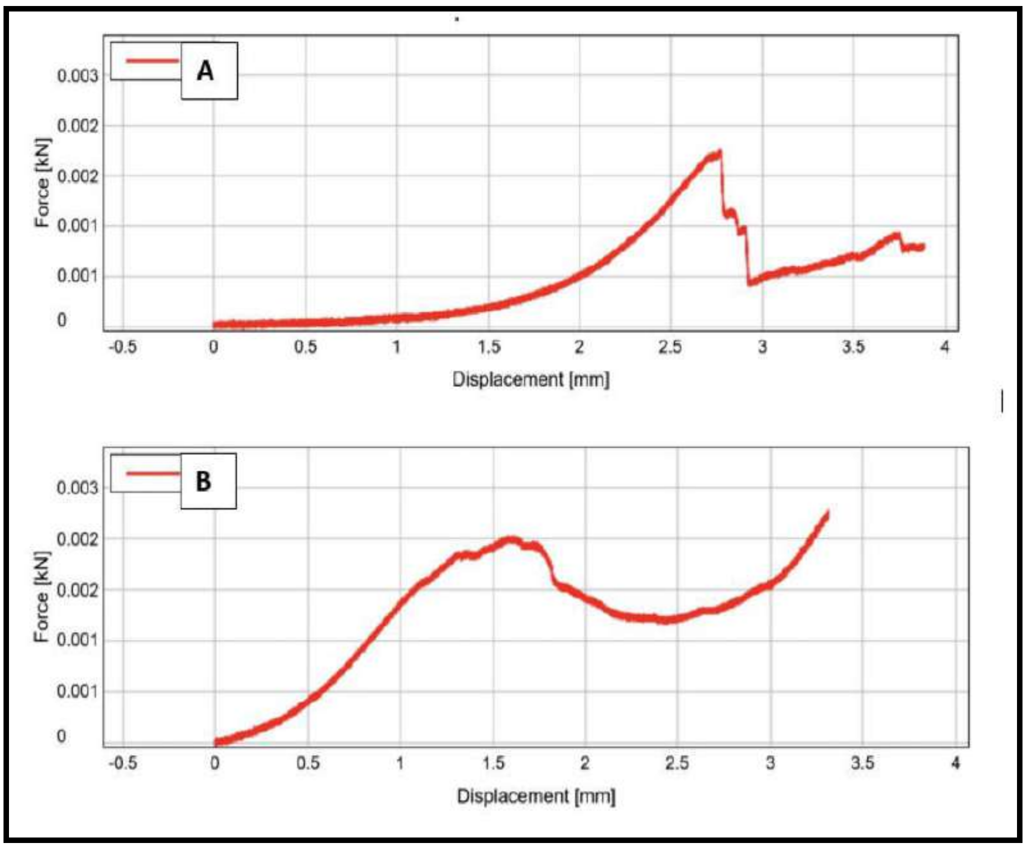

Figure 6:

DISCUSSION

This study outlines an efficient and economical method for producing biomimetic nano-hydroxyapatite (nHAp) powder within a shorter processing timeframe compared to conventional methods. The research includes an assessment of phase purity and crystallographic characteristics for nHAp samples subjected to 80°C drying and subsequent calcination at 900°C, conducted at different incubation time intervals.

An injectable hydrogel containing a substantial amount of polysaccharide and hydrogel/hydroxyapatite (GH) was synthesized based on a previously documented procedure. The hydrogel's injectability was assessed using a 26-gauge needle . Remarkably, the hydrogel not only passed through the needle successfully but also rapidly adhered at room temperature in less than 20 seconds. This feature prevented any additional harm to the wound that might occur with the use of conventional bone graft materials . Additionally, the hydrogel displayed self-healing properties when subjected to physical defects , with the damaged pores being repaired within a span of 3 hours .

SEM image indicates formation of nano size spherical particles. EDAX spectra confirms composition of elements present in the prepared nanoparticles. EDAX spectra gives Ca 17.8 wt% and P 10.7 wt% which gives Ca/P ratio of 1.66. This Ca/P ratio of 1.66 is very similar to composition of HAp in the bones of the human body. Thus SEM and EDAX confirms formation of nano HAp and CA/P ratio similar to the human body. Curcumin and nano HAP at 2% crosslinking formed an injectable gel which became very stable after reaching body temperature.

This concentration is taken for further characterization. Morphology of nanoparticle incorporated hydrogel at 2% cross linking is shown .

Presence of nanoparticles in hydrogel was confirmed from SEM image. The compression modulus of injectable gel in control and after nanoparticles loaded is shownIt was evident that with nanoparticle loading gel strength increased. This could serve as stronger material for bone regeneration in periodontitis treatment.

Bone grafts have long been employed to facilitate bone regeneration, and their effectiveness hinges on their bioactivity and capacity to stimulate osteogenic processes. Hydrogels used in bone tissue engineering represent a promising solution for addressing bone defects. They serve as a suitable platform for integrating extracellular matrix (ECM), stem cells, and osteoinductive components, thereby efficiently promoting bone healing. Additionally, hydrogels can be further customized to emulate bone properties and can be administered into bone defect areas through minimally invasive procedures.

In this study, a biomimetic bone tissue engineering material was developed using a readily accessible approach. This involved introducing nano hydroxyapatite (HAP) and silk fibroin nanoparticles (SN) into a hydrogel composed of Gelatin Methacryloyl (GelMA) encapsulating mesenchymal stem cells (MSCs). The resulting injectable GelMA-HAP-SN hydrogel demonstrated impressive capabilities for bone regeneration, both in vivo and in vitro

LIMITATIONS

It is important to acknowledge the limitations of this study. Firstly, the in vitro nature of the experiments means that the effectiveness of the developed hydrogel for periodontitis treatment has not been tested in an in vivo model. Additionally, the study focused on the mechanical and structural properties of the hydrogel, and further research is needed to assess its biological properties and its actual effectiveness in promoting periodontal tissue regeneration in living organisms.

FUTURE SCOPE

Future research in this field could involve in vivo studies to evaluate the therapeutic efficacy of the developed hydrogel in animal models or clinical trials in humans. Moreover, additional investigations into the release of curcumin from the hydrogel and its antibacterial and anti-inflammatory effects on periodontal tissues could provide valuable insights.

CONCLUSION

In conclusion, this study successfully developed an injectable hydrogel incorporated with curcumin and nano-hydroxyapatite, showing potential for periodontitis treatment. The synthesis of stable nHAp nanoparticles and the characterization of the hydrogel's mechanical properties are promising steps towards its application in periodontal tissue regeneration. However, further research and testing are required to validate its effectiveness in vivo.

ACKNOWLEDGEMENT

We sincerely thank Saveetha Dental College,Saveetha Institute of Medical and Technical Sciences(SIMATS),India for providing necessary facilities for carrying out the present work.

Conflict of Interest:

The authors declare no conflict of Interest

Author contribution

Juvairiya Fathima .A:Literature data,data collection,manuscript writing.

Mrs.S.Sangeetha:Study design,data verification,manuscript correcting.

REFERENCES:

1. Baranov N, Popa M, Atanase LI, Ichim DL. Polysaccharide-Based Drug Delivery Systems for the Treatment of Periodontitis. Molecules. 2021 May 6;26(9):2735.
2. Nasim I, Rajesh Kumar S, Vishnupriya V, Jabin Z. Cytotoxicity and anti-microbial analysis of silver and graphene oxide bio nanoparticles. Bioinformation. 2020 Nov 30;16(11):831–6.
3. Ezhilarasan D, Varghese SS. Porphyromonas gingivalis and dental stem cells crosstalk amplify inflammation and bone loss in the periodontitis niche. J Cell Physiol. 2022 Oct 1;237(10):3768–77.
4. View of A Review on plant mediated selenium nanoparticles and its applications [Internet]. [cited 2024 Jan 7]. Available from: http://dx.doi.org/10.47750/jptcp.2022.870
5. Injectable chitosan-quince seed gum hydrogels encapsulated with curcumin loaded-halloysite nanotubes designed for tissue engineering application. Int J Biol Macromol. 2021 Apr 30;177:485–94.
6. Hassanzadeh A, Ashrafihelan J, Salehi R, Rahbarghazi R, Firouzamandi M, Ahmadi M, et al. Development and biocompatibility of the injectable collagen/nano-hydroxyapatite scaffolds as in situ forming hydrogel for the hard tissue engineering application. Artif Cells Nanomed Biotechnol [Internet]. 2021 Jan 1 [cited 2023 Oct 6]; Available from: https://www.tandfonline.com/doi/abs/10.1080/21691401.2021.1877153
7. Omidian H, Chowdhury SD. Advancements and Applications of Injectable Hydrogel Composites in Biomedical Research and Therapy. Gels. 2023 Jun 30;9(7):533.
8. Injectable hydrogel-loaded nano-hydroxyapatite that improves bone regeneration and alveolar ridge promotion. Materials Science and Engineering: C. 2020 Nov 1;116:111158.
9. Accelerated synthesis of biomimetic nano hydroxyapatite using simulated body fluid. Mater Chem Phys. 2016 Sep 1;180:166–72.
10. A self-healing, magnetic and injectable biopolymer hydrogel generated by dual cross-linking for drug delivery and bone repair. Acta Biomater. 2022 Nov 1;153:159–77.
11. Rajasekar A, Varghese SS. Microbiological Profile in Periodontitis and Peri-Implantitis: A Systematic Review. J Long Term Eff Med Implants. 2022;32(4):83–94.