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DEVELOPMENT OF INJECTABLE HYDROGEL INCORPORATED WITH BONE LIKE B-TCP FOR PERIODONTITIS TREATMENT

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Abstract

Periodontitis, a common inflammatory disease affecting the supporting structures of the teeth, requires effective treatment strategies to promote tissue regeneration and prevent further deterioration. In this study, an injectable hydrogel incorporated with nano hydroxyapatite (nHAp) was developed for the treatment of periodontitis. The hydrogel was prepared using gelatin solution, and varying concentrations of glutaraldehyde were utilized as a crosslinking agent to optimize gel formation. The nHAp was synthesized using simulated body fluid as the growth medium to mimic human blood plasma ion concentrations. The resulting nanoparticles loaded gel was freeze-dried and characterized using scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDAX), and X-ray diffraction (XRD). SEM analysis revealed the formation of nano-sized spherical particles, while EDAX spectra indicated a Ca/P ratio of 1.66, closely resembling the composition of hydroxyapatite in human bone. Crosslinking with glutaraldehyde demonstrated enhanced gel stability, particularly at 2% crosslinking concentration and body temperature. XRD peaks confirmed the successful incorporation of nHAp into the hydrogel matrix. Compression tests showed increased gel strength with nanoparticle loading. Overall, the developed injectable hydrogel incorporated with nanohydroxyapatite shows promise as a potential treatment for periodontitis, offering improved tissue regeneration and stability. This research contributes to the development of advanced biomaterials for periodontal therapy, with potential applications in clinical practice.

Keywords: Periodontitis, Injectable Hydrogel, Nano Hydroxyapatite, Biomaterials, Tissue Regeneration.

INTRODUCTION

Microbes have the ability to colonize human oral surfaces and cause various diseases as easily as possible(Mosaddad et al., 2019; Varshan & Prathap, 2022). One such disease caused due to accumulation of oral diseases is Periodontitis which is a chronic inflammatory disease affecting tissues surrounding teeth causing bone loss(Prathap & Lakshmanan, 2022; Ray, 2023). Half of the adult population worldwide have at least one tooth with periodontitis. It was found that 49.5% of Americans had periodontitis, and 9.1% had severe periodontitis. This oral disease deals with loss of bone. Hence the requirement to fill to the bone cavity with the better biocompatible material has increased(Kumaresan et al., 2022; Yamada & Egusa, 2018). The HA with the chemical formula of Ca10(OH) 2(PO4) 6 is very identical to the inorganic portion of bone and also have high stability and minimal solubility. n-HAp composite scaffolds that are highly biocompatible have a great potential role in bone regeneration(Bhat, Uthappa, Altalhi, Jung, & Kurkuri, 2021; Varshan & Prathap, 2022). Loading bioactive factors and drugs onto n-HAp composites has emerged as a promising strategy for bone defect repair in bone tissue engineering through osteoconduction or by acting as a scaffold for filling of defects and also as a good alternative to the use of auto- and allografts to guide and support tissue regeneration in critically-sized bone defects.

Nanohydroxyapatite can be delivered in the injectable form by incorporating with hydrogel(Pan et al., 2020; Prathap & Jayaraman, 2022). Hydrogels are promising for a variety of medical applications due to their high-water content and mechanical similarity to natural tissues(Means & Grunlan, 2019; Yuvaraj, Sangeetha, & Kavitha, 2020). When made injectable, hydrogels can reduce the invasiveness of application, which in turn reduces surgical and recovery costs. Injectable hydrogel systems can offset difficulties with conventional hydrogel-based drug delivery systems and increase the ease of drug delivery(Mathew, Uthaman, Cho, Cho, & Park, 2018). It has demonstrated a great potential as three-dimensional cell culture scaffolds in cartilage and bone tissue engineering, owing to their high-water content, similarity to the natural extracellular matrix (ECM), porous framework for cell transplantation and proliferation, minimal invasive properties, and ability to match irregular defects(USHANTHIKA & MOHANRAJ, 2020). Hydroxyapatite incorporated with hydrogel in the injectable form has the potential to both maintain dimensional alveolar ridge, as well as to promote soft tissue healing. The incorporation of mineralized collagen fibers within the hydrogel further increased the mechanical properties and osteoconductive of the hydrogels. Similarly, gelatin gum-based hydrogel with superior osteogenesis holds promise for treating infectious bone defects caused by refractory periradicular periodontitis(Prathap & Lakshmanan, 2022; Xu et al., 2020).

Further, in the study conducted by Piyachat Chuysinuan, the potential use of injectable HA fibroin-alginate hydrogel as dental scaffolding material was shown.

Thus, the aim of this study is to develop an injectable hydrogel incorporated with bone like beta -type carbonated nano hydroxyapatite for periodontitis treatment. The objective of incorporating nano hydroxyapatite into injectable hydrogel is to enhance periodontal tissue regeneration and improve periodontitis treatment outcomes.

MATERIALS AND METHODS

Preparation of Bone like Hydroxyapatite Nanoparticle (nHAp)

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).[1,16] 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 step-wise 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 into 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 900ºC (as nHAp is stable up to 900ºC 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 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 stirred for 2 hr. Crosslinking agent glutaraldehyde was added at various percentage ranging from 0.5,1,1.5, and 2 % v/v.

Characterization

The developed nanoparticles loaded gel was freezed at -80ºC 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.

XRD

Phase purity and crystallographic studies of the nHAp powders were carried out using a powder X-ray diffraction (XRD) (D8 Advance Powder XRD, Bruker) in continuous scan mode at a speed of 10º to 90º 2 Theta at scanning rate of 0.04 2-theta step size with 1 sec per step. A Cu-Ka tube operated at 40 KV and 30 mA was used for the generation of X-rays of wavelength 1.5406 Å in a Guiner geometry.

Compression Test

Mechanical analysis was completed using a Universal testing machine (Electroplus 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 37ºC 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

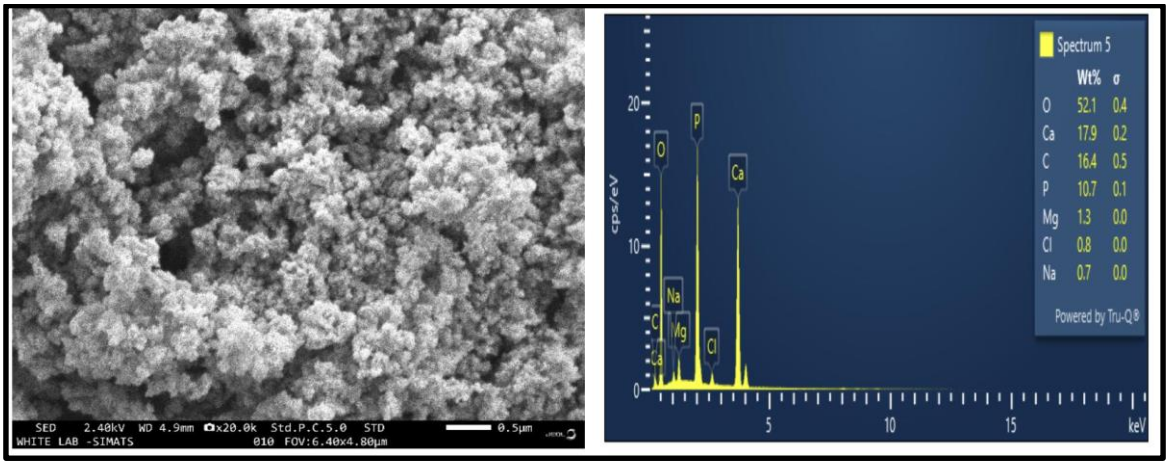

Figure 1: SEM Image and EDAX of Nano HAp Particles

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 bone of human body. Thus SEM and EDAX confirms formation of nano HAp and CA/P ratio similar to the human body.

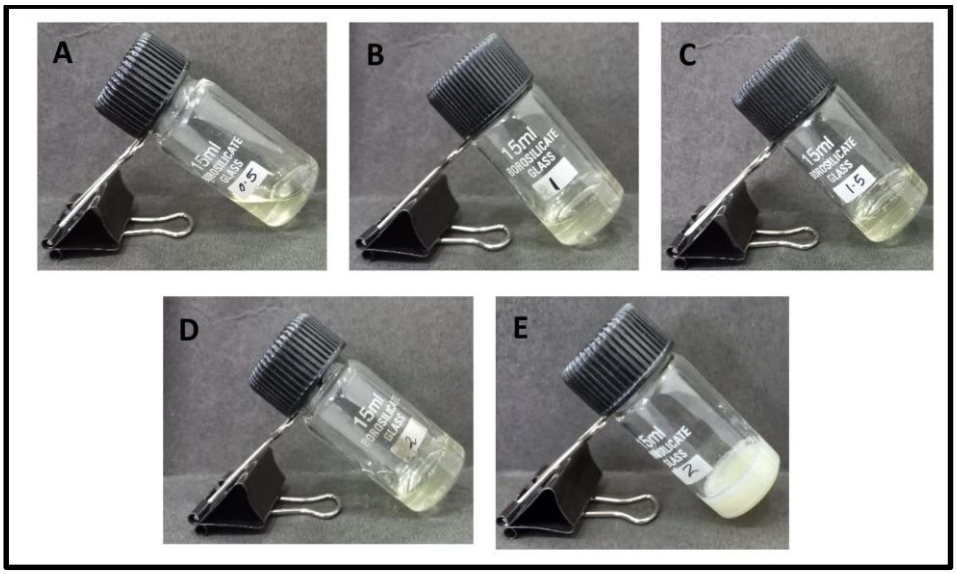

Figure 2: Optimization of Gelation with different % of Cross Linker A) 0.5 %; B) 1%: C) 1.5%; D) 2 % and E) 2% Gel Loaded with 10 % w/v of Nano HAp Particles

Formation of injectable gel with different percentage of cross linker is shown in Fig. 2. At 0.5% it was not forming stable gel. With increase in concentration strong gel formation was observed. at 2% crosslinking it formed injectable gel which became very stable after reaching body temperature. This concentration for taken for further characterization.

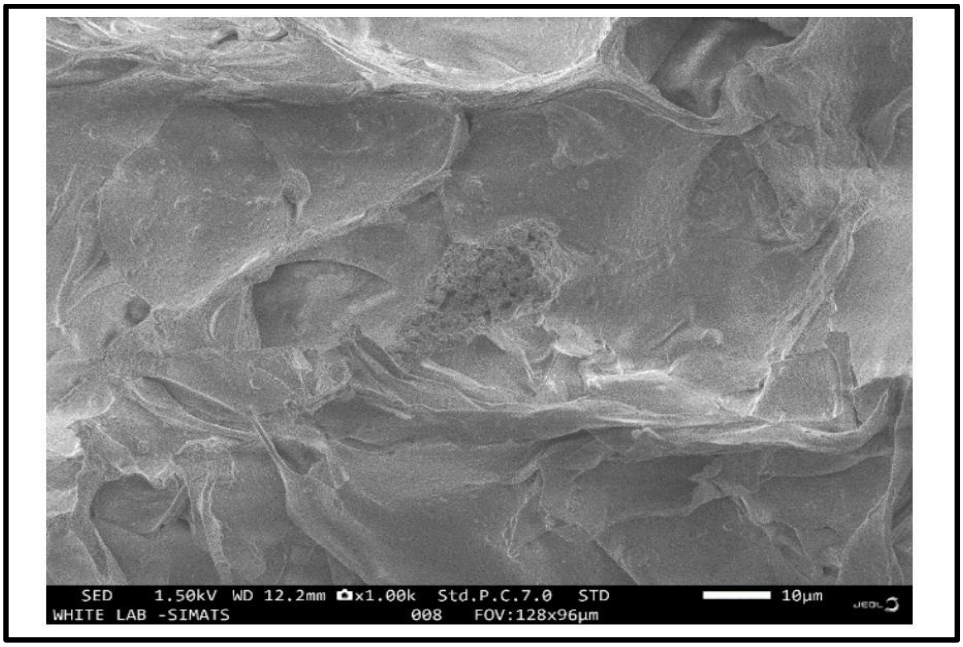

Figure 3: SEM Image of Nanoparticle Incorporated Injectable Hydrogel

Morphology of nanoparticle incorporated hydrogel at 2% cross linking is shown in Fig 3. Presence of nanoparticles in hydrogel was confirmed from SEM image.

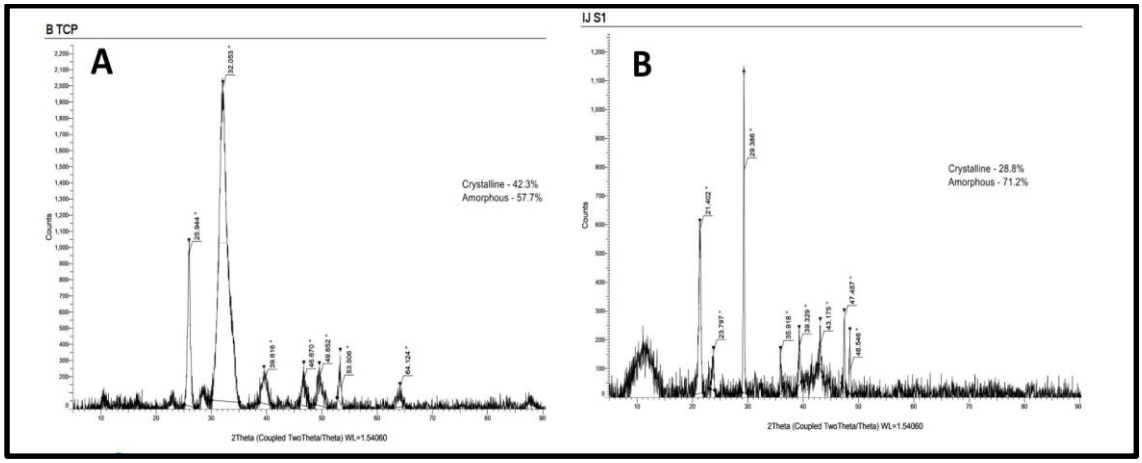

Figure 4: XRD of A) HAp Nanoparticles and B) Hydrogel With Nanoparticles

XRD pattern of nano HAP and hydrogel with nanoparticles is given in Fig 4. Peaks at 25, 32,39, 46,49,53 two Theta values confirm the formation of HAp similar to bone structure in human. The similar pattern in hydrogel confirms the loading of nano HAp in injectable hydrogel formed.

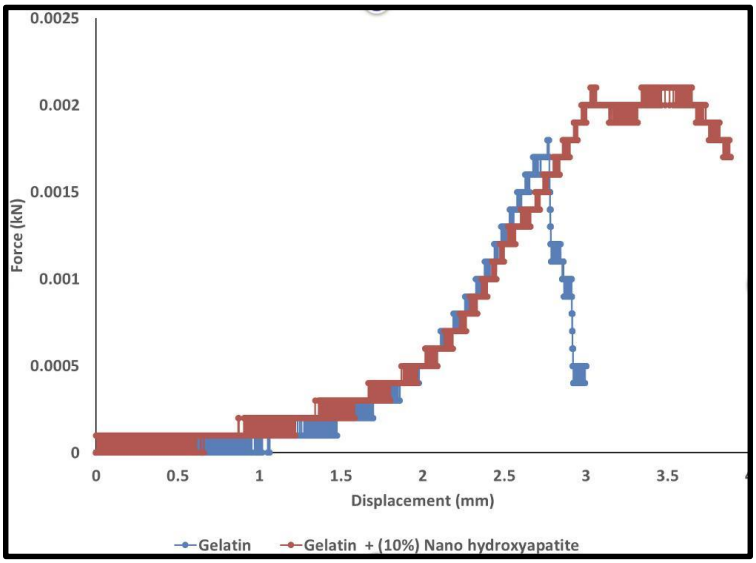

Figure 5: Compression Strength using UTM of Nanoparticles Loaded Injectable Gel at 2 % Cross Linking

The compression modulus of injectable gel in control and after nanoparticles loaded is shown in Fig 5. It was evident that with nanoparticle loading gel strength increased. This could serve as stronger material for bone regeneration in periodontitis treatment

DISCUSSION

The development of injectable hydrogel formulations incorporated with nano hydroxyapatite (nHAp) presents a promising avenue for the treatment of periodontitis and other bone-related disorders(Geng et al., 2023). The comprehensive review of existing research studies highlights the efficiency and potential of such formulations in

promoting tissue regeneration and addressing various challenges associated with bone defects and infectious conditions.

The results obtained from various research studies underscore the significant impact of injectable hydrogel formulations containing nHAp on bone regeneration and tissue healing(Fendi, Abdullah, Suryani, Usman, & Tahir, 2024). For instance, studies involving rat models demonstrate enhanced bone formation and soft tissue healing following the injection of hydrogel-nHAp composites into tooth extraction sites. These findings suggest that such formulations have the capacity to promote both dimensional stability of the alveolar ridge and accelerated soft tissue healing, crucial aspects in periodontal therapy. Moreover, the development of bilayered hydrogel composites combining naturally derived and synthetic polymers with nHAp further reinforces the efficacy of injectable hydrogel systems in promoting tissue regeneration. These composites facilitate the regeneration of osteochondral tissue in rabbit knee defects, indicating their potential for clinical applications in orthopedic and periodontal surgeries.

Additionally, the antibacterial properties of injectable hydrogel formulations, as demonstrated by GG-based hydrogels, hold promise for treating infectious bone defects associated with refractory periradicular periodontitis. This highlights the multifaceted benefits of incorporating antibacterial agents into hydrogel-nHAp systems, addressing both regenerative and infectious aspects of periodontal diseases. Furthermore, the ability of hydrogels enriched with nHAp to promote osteogenic differentiation of mesenchymal stem cells underscores their potential for bone regeneration applications.

This capability, coupled with the injectable nature of these formulations, offers a versatile and minimally invasive approach for addressing various bone-related disorders, including osteoporosis and tumor-associated bone regeneration. Overall, the discussion emphasizes the significant therapeutic potential of injectable hydrogel formulations incorporated with nHAp in the field of periodontal therapy and bone tissue engineering(Alkhursani et al., 2022). Further research and clinical trials are warranted to validate the efficacy, safety, and clinical applicability of these formulations, paving the way for their translation into clinical practice and improving outcomes for patients suffering from periodontitis and other bone-related conditions.

CONCLUSION

In conclusion, the development of an injectable hydrogel incorporated with nanohydroxyapatite (nHAp) presents a promising solution for treating periodontitis. This approach harnesses the regenerative properties of nHAp within a hydrogel matrix, offering enhanced mechanical support and a biomimetic environment conducive to tissue regeneration.

The injectable nature of the composite facilitates minimally invasive delivery and targeted treatment, with potential for sustained therapeutic release. While promising, further research is needed to optimize the formulation, ensure safety, and evaluate long-term efficacy. Nonetheless, this innovative approach represents a significant advancement in periodontal therapy, with potential to improve treatment outcomes and address the challenges associated with periodontitis. Future studies will be crucial for translating these findings into clinical practice and improving patient care.

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