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DEVELOPMENT OF NANO HYDROXYAPATITE (nHap) AND CLOVE ESSENTIAL OIL INCORPORATED HYDROGEL FOR PERIODONTAL TREATMENT

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Abstract

This study introduces a novel approach for periodontal treatment by incorporating nano hydroxyapatite (nHap) with clove essential oil within a hydrogel matrix. nHap powder was synthesized using a biomimetic technique in simulated body fluid buffer, followed by drying and grinding. A hydrogel was formulated by dissolving 10% w/v gelatin in distilled water, with the addition of clove essential oil. The nHap powder was integrated into the hydrogel and cross-linked using various concentrations of glutaraldehyde. Morphological analysis via scanning electron microscopy (SEM) revealed spherical hydroxyapatite crystals, akin to bone tissue. Elemental composition analysis through energy-dispersive X-ray spectroscopy (EDAX) and X-ray diffraction (XRD) confirmed a composition resembling bone tissue. Compression strength, assessed using a universal testing machine (UTM), demonstrated stability and potential for bone regrowth. This innovative material, combining nHap with clove essential oil in a hydrogel matrix, exhibits promising properties for bone growth and regeneration. These findings underscore its potential as a therapeutic avenue in periodontal treatment, offering new perspectives in oral healthcare.

Keywords: Nano Hydroxyapatite, Clove Essential Oil, Hydrogel Matrix, Periodontal Treatment, Bone Regeneration, Biomimetic Synthesis.

INTRODUCTION

Periodontitis is a severe form of gum disease that affects the supporting structures of the teeth(Könönen, Gursoy, & Gursoy, 2019; Varshan & Prathap, 2022). It is characterized by inflammation and infection of the gums, which can lead to the destruction of the tissues and bone that support the teeth. Periodontitis is a progressive condition that, if left untreated, can result in tooth loss(Buckley & Growley, 1984; Prathap & Lakshmanan, 2022). The primary cause of periodontitis is the buildup of plaque, a sticky film of bacteria that forms on the teeth. When plaque is not adequately removed through regular brushing and flossing, it can harden into tartar or calculus, which cannot be removed by routine oral hygiene practices(Bass, 1948; Kumaresan et al., 2022). Tartar harbors bacteria and toxins that can irritate and infect the gums, triggering an immune response that leads to inflammation(Santacroce et al., 2023). Symptoms of periodontitis may include swollen or red gums, bleeding gums (especially during brushing or flossing), receding gums, persistent bad breath, a bad taste in the mouth, loose teeth, and changes in the bite. However, in some cases, periodontitis may not cause noticeable symptoms until it reaches an advanced stage(Abbott, 2004). Many treatment modalities have been administered, dental cleaning include procedures like scaling and root planning where there is removal of the accumulated plaque and tarts and antiseptics containing chlorhexidine can reduce the inflammation and has and antibacterial effect(Barzegar et al., 2022; Kumaresan et al., 2022). Surgical treatments like pocket reduction surgeries are administered when

scaling and root planning is ineffective, here the gums are lifted and tarts are removed then they are sutured back with a reduction in the gum length for the easy maintenance and other treat like laser therapy can be administered. But there is need for a better treatment which gives a promising result and one such is bone regeneration(Dimitriou, Jones, McGonagle, & Giannoudis, 2011; Prathap & Jayaraman, 2022); Bone is one of the important hard tissues which constitutes both organic (45%) and inorganic (65%, mainly made of nHap) compounds. Bone regeneration is procedure which mainly centers the regrowth of bone and their surrounding tissues(Lopes, Martins-Cruz, Oliveira, & Mano, 2018; USHANTHIKA & MOHANRAJ, 2020). This procedure can be used in dentistry for the regeneration of bone in periodontitis, where there is tooth loss. Bone regeneration can be promoted by nano hydroxyapatite (nHap) Ca10 (OH)2(PO4)6 scaffolds, which contributes for the hardness in the bone and enables the periodontal ligament fibers to reattach the cementum and the alveolar bone and also helps for the bone growth, which is one of the main factors(Shanmugam & Sahadevan, 2018; Yuvaraj, Sangeetha, & Kavitha, 2020). In this study, nano hydroxyapatite (nHap) was fabricated using biomimetic mineralization with clove essential oil as template and incorporated in injectable hydrogel form. Generally essential oil like tea tree oil, peppermint oil, clove essential oil has been used for the periodontitis against the oral bacteria(Jain, Selvi, Arumugam, & Jayaseelan, 2021; Thosar, Basak, Bahadure, & Rajurkar, 2013). Traditionally clove essential oil contains eugenol which a has an analgesic (pain relieving) and anti-inflammatory property and apart from this they also exhibit antioxidant property, by which they relive the pain and subsite the inflammation. The nHap scaffolds will increase the storage modulus which in turn support the bone ingrown and bears a low bone defect and aids the regeneration process(Huang et al., 2021). There is better hemocompatibility of nHap&clove essential oil by the protein adsorption and platelet adhesion studies. The scaffold also has a stimulative effect on periodontal ligament stem cell which proliferates and later developed and locks the periodontal cells , this results in cell growth and support the newly developed alveolar bone and cementum formation. The nHap will help in growth of new vessels and collagen which in turn promotes the bone healing and regeneration. The nHap which is incorporated with the hydrogel increases the biocompatibility and lock the periodontal cells and helps in new alveolar bone, cell growth which ultimately results in the bone growth and regeneration, but during the administration of the injection there is chance that the patient might have minor discomfort. The nanohydroxyapatite incorporated with injectable hydrogel and clove essential oil produces cytotoxicity towards the fibroblast and osteoblast cells.

Previous research which had been conducted on rats with nHap with injectable hydrogel promoted the alveolar bone regeneration and new bone formation (Damiri et al., 2024). There is an increase in 50% alveolar rigidity and soft tissue healing. The alveolar bone which is formed by the nHap is feasible and they are also thermosensitive. The nHap with Gum Arabic /k carrageenan had a substantial interaction, expressed a compressive strength and osteogenic differentiation which in turn can be very helpful for the regeneration. This biomimetic approach promotes the alveolar bone growth and tooth formation(Girija, Jayaseelan, & Arumugam, 2018; Upadhyay et al., 2020). The aim of this study is to develop a nano hydroxyapatite with clove essential oil incorporated in hydrogel against the periodontitis.

MATERIALS AND METHOD

Materials

The nanoparticles were prepared in lab of Saveetha Dental College and the clove essential oil was purchased from the nearby whole sale shops.

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). 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 calcined 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 Injectable Hydrogel

Gelatin solution 10% w/v is prepared in distilled water. Prepared nano HAp was added into the gelatin solution at 10 w/w% 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^{o}C for 12 hrs. 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 10^o to 90^o 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 Strength

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^{o}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

SEM and EDAX

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 nanoHAp and CA/P ratio similar to the human body.

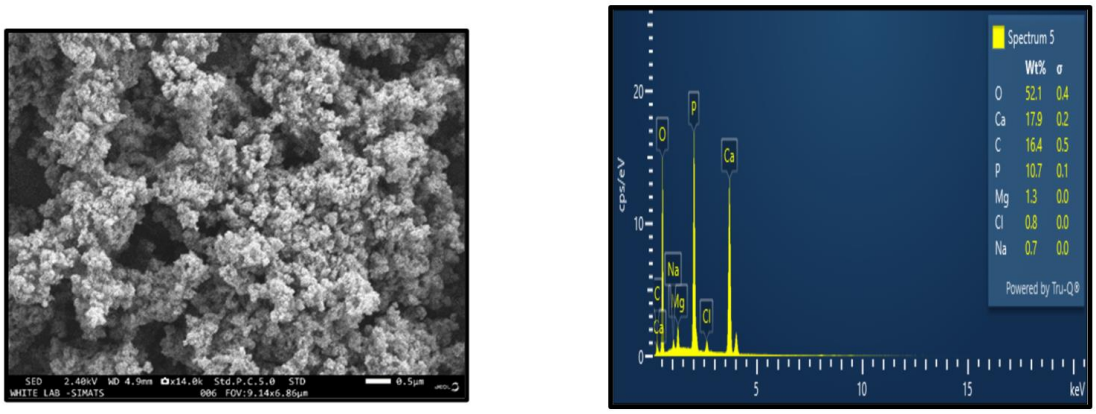

Figure 1: (a) SEM image and (b) EDAX of nano HAp particles

Optimization Injectable Hydrogel

Formation of injectable gel with a different percentage of essential oil and nano particles is shown in Fig. 2. At 0.5% it was not forming a stable gel. With increase in concentration strong gel formation was observed. at 2% crosslinking it formed an injectable gel which became very

stable after reaching body temperature. This concentration for taken for further characterization

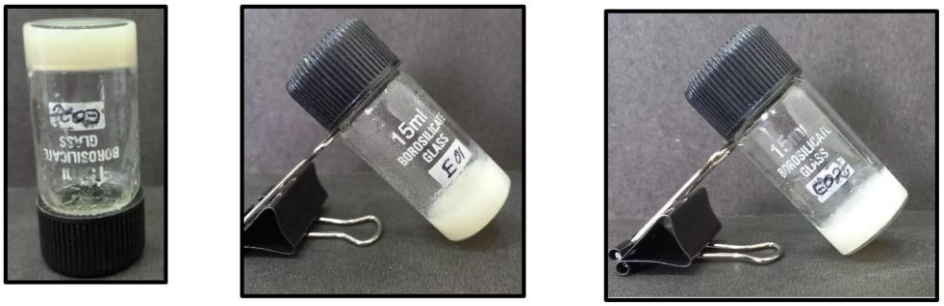

Figure 2: Optimization of Gelation with Different % Clove Oil Loading with 2 % of Cross Linker A) 0.5 % Oil Loading; B) 10% Oil Loading: C) 2% Gel Loaded with 10 % w/v of Nano HAp Particles and Clove Oil

SEM of Nanoparticles and Clove Essential Oil in Hydrogel

Morphology of nanoparticles incorporating hydrogel at 2% cross linking showed better compatibility and the properties were enhanced and not much altered. This is shown in Fig 3. Presence of nanoparticles and oil droplets in hydrogel was confirmed from SEM image.

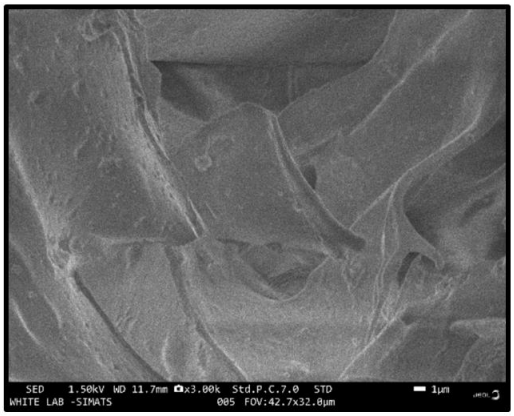

Figure 3: SEM Image of Nanoparticle and Clove Oil Loaded Injectable Hydrogel

XRD

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 humans. The similar pattern in hydrogel confirms the loading of nano HAp in injectable hydrogel form.

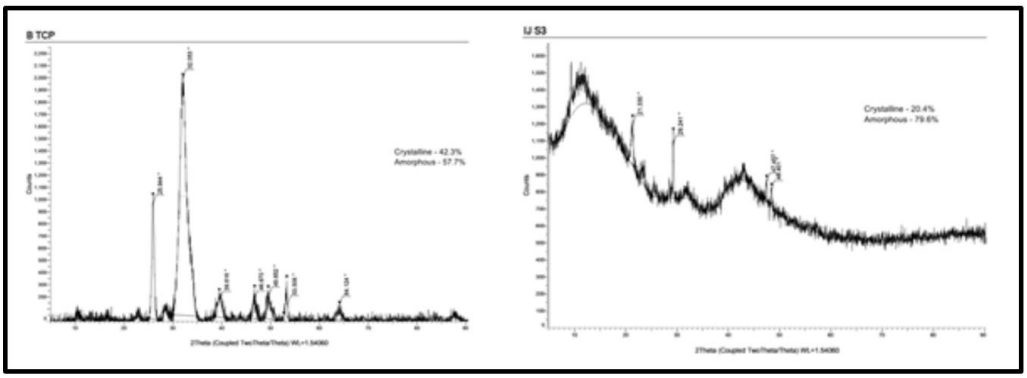

Figure 4: XRD of A) HAp Nanoparticles and B) Hydrogel with Nanoparticles and Clove Oil

Compression Strength

The compression modulus of injectable gel in nanoparticles loaded and with oil loading is shown in Fig 5. With clove oil loading gel strength slightly decreased. It was evident that with nanoparticle loading gel strength increased. This could serve as stronger material for bone regeneration in periodontitis treatments.

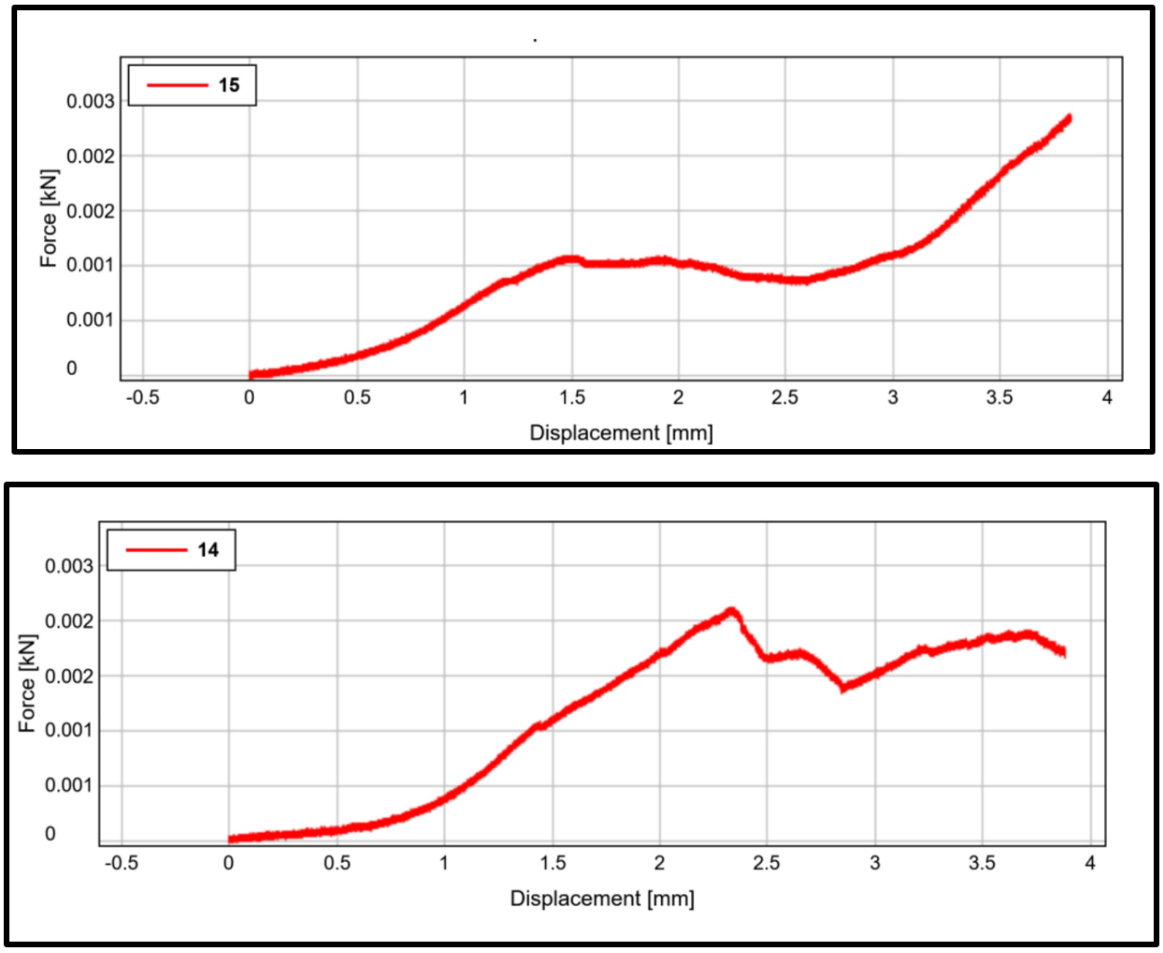

Figure 5: Compression Strength using UTM of Nanoparticles Loaded Injectable Gel at 2 % Cross Linking (A) and B) Clove Oil and Nanoparticles Loaded Injectable Gel

DISCUSSION

From the results it can be seen that the formed nanoparticles exhibited a crystalline nature, which can be further confirmed by XRD analysis which depicted a crystalline arrangement and when incorporated with hydrogel their crystallinity gets reduced(Sethi et al., 2022).By the EDAX we are able to analyze their composition ,which detected that they are nearly same as body composition and so they produce less toxicity and also biocompatible in nature. Hence the body can readily accept it and can promote bone regeneration; Apart from this they also exhibit tissue regeneration and angiogenesis. The compression strength of the hydrogel incorporated with nanoparticle and clove essential oil was found to be decreased when compared to nanoparticle with hydrogel but their regeneration property remained the same. But we found that incorporation of any material with hydrogel will tend to decrease their compressive strength(Wang et al., 2019).

Sem Analysis

The SEM image of zinc oxide nanoparticles under high magnification were found to be exhibit weak particle separation and they were not found in the nano range, Another studies showed that the sem image of the chitosan particles were based on the molecular weight and the TEM results revealed that the particle size of chitosan /gum arabic particles had a particles size ranging from 200-300 nm which proved that they can aid for the bone regeneration. The SEM analysis yielded an approximate particle size of 13 nm. The aggregation of the particles caused the Fe-MNP to change into irregular particles as the preparation temperature rose; The morphology of the Fe3O4 MNP sample prepared at 150 °C was characterized using a Scanning Electron Microscope (SEM) and found that The van der Waals force acting in the solid phase is the reason for the spherical agglomerated structure observed in the Fe-MNP, according to the data. Comparing the Fe-MNP's spherical structure to other shapes like nano-disk or nano-cube geometries, it offers a better internationalization rate and cellular take-up.

XRD Analysis

These are the XRD patterns. Six primary reflections were found for GelMA. The polymer network was responsible for the reflections at 46, 57, 67, 75, and 84°, as well as the maximum intensity reflection at 2θ = 32°. The first reflection (001 reflection) at 2θ = 6.5° for the Lap sample (Figure 3(b)) was linked to a basal spacing of 1.36 nm, which is typical for Lap. There were also more reflections at 20°, 29°, 35°, 55°, and 61°. The SEM image of praseodymium oxide collagen 3-D pro-vasculogenic biomatrix exhibited a spherical morphology and the X Ray diffraction revealed the crystallin property of the oxide,the sharp peaks was observed at 2Ɵ degree values were 27.8,32.3,46.5 and 55.3 corresponds to hkl values of (1 1 1), (2 0 0), (2 2 0), (3 1 1) depicts the high crystalline nature of the nanoparticles.

In a study the findings show that when specimens are cured in saturated limewater, the addition of Al_2O_3 nanoparticles up to a maximum of 2.0% results in concrete with better compressive strength and setting time. For cured specimens in water, 1.0 weight percent replacement is the ideal amount. While concrete without nanoparticles cured in water has a lower strength than concrete with nanoparticles cured in limewater, specimens containing nanoparticles cured in saturated limewater have a more robust gel formation surrounding the Al_2O_3 nanoparticles, resulting in a faster setting time and higher strength.

LIMITATIONS

Maintaining the stability and bioactivity of both nano hydroxyapatite and clove essential oil within the hydrogel matrix over time can be challenging. Nano-sized materials are prone to agglomeration and precipitation, while essential oils are volatile and susceptible to degradation. Achieving controlled release kinetics of clove essential oil from the hydrogel is essential for sustained therapeutic effects. Balancing release kinetics to ensure optimal antibacterial and anti-inflammatory effects without causing cytotoxicity or irritation is a delicate task. The hydrogel must possess suitable mechanical properties to withstand the dynamic environment of the oral cavity, including mastication forces and saliva flow. Achieving the desired balance between rigidity and flexibility while maintaining adhesion to periodontal tissues is challenging.

FUTURE SCOPES

Continued research into novel formulations and delivery systems can enhance the efficacy and stability of the hydrogel. Incorporating advanced materials, such as biocompatible polymers or nanocarriers, may improve controlled release kinetics and bioavailability of active ingredients(Jacob et al., 2021). Integration of bioactive materials, such as growth factors, antimicrobial peptides, or stem cells, into the hydrogel matrix may promote tissue regeneration, wound healing, and modulation of the host immune response, offering potential for adjunctive periodontal therapies.

CONCLUSION

Hydrogel incorporated with nanoparticles and clove essential oil exhibited a promising material against the bone regeneration due to their anti-inflammatory and antipyretic property due to the eugenol present in clove essential oil; Based on the SEM, XRD and EDAX we come to know that they exhibit biocompatibility and bone regeneration by their boldly composition. The development of nano hydroxyapatite (nHap) and clove essential oil incorporated hydrogel for periodontal treatment presents both challenges and promising future scopes. Despite the current limitations surrounding biocompatibility, stability, controlled release, mechanical properties, and regulatory approval, ongoing research efforts offer opportunities for advancement and innovation in dental therapeutics.

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