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DEVELOPMENT OF NANOHYDROXYAPATITE AND CURCUMIN BASED BILAYER SCAFFOLD FOR PERIODONTITIS TREATMENT

Hirshasri[1], Mrs. S. Sangeetha[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

[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

[1]152201077.sdc@saveetha.com and [2]sangeethas.sdc@saveetha.com

ABSTRACT

Periodontitis is a chronic inflammatory disease that affects the supporting tissues surrounding the teeth, including the gums, periodontal ligament, and alveolar bone .The development of nanohydroxyapatite (nHA) and curcumin-based bilayer scaffolds represents a novel strategy for periodontitis treatment. To develop a bilayer scaffold using nano hydroxyapatite and curcumin aims to provide an alternative and potentially more effective treatment for periodontitis.Synthesis of nanohydroxyapatite followed by crosslinking with gelatin hydrogel and incorporation of curcumin as a bilayer scaffold and investigating them through characterisation studies.The development of a bilayer scaffold involves the combination of nHA and curcumin in a layered structure. The purpose of the bilayer design is to provide a controlled release of curcumin, which can help reduce inflammation and promote tissue regeneration. The nHA layer provides mechanical support and mimics the mineralized matrix, while the curcumin layer delivers therapeutic benefits.The development of a nanohydroxyapatite and curcumin-based bilayer scaffold shows promise for the treatment of periodontitis. The incorporation of curcumin in the scaffold offers additional therapeutic benefits. For alveolar bone regeneration it will fulfill the need of biocompatible, biomimic, regenerative material.

Keywords: periodontitis, bone regeneration, curcumin, bilayer scaffold, mineralised matrix, biocompatible

INTRODUCTION

The prevalent chronic inflammatory condition known as periodontitis causes gingival atrophy and alveolar bone abnormalities in addition to the degradation of periodontal tissue.(1)Up to 70% of the world's population, including adolescents, adults, and elderly people, are affected by disorders of the periodontal tissues, which represent a serious clinical issue in dentistry. Periodontal diseases are more likely to develop as a result of a number of factors, including smoking, poor oral hygiene, illnesses, aging, drugs, and stress. Periodontal disease damages the periodontium tissues as it advances, which results in tooth loss. Reduced inflammation and regeneration of disease-related tissue loss are the ultimate goals of periodontal regeneration therapy.

(2). Nanoparticles play a crucial role in the field of biomedicine and are commonly synthesized through both physical and chemical techniques.(3)Due to its improved osseointegrative capabilities, nanohydroxyapatite (nano-HA) represents a significant class of bone transplant materials. revealed that n-HA makes a tight connection with newly deposited bone while also stimulating osteoblast activity with new bone production. The chemical makeup of n-HA material set it apart from microcrystalline hydroxyapatite biomaterials. The chemical formula for natural bone is [Ca10(PO4)6(OH)2] with a calcium to phosphate ratio of 1.67.(4)Nanoparticles wield significant influence on societal dynamics.(5)The utilization of plant-derived nanoparticles offers several advantages compared to traditional physico-chemical approaches. Unlike conventional methods such as the chemical reduction process, which involves the use of hazardous chemicals for nanoparticle synthesis, enhancing plant materials for this purpose presents a more sustainable and environmentally friendly alternative.(6)

Due to their overall efficacy and safety, some herbal medications have recently attracted interest for the treatment of periodontitis. It has been demonstrated that the bioactive polyphenol curcumin, which is derived from Curcuma longa, possesses antioxidant, antibacterial, anti-inflammatory, and analgesic activities. Numerous research have evaluated curcumin's effectiveness against periodontal diseases(7)As a natural plant extract, curcumin has the characteristics of high safety and low toxicity. The United States Food and Drug Administration (FDA) confirmed curcumin as a compound “generally recognized as safe.(8)Natural anti-inflammatory curcumin has a number of biological and therapeutic qualities. Numerous illnesses have been explored for its therapeutic potential, but only a small number of studies have assessed the effectiveness of curcumin as a local drug delivery agent and in the management of periodontitis(9).

Regenerative treatments like guided tissue regeneration (GTR), enamel matrix derivatives (EMDs), and platelet-rich plasma (PRP) have shown promising results, but there is a lack of comprehensive understanding regarding their long-term outcomes and prognostic implications.(10)In recent research, there has been a notable emphasis on tissue engineering approaches aimed at regenerating osteochondral defects, employing various scaffold designs. These scaffolds have evolved from single-layer structures to dual-layer constructs, serving the purpose of addressing both the cartilage-bone interface and providing individual support to each tissue component.(11)

Scaffolds serve as essential artificial frameworks, enabling cells to proliferate and retain their specialized functions. An optimal scaffold should closely replicate the natural extracellular environment of the target tissue for regeneration. Therefore, the choice of scaffold material stands out as a critical factor in achieving success in tissue engineering methodologies.(12)

AIM

To develop a bilayer scaffold using nanohydroxyapatite and curcumin aims to provide an alternative and potentially more effective treatment for periodontitis

MATERIALS AND METHOD

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 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 drop-wise 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 loaded bilayer scaffold

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 -80^{o}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.

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 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

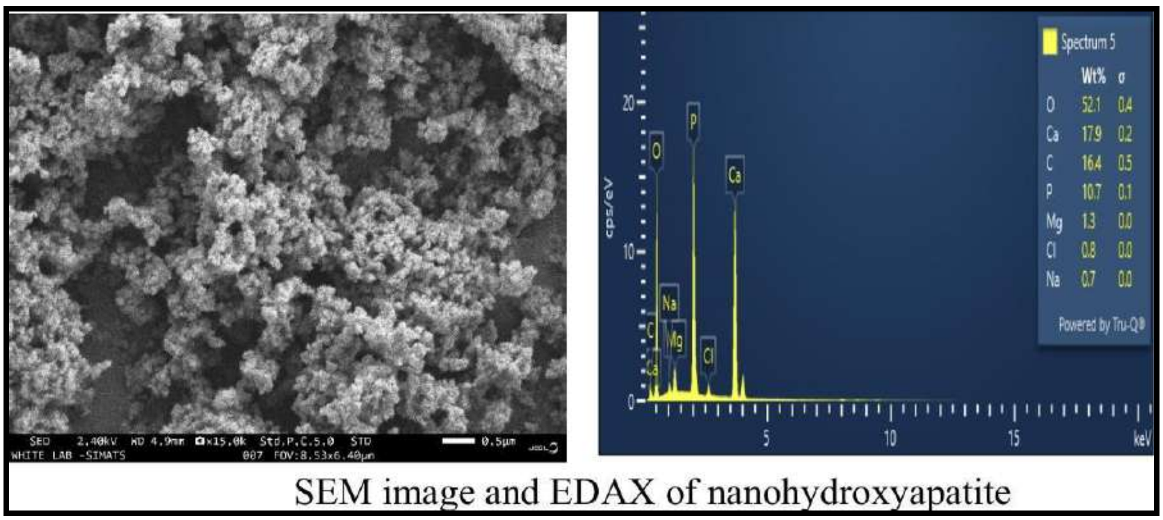

Fig 1: SEM image and EDAX of nano HAp particles

Fig 2: Bilayer scaffold - Curcumin and nHAP loaded gel

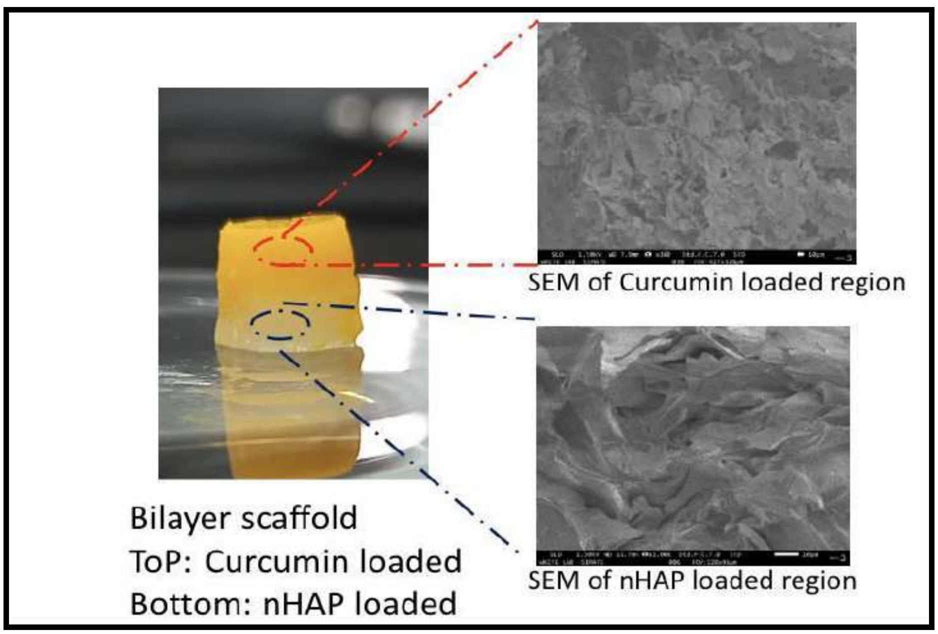

Fig 3: Bilayer scaffold ToP: Curcumin loaded and Bottom: nHAP loaded; SEM of Curcumin loaded region and SEM of nHAP loaded region

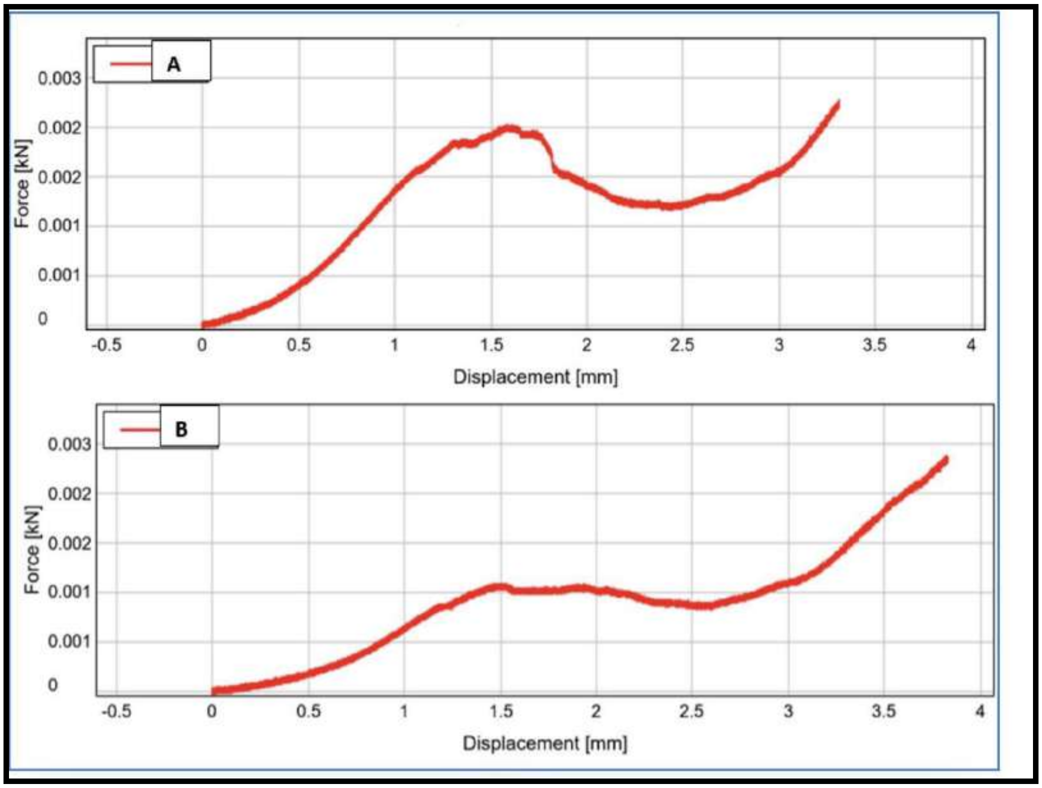

Fig 4: Compression strength using UTM of injectable gel at 2 % cross linking and B) Bilayer scaffold with nano HAp.

DISCUSSION

SEM IMAGE OF EDAX AND HYDROXYAPATITE

In fig 1 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 human body.

Nano hydroxyapatite possesses the capability to enhance the remineralization of tooth structures. Due to its hydrophilic nature and increased surface area compared to conventional hydroxyapatite crystals, it exhibits superior wettability. As a result, it can create a thin yet robust layer on the enamel surface that forms a strong bond with the tooth structure.(13).

Bilayer scaffold - Curcumin and nHAP loaded gel

The bilayer scaffold comprised of top layer of curcumin and bottom layer nanohydroxyapatite

In a similar study A bilayer membrane was created to simulate periodontal tissue in dental defects. The top layer of these membranes features a nanofiber structure resembling the extracellular matrix found in soft tissue. This top layer functions as a barrier to prevent fibroblast migration. Meanwhile, the lower layer offers a suitable porous microenvironment for promoting bone tissue regeneration, thanks to the inclusion of Si-doped nanohydroxyapatite, which enhances bioactivity.(14)

SEM of Curcumin loaded region and SEM of nHAP loaded region

Curcumin, a natural polyphenolic compound found in turmeric, has gained attention for its anti-inflammatory, antioxidant, and antimicrobial properties. It has also demonstrated potential in promoting tissue regeneration and wound healing. When loaded onto the bilayer scaffold, curcumin can serve as a bioactive agent that not only combats inflammation at the implant site but also stimulates tissue regeneration processes. This combination of nHA and curcumin can offer a multifaceted approach to improving the scaffold's performance.(15).

Compression strength using UTM of injectable gel at 2 % cross linking and B) Bilayer scaffold with nano HAp.

It was evident that with nanoparticle loading gel strength increased. This could serve as stronger material for bone regeneration in periodontitis treatment

CONCLUSION

The development of a nanohydroxyapatite and curcumin-based bilayer scaffold shows promise for the treatment of periodontitis. The incorporation of curcumin in the scaffold offers additional therapeutic benefits. For alveolar bone regeneration it will fulfil the need of biocompatible, biomimic, regenerative material.

FUTURE SCOPE OF THE STUDY

Conduct preclinical studies using appropriate animal models to evaluate the safety, efficacy, and long-term effects of the scaffold. If successful, move on to clinical trials to assess its performance in human subjects, including its impact on clinical parameters, radiographic outcomes, and patient-reported outcomes.

AUTHOR CONTRIBUTIONS:

Ms.Hirshasri A.G : Literature search, data collection, manuscript writing.

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

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CONFLICT OF INTEREST:

None to declare.

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