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ISSN 1462 2815

3D SCAFFOLD LOADED WITH CLOVE OIL AND NANO HAP FOR PERIODONTITIS TREATMENT

Divya Dharshini S [1], Taniya Mary Martin [2], K Meenakshi Sundaram [3], Lavanya Prathap [4], Anandakumar Ponnnala [5] and S Sangeetha [6]*

[1, 6] Department of Anatomy, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu - 600077, India.
*Corresponding Author Email: sangeethas.sdc@saveetha.com
[2, 3, 4, 5] Biomedical Research Unit and Lab Animal Centre (BRULAC), Department of Anatomy, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu - 600077, India.

DOI: 10.5281/zenodo.10977896

Abstract

Periodontitis is a prevalent inflammatory gum disease which leads to tooth loss if not managed effectively. This study focuses on developing an innovative therapeutic approach for periodontitis treatment through the design and fabrication of a 3D-printed hydrogel scaffold loaded with nano hydroxyapatite (nHA) and clove essential oil (CEO). This composite scaffold aims to address the challenges of periodontal defects by providing localized drug delivery and promoting tissue regeneration. The incorporation of CEO, known for its anti-inflammatory and antimicrobial properties, targets microbial infections and mitigates inflammation, while nHA, a bioactive form of calcium phosphate, supports bone regeneration and enhances periodontal tissue healing. The scaffold's physical, chemical, and mechanical properties were comprehensively characterized through scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and Energy Dispersive X-Ray Analysis (EDAX). Results demonstrated a porous structure, well-dispersed nHA particles within the scaffold matrix, and successful incorporation of characteristic functional groups from both clove oil and nHA. Mechanical testing confirmed the scaffold's adequate strength and stability. Overall, the proof of concept for this 3D-printed hydrogel scaffold showcases promising potential for synergistic effects between nHA and CEO, addressing both bone regeneration and microbial control in the effective management of periodontitis, thereby presenting a novel and advanced therapeutic strategy for future clinical applications.

Keywords: Nanohydroxyapatite, Periodontitis, Clove Essential Oil, Injectable Hydrogel, 3D Printing Application.

INTRODUCTION

Nano hydroxyapatite (nHA) holds significant promise in various biomedical applications due to its biocompatibility and structural similarity to non-organic bone tissue. Its versatility stems from its ability to mimic the natural composition of bones, making it an ideal candidate for use in regenerative medicine. One notable application is in the treatment of periodontitis, a widespread and destructive gum infection affecting the tissues surrounding teeth(1). This disease poses a substantial global health burden, with nearly 3.5 billion individuals grappling with dental caries and periodontal diseases. Clove oil, derived from the cloves of the Syzygium aromaticum plant, possesses inherent antimicrobial properties that make it a valuable agent in combating oral infections(2). Its natural ability to impede bacterial growth and address mouth and throat infections aligns with the goals of periodontitis management, making it a compelling component for inclusion in therapeutic strategies. In response to the urgent need for effective periodontitis treatments, researchers have embarked on a groundbreaking study aiming to develop a 3D-printed hydrogel scaffold loaded with both nano hydroxyapatite and clove essential oil (3). This innovative approach not only leverages the regenerative properties of nHA but also harnesses the antimicrobial prowess of clove oil. The hydrogel scaffold, designed using biocompatible materials, serves as a delivery system for these bioactive agents, facilitating localized treatment and tissue regeneration within periodontal defects (4). Characterization techniques such as scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and Energy Dispersive X-Ray Analysis (EDAX) have been employed to assess the physical, chemical, and mechanical properties of the 3D-printed scaffold (5). Preliminary findings indicate a porous structure, well-dispersed nHA particles, and successful incorporation of characteristic functional groups from both clove oil and nHA. The ultimate goal of this study is to lay the foundation for a future where 3D-printed hydrogel scaffolds, loaded with nano hydroxyapatite and clove essential oil, become a viable and effective therapeutic intervention for periodontitis. This approach holds the potential to revolutionize the treatment landscape, offering a synergistic solution that addresses both microbial control and bone regeneration in the context of periodontal diseases(6).

Nano-hydroxyapatite (nano-HA) stands as a versatile material with diverse applications within the realm of dentistry, owing to its remarkable biocompatibility and structural resemblance to nonorganic bone tissue. Its utilization spans various dental domains, including implantology, surgery, periodontology, esthetics, and preventive dentistry(7). Researchers have been motivated to develop nanohydroxyapatite (nHA) materials, moving beyond the limitations associated with bulk HA or microparticle HA. The primary goal is to address these challenges and, concurrently, enhance bone integration and regeneration, particularly in the context of bony defects. Periodontitis, a prevalent inflammatory disease, constitutes a significant threat to periodontal tissues, ultimately leading to tooth movement and loss. Current clinical treatments predominantly center on plaque removal and local inflammation control, employing methods such as scaling, root planing, and various surgical interventions(8). However, existing partial-mouth periodontal examination protocols may underestimate the true prevalence of periodontitis by more than 50%, as highlighted by recent studies. This underscores the need for more accurate diagnostic approaches to capture the full extent of the disease burden. Injectable hydrogels emerge as a promising frontier in the domain of oral bone regeneration. Their unique properties make them an attractive option for filling defects with irregular shapes and contours conservatively. This represents a notable advancement in the field, providing a potential solution for addressing the complexities of bone regeneration within the oral cavity(9). The consequences of periodontitis extend beyond mere oral health, significantly impacting critical aspects of daily life such as mastication, food intake, and aesthetics. This inflammatory condition is a prominent cause of tooth loosening, movement, and eventual loss, emphasizing the urgency of effective interventions. While current treatment methods, including initial periodontal therapy, surgery of the periodontal flap, and bone tissue regeneration, aim to lower the depth of periodontal pockets and partially restore periodontal attachment, their efficacy remains limited, prompting the need for further investigation(10). In conclusion, the multifaceted applications of nano-hydroxyapatite and the ongoing advancements in nanomaterial development hold promise for addressing the challenges posed by periodontitis. The exploration of injectable hydrogels represents a significant step forward in conservative bone regeneration strategies. However, the complexities of periodontitis necessitate continued research efforts to develop comprehensive and enduring solutions for the preservation of periodontal health(11). As the field progresses, a more nuanced understanding of the disease and innovative treatment modalities may usher in a new

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era of improved outcomes and patient well-being. The advantages of nano hydroxyapatite are they have closer contact with the surrounding network, has properties that are more quickly absorbed and a high number of molecules on the surface(12). The attachment of osteoblasts and osteoclasts is more formed on the nanocrystalline hydroxyapatite than conventional hydroxyapatite. They have good bioaffinity which could help in the integration of bone, the expression of collagen I, and the differentiation of osteoblasts. Enhancing the biocompatibility of hydrogels created from blends of natural and synthetic biopolymers can result in the creation of the best scaffold match to the extracellular matrix (ECM) for bone and periodontal tissue regeneration(13). Additionally, adding various nanoparticles can increase the scaffold hydrogel stability and provide a number of biological effects. polysaccharide hydrogel scaffolds have several functions, such as controlling drug delivery, and can be carrying bioactive molecules. Injectable polysaccharide hydrogel can be applied to bony defects and cross-linked in situ; therefore, they are preferred for repairing irregular periodontal defects. However, the main downside of a scaffold-based polysaccharide hydrogel is that it has weak mechanical property(14). Some inorganic nanocomponents could be used and incorporated to improve the mechanical property, such as inorganic hydroxyapatite nanoparticles; however, this affects the injectability of the hydrogel. Before being implanted, preformed polysaccharide hydrogel scaffolds have a predesigned size and morphology. Several approaches are used to create a preformed polysaccharide hydrogel scaffold, including the lyophilization process, casting process, and 3D printing process(15). The most promising method for promoting and guiding tissue regeneration is 3D printing. Optimized GH stent has the potential to both maintain dimensional alveolar ridge, as well as to promote soft tissue healing. Moreover, using the hydroxyapatite-containing hydrogel platform has the potential to promote bone and soft tissue regeneration. Presently used scaffolds are difficult to modify, have poor mechanical properties, and are not rapidly degraded. Furthermore, bon defects caused by tooth extraction are irregular, and not conducive to adhesion and fixation of grafted material, thus making it difficult for bio scaffolds to promote alveolar ridge preservation(16). Conventional polymer hydrogels have poor cell orientation and adhesion, and lack the proteins required for osteoblast action, hence they cannot bind directly to host bone that greatly diminishes the capability of hydrogel scaffolds. During the past few decades, injectable hydrogels' application for reconstructing bone defects with irregular size and shape has attracted extensive attention. The current study's objective was to develop injectable hydrogel containing PEEK to regenerate critical size bone defects in cranial region. These surgically challenging defects must be carefully managed to reconstruct the lost area to achieve the desired function and esthetic(17). Injectable in situ forming hydrogels are promising approaches that can provide easy handling, facile and homogenous distribution of hydrogel in irregular and large defects before complete gelation of the hydrogel. This research is vital due to the absence of a study material in hydrogel form incorporating both nano hydroxyapatite (nHA) and clove essential oil for the treatment of periodontitis, particularly through the innovative technique of 3D printing(18). The lack of existing studies focusing on this specific combination highlights the need for exploration in this promising area of research. The study aims to provide a proof of concept for 3D printing using a hydrogel loaded with nano hydroxyapatite and clove essential oil, presenting a novel approach for potential use in periodontitis treatment. The primary objective of this research is to formulate a hydrogel that combines the regenerative properties of nano hydroxyapatite with the antimicrobial and anti-inflammatory characteristics of clove essential oil(19). This dual-functional hydrogel aims to address the complexities of periodontitis by promoting tissue regeneration and controlling microbial infections. By incorporating both nHA and clove essential oil into the hydrogel matrix, the study aims to achieve a synergistic effect that enhances the overall therapeutic potential of the material. The choice of 3D printing as the fabrication technique is strategic, as it allows for precise control over the structure and composition of the hydrogel scaffold. This method facilitates the creation of a customized 3D structure that can be tailored to fit the specific requirements of periodontal defects(20). The integration of nano hydroxyapatite and clove essential oil into the hydrogel matrix, along with the 3D printing approach, aims to enhance the localized drug delivery and tissue regeneration capabilities of the material. The study involves comprehensive characterization of the prepared hydrogel, utilizing techniques such as scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and Energy Dispersive X-Ray Analysis (EDAX). These analyses will provide insights into the physical, chemical, and mechanical properties of the 3D-printed hydrogel scaffold, confirming the successful incorporation of nano hydroxyapatite and clove essential oil. The significance of this research lies in its potential to introduce a groundbreaking approach for periodontitis treatment, addressing the limitations of current methods. The combination of 3D printing technology with a hydrogel loaded with nano hydroxyapatite and clove essential oil opens new avenues for localized drug delivery, improved tissue regeneration, and enhanced outcomes in periodontal defect management. This study not only contributes to the advancement of therapeutic strategies for periodontitis but also establishes a foundation for future investigations and clinical applications in the field of dental medicine(21).

MATERIALS AND METHOD

Preparation of bone like hydroxyapatite Nanoparticle (nHAp)

Synthesis of Nano hydroxyapatite:

In this study, a simulated body fluid (SBF) with ion concentrations akin to human blood plasma, as previously reported by Leena et al. (2016), was employed as the growth medium for nano-hydroxyapatite (nHAp). The synthesis of nHAp at an expedited incubation time involved the step-wise addition of measured amounts of CaCl2 (8.7 M) and Na2HPO4 (3.5 M) to 1000 ml of SBF, with the latter exceeding the reported quantity by 3.5 times. This adjustment aimed to maintain a Ca/P ratio of 2.5, mirroring natural human bone plasma (HBP) and preventing the precipitation of higher resorbable phases of calcium phosphates (CaP). Specifically, 0.4935 g of Na2HPO4 was added to 980 ml of SBF, and then 0.9695 g of CaCl2 was added to the remaining 20 ml of SBF. After complete mixing, 20 ml of CaCl2 solution in SBF was added drop-wise to 980 ml of SBF containing Na2HPO4 under continuous stirring, resulting in a pH decrease to 7.25. The precipitates formed after a 12-hour incubation were filtered, washed six times with ultrapure water, and subsequently dried at 80°C for 24 hours. The dried samples were calcinated at 900°C for 2 hours in a muffle furnace to assess thermal stability and phase changes, followed by crushing to obtain the final nano-hydroxyapatite powder(22).

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Preparation of 3D printable hydrogel

A gelatin solution at a concentration of 10% w/v was meticulously prepared using distilled water. Subsequently, the synthesized nano-hydroxyapatite (nHAp) was incorporated into the gelatin solution at a concentration of 10% w/w, and the mixture was stirred for a duration of 2 hours to ensure uniform dispersion. To induce crosslinking within the gelatin-nHAp composite, varying percentages (0.5%, 1%, 1.5%, and 2% v/v) of the crosslinking agent glutaraldehyde were introduced. This step aimed to enhance the structural integrity and stability of the resulting hydrogel(23). After the optimization of the crosslinking agent concentration, clove oil, recognized for its anti-inflammatory and antimicrobial properties, was introduced into the gelatin solution at an optimized concentration of 5% v/v. The subsequent crosslinking process further solidified the composite structure, creating a hydrogel infused with nano-hydroxyapatite and clove oil. This sequential procedure not only establishes the framework of the gelatin-based hydrogel but also highlights the deliberate steps taken to incorporate nano-hydroxyapatite, crosslinking agent, and clove oil to enhance the composite's properties. The unique combination of these elements holds significant potential for applications in periodontitis treatment, providing a multifunctional and biocompatible material for localized drug delivery and tissue regeneration(24).

RESULTS

The nanoparticles loaded gel underwent a freeze-drying process by being frozen at -80°C for a duration of 12 hours. Subsequently, freeze-drying was employed to remove the frozen water content and preserve the structure of the gel(25). The resulting material was then subjected to characterization using scanning electron microscopy (SEM). The morphological features of both the nanoparticles and the hydrogel were examined, and Energy Dispersive X-ray Analysis (EDAX) spectra were obtained. The SEM analysis and EDAX spectra were conducted using a JEOL JSM IT 800 instrument, with a 30-second platinum coating applied to enhance imaging and analysis. Additionally, Fourier-transform infrared spectroscopy (FTIR) was employed to investigate the molecular composition of the developed material. FTIR spectra were recorded using a Bruker Alpha II model, covering the wavenumber range from 500 to 400 cm^-1 at a resolution of 4 cm^-1, and an averaging of 32 scans was performed. This analytical technique allowed for the identification of functional groups and chemical bonds present in the nanoparticles loaded gel. These characterization techniques, including SEM, EDAX, and FTIR, offer comprehensive insights into the physical structure, elemental composition, and molecular interactions within the developed gel loaded with nanoparticles. Such analyses are crucial for understanding the material's properties and potential applications in the context of periodontitis treatment, ensuring its suitability for targeted drug delivery and tissue regeneration(26).

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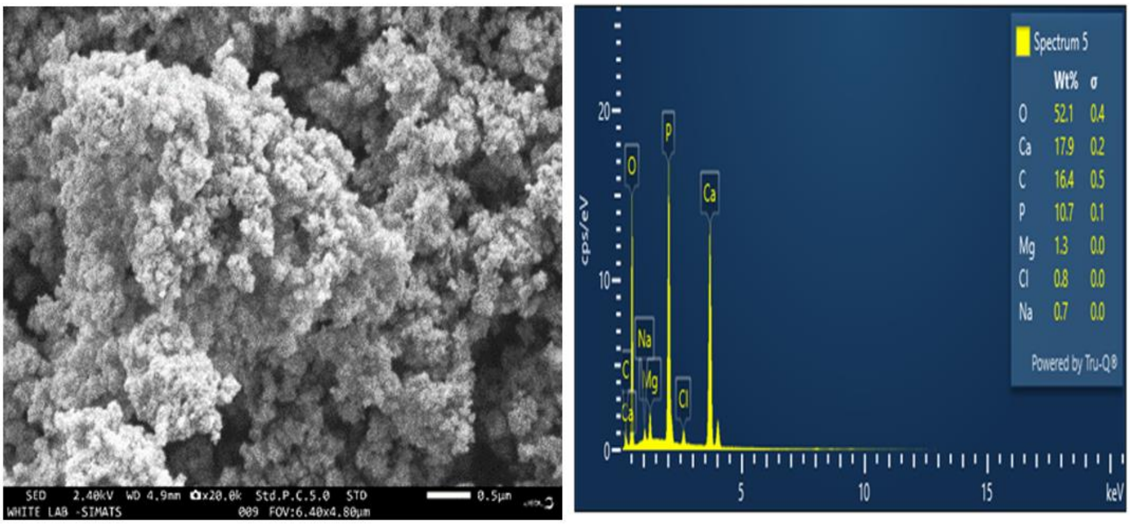

Fig 1: SEM image and EDAX of nano HAp particles

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 human body.

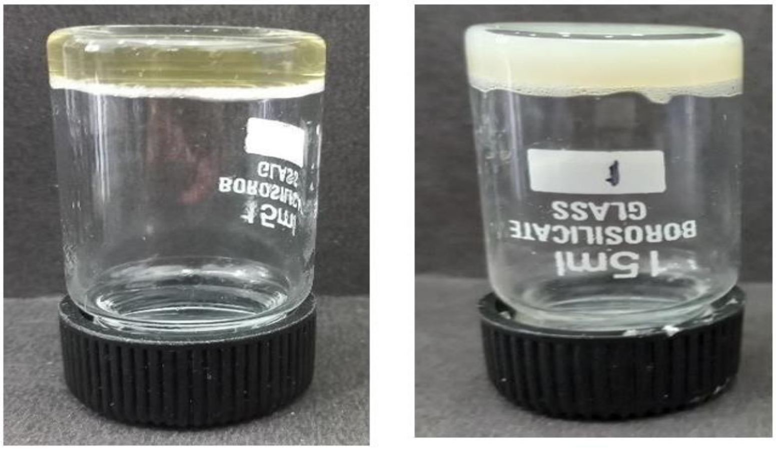

Fig 2: Optimization of gelation with 2 % of cross linker A) Hydrogel at 2%; B) Clove oil and nano HAp loaded hydrogel stable at room temperature

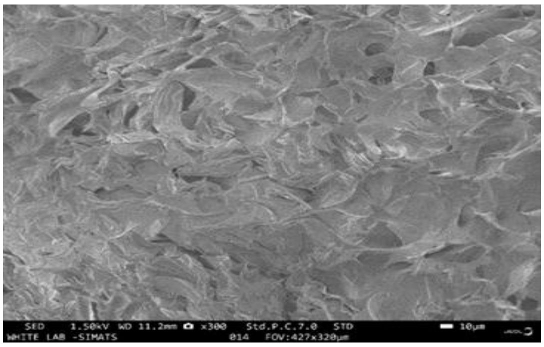

Fig 3: SEM image of clove oil and nanoparticle incorporated hydrogel

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

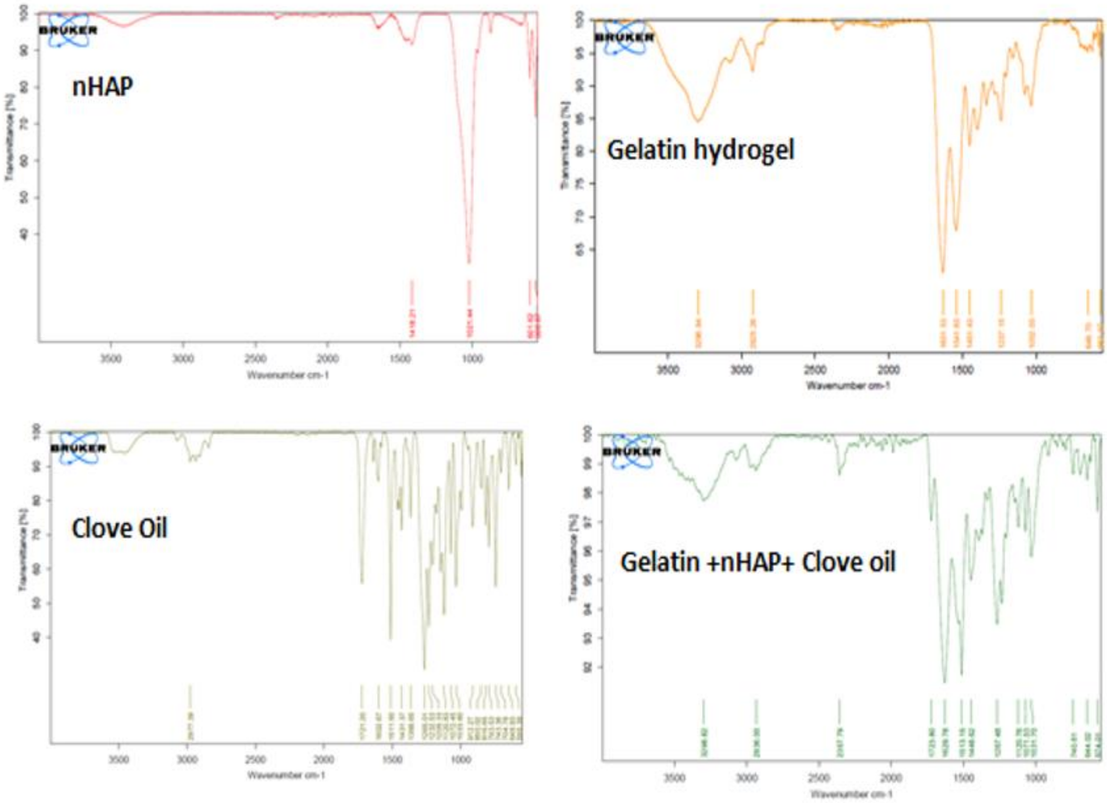

Fig 4: IR spectra of A) nano Hap; B) Gelatin; C) Clove Oil D) Hydrogel formed with gelatin, nano HAP and Clove oil

FTIR spectra shown in Fig 4 confirms effective loading of nano HAP and Clove oil in hydeogel for 3D printing

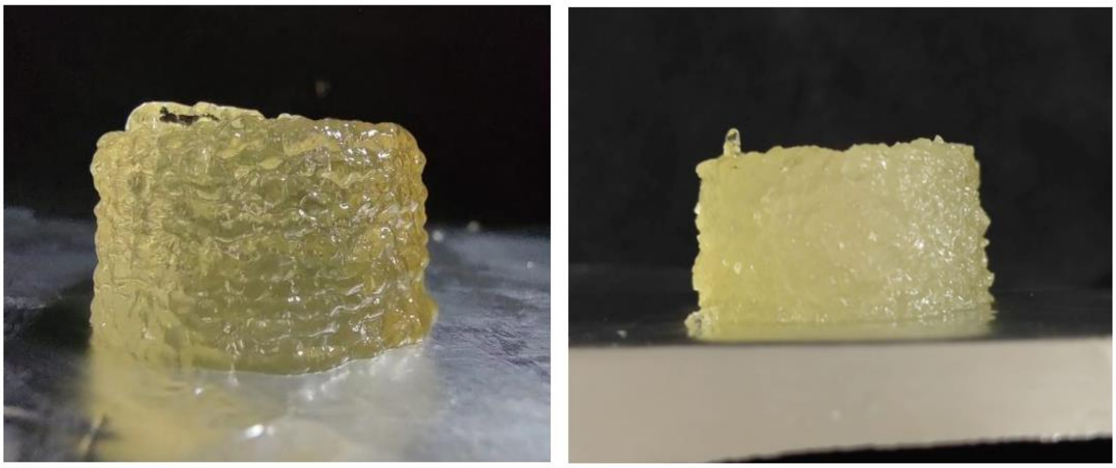

Fig 5: 3D printed Scaffold: A) Gelain hydrogel and b) nHAP and clove oil loaded gelatin hydrogel

3D printed hydrogel with nano Hap and clove oil loaded hydrogel is shown in Fig 5. Stable 3D structure at room temp obtained after 3D printing.

DISCUSSION

The evolving landscape of medical technologies has paved the way for innovative approaches to personalized and precise medicine delivery, particularly in the realm of periodontitis treatment(27). The advent of 3D printing technology stands as a promising avenue in this regard, offering a platform for the development of tailored medicine delivery systems (28). One notable application involves the integration of nanohydroxyapatite (nHAp) and clove essential oil into a 3D printed hydrogel, presenting a novel and potentially transformative strategy for achieving enhanced therapeutic outcomes. Nanoparticles, whether produced through physical or chemical processes, have become integral in the field of biological medicine. These minute entities find utility in various medical applications and are naturally synthesized by organisms such as plants, fungi, and bacteria (29). In the context of periodontitis, nanohydroxyapatite emerges as a key player, renowned for its bioactive properties and its dual capabilities in antibacterial action and bone regeneration. By promoting the development of new bone cells and inhibiting bacterial growth, nanohydroxyapatite, when incorporated into a hydrogel matrix, becomes a critical element in the restoration of damaged periodontal tissues, fostering tissue regeneration while preventing further bacterial intrusion (30). Complementing the regenerative properties of nanohydroxyapatite, clove essential oil brings a distinct set of qualities to the treatment strategy. Recognized for its antibacterial and anti-inflammatory attributes, clove essential oil becomes instrumental in reducing bacterial load, managing inflammation, and safeguarding periodontal tissues against additional harm. This multifaceted approach addresses not only the microbial aspect of periodontitis but also mitigates the inflammatory response, contributing to an overall comprehensive therapeutic effect (31). The effectiveness of the treatment is further elevated through the strategic utilization of a 3D printed hydrogel. This innovative delivery system facilitates a controlled and sustained release of the loaded medications, optimizing the therapeutic impact (32). Tailoring the hydrogel to possess specific porosity and permeability characteristics ensures that the loaded drugs reach the affected area with precision, enhancing the therapeutic potential of the treatment (33). The ability to customize hydrogels through 3D printing technology presents a significant advantage, allowing for the creation of structures precisely molded to fit the unique contours of a patient's periodontal lesion. This individualized treatment approach not only enhances adaptability but also augments therapeutic efficacy (34).
In summary, the development of a 3D printed hydrogel infused with nanohydroxyapatite and clove essential oil represents a pioneering and advanced approach to addressing the complex challenges of periodontitis. This innovative method has the potential to revolutionize patient outcomes in periodontal therapy by providing controlled drug delivery, promoting tissue regeneration, and effectively preventing bacterial growth. As the synergy between 3D printing technology and bioactive compounds continues to unfold, the future holds promise for more personalized and effective treatment strategies in the realm of periodontal health(35).
In conclusion, the utilization of 3D printing technology to create a personalized medicine delivery system in the form of a 3D printed hydrogel loaded with nanohydroxyapatite (nHAp) and clove essential oil holds great promise for advancing periodontitis treatment. The synergistic combination of nHAp and clove oil addresses the multifaceted challenges posed by periodontitis, offering antibacterial, anti-inflammatory, and tissue-regenerative properties(36). The controlled drug delivery facilitated by the 3D printed hydrogel enhances the effectiveness of the treatment by

ensuring sustained release and targeted delivery of therapeutic agents. The incorporation of nanohydroxyapatite contributes to the promotion of new bone cell development and prevents bacterial growth, fostering tissue regeneration and halting further damage. Clove essential oil, with its recognized antibacterial and anti-inflammatory qualities, complements this effect by reducing bacterial load and managing inflammation, ultimately safeguarding periodontal tissues(37).

CONCLUSION

The individualization of treatment through tailored hydrogels with specific porosity and permeability characteristics, enabled by 3D printing technology, further enhances adaptability and therapeutic efficacy. This innovative approach represents a significant advancement in periodontal therapy, offering a holistic solution that not only controls drug delivery but also supports tissue regeneration and combats bacterial growth. Overall, the 3D printed hydrogel loaded with nHAp and clove essential oil emerges as a groundbreaking method with the potential to revolutionize patient outcomes and pave the way for more effective and personalized treatment strategies in the field of periodontitis(38).

References

1) Alipour, Mahdieh, Marjan Ghorbani, Masume Johari Khatoonabad, and Marziyeh Aghazadeh. 2023. “A Novel Injectable Hydrogel Containing Polyetheretherketone for Bone Regeneration in the Craniofacial Region.” Scientific Reports 13 (1): 864.

2) Alkhursani, Sheikha A., Mohamed Mohamady Ghobashy, Samera Ali Al-Gahtany, Abeer S. Meganid, Shady M. Abd El-Halim, Zubair Ahmad, Farhat S. Khan, Gamal Abdel Nasser Atia, and Simona Cavalu. 2022. “Application of Nano-Inspired Scaffolds-Based Biopolymer Hydrogel for Bone and Periodontal Tissue Regeneration.” Polymers 14 (18). https://doi.org/10.3390/polym14183791.

3) Bordea, Ioana Roxana, Sebastian Candrea, Gabriela Teodora Alexescu, Simion Bran, Mihaela Băciuț, Grigore Băciuț, Ondine Lucaciu, Cristian Mihail Dinu, and Doina Adina Todea. 2020. “Nano-Hydroxyapatite Use in Dentistry: A Systematic Review.” Drug Metabolism Reviews 52 (2): 319–32.

4) Eke, Paul I., Wenche S. Borgnakke, and Robert J. Genco. 2020. “Recent Epidemiologic Trends in Periodontitis in the USA.” Periodontology 2000 82 (1): 257–67.

5) Ezhilarasan, Devaraj, and Sheeja S. Varghese. 2022. “Porphyromonas Gingivalis and Dental Stem Cells Crosstalk Amplify Inflammation and Bone Loss in the Periodontitis Niche.” Journal of Cellular Physiology 237 (10): 3768–77.

6) Leena, Maria, Deepti Rana, Thomas J. Webster, and Murugan Ramalingam. 2016. “Accelerated Synthesis of Biomimetic Nano Hydroxyapatite Using Simulated Body Fluid.” Materials Chemistry and Physics 180 (September): 166–72.

7) Liang, Yongxi, Xianghong Luan, and Xiaohua Liu. 2020. “Recent Advances in Periodontal Regeneration: A Biomaterial Perspective.” Bioactive Materials 5 (2): 297–308.

8) Lowe, Baboucarr, John G. Hardy, and Laurence J. Walsh. 2020. “Optimizing Nanohydroxyapatite Nanocomposites for Bone Tissue Engineering.” ACS Omega 5 (1): 1–9.

9) Nasim, Iffat, S. Rajesh Kumar, V. Vishnupriya, and Zohra Jabin. 2020. “Cytotoxicity and Anti-Microbial Analysis of Silver and Graphene Oxide Bio Nanoparticles.” Bioinformation 16 (11): 831–36.

10) Pan, Yongsheng, Yue Zhao, Rong Kuang, Hou Liu, Duo Sun, Tianjiao Mao, Kexin Jiang, et al. 2020. “Injectable Hydrogel-Loaded Nano-Hydroxyapatite That Improves Bone Regeneration and Alveolar Ridge Promotion.” Materials Science & Engineering. C, Materials for Biological Applications 116 (November): 111158.

11) Rajasekar, Arvina, and Sheeja S. Varghese. 2022. “Microbiological Profile in Periodontitis and Peri-Implantitis: A Systematic Review.” Journal of Long-Term Effects of Medical Implants 32 (4): 83–94.

12) Nithya, P., Jeyaram, C., Sundaram, K. M., Chandrasekar, A., & Ramasamy, M. S. (2014). Anti-dengue viral compounds from Andrographis paniculata by insilico approach. World Journal of Alternative Medicine, 1(2), 10-16.

13) Subramanian, U., Kishorekumar, M. S., Muthuraman, S., Munusamy, A. P., & Sundaram, R. (2018). Marine algal secondary metabolites promising anti-angiogenesis factor against retinal neovascularization in CAM model. *Research and Reviews: A Journal of Life Sciences*, *8*, 19-25.

14) Saravanan, K. M., & SUNDARAM, K. M. (2021). Effect of bromocriptine in diabetes mellitus: a review. *Uttar pradesh journal of zoology*, 1166-1170.

15) Meenakshi Sundarm, K., Devi, U., & Manivannan, R. (2015). In silico Discovery of Seaweed Molecules against Matrixmet all oproteinase-26. *Journal of Advanced Bioinformatics Applications and Research*, *6*(2), 52-61.

16) Akshaya, A., & Ganesh, K. (2022). Knowledge, Prevalence, and Impact on Social Distancing on Coronavirus (COVID-19) Infection Spread in Young, Adult, and Aged Population: A Survey. *ECS Transactions*, *107*(1), 14063. https://ui.adsabs.harvard.edu/abs/2022ECSTr.10714063A/abstract

17) Ambika, S., Manojkumar, Y., Arunachalam, S., Gowdhami, B., Meenakshi Sundaram, K. K., Solomon, R. V., . . . Sundararaman, M. (2019). Biomolecular interaction, anti-cancer and anti-angiogenic properties of cobalt (III) Schiff base complexes. *Scientific reports*, *9*(1), 2721. DOI: 10.1038/s41598-019-39179-1

18) Amirsasan, R., Akbarzadeh, M., & Akbarzadeh, S. (2022). Exercise and colorectal cancer: prevention and molecular mechanisms. *Cancer Cell International*, *22*(1), 247. https://link.springer.com/content/pdf/10.1186/s12935-022-02670-3.pdf

19) Arends, J., Bachmann, P., Baracos, V., Barthelemy, N., Bertz, H., Bozzetti, F., . . . Kaasa, S. (2017). ESPEN guidelines on nutrition in cancer patients. *Clinical nutrition*, *36*(1), 11-48. https://pubmed.ncbi.nlm.nih.gov/27637832/

20) Balaji, A., Bhuvaneswari, S., Raj, L. S., Bupesh, G., Meenakshisundaram, K. K., & Saravanan, K. M. (2022). A review on the potential species of the zingiberaceae family with anti-viral efficacy towards enveloped viruses. *J Pure Appl Microbiol*, *16*(2), 796-813. https://www.sciencedirect.com/science/article/abs/pii/S2214785321010774

21) Benedetti, F. (2013). Placebo and the new physiology of the doctor-patient relationship. *Physiological reviews*, *93*(3), 1207-1246. https://pubmed.ncbi.nlm.nih.gov/23899563/

22) Bhandari, S. V., Nagras, O. G., Kuthe, P. V., Sarkate, A. P., Waghamare, K. S., Pansare, D. N., . . . Belwate, M. C. (2023). Design, Synthesis, Molecular Docking and Antioxidant Evaluation of Benzimidazole-1, 3, 4 oxadiazole Derivatives. *Journal of Molecular Structure*, *1276*, 134747. https://ui.adsabs.harvard.edu/abs/2023JMoSt127634747B/abstract

23) Borniger, J. C. (2019). Central regulation of breast cancer growth and metastasis. *Journal of cancer metastasis and treatment*, *5*. https://pubmed.ncbi.nlm.nih.gov/31773065/

24) Ch Beck, G., Brinkkoetter, P., Hanusch, C., Schulte, J., van Ackern, K., van der Woude, F. J., & Yard, B. A. (2004). Clinical review: Immunomodulatory effects of dopamine in general inflammation. *Critical Care*, *8*(6), 485. https://doi.org/10.1186/cc2879

25) Chockalingam, S., Sasanka, K., Babu K, Y., Ramanathan, V., & Ganapathy, D. (2020). Role of Bruxism in Prosthetic Treatments-A Survey. *Indian Journal of Forensic Medicine & Toxicology*, *14*(4). https://agupubs.onlinelibrary.wiley.com/doi/abs/10.1029/2001JD900133

26) Fleming, B., Edison, P., & Kenny, L. (2023). Cognitive impairment after cancer treatment: mechanisms, clinical characterization, and management. *bmj*, *380*. https://bmjecomm.bmj.com/content/380/bmj-2022-071726

27) Han, X., Li, B., Ye, X., Mulatibieke, T., Wu, J., Dai, J., . . . Xue, J. (2017). Dopamine D2 receptor signalling controls inflammation in acute pancreatitis via a PP2A-dependent Akt/NF-κB signalling pathway. *British Journal of Pharmacology*, *174*(24), 4751-4770. https://bpspubs.onlinelibrary.wiley.com/doi/full/10.1111/bph.15026

28) Vijaya Anand, A., Bharathi, V., Bupesh, G., Lakshmi, J., Meenakshi Sundaram, K., & Saradhadevi, M. (2021). Identification of novel potent pancreatic lipase inhibitors from Ficus racemosa. *Biomedicine*, *41*(1), 23-30. https://doi.org/10.51248/.v41i1.528

29) Wu, Y., Hu, Y., Wang, B., Li, S., Ma, C., Liu, X., . . . Yang, S. (2020). Dopamine uses the DRD5-ARRB2-PP2A signaling axis to block the TRAF6-mediated NF-κB pathway and suppress systemic inflammation. *Molecular cell*, *78*(1), 42-56. e46. https://www.cell.com/molecular-cell/pdf/S1097-2765(20)30043-5.pdf

30) Yin, T., He, S., Shen, G., Ye, T., Guo, F., & Wang, Y. (2015). Dopamine receptor antagonist thioridazine inhibits tumor growth in a murine breast cancer model. *Molecular medicine reports*, *12*(3), 4103-4108. https://www.spandidos-publications.com/mmr/12/3/4103

31) Sundaram, K. K. M., Bupesh, G., & Saravanan, K. M. (2022). Instrumentals behind embryo and cancer: a platform for prospective future in cancer research. *AIMS Molecular Science*, *9*(1), 25-. https://www.aimspress.com/article/doi/10.3934/molsci.2022002?viewType=HTML

32) Huang, H., Wu, K., Ma, J., Du, Y., Cao, C., & Nie, Y. (2016). Dopamine D2 receptor suppresses gastric cancer cell invasion and migration via inhibition of EGFR/AKT/MMP-13 pathway. *International immunopharmacology*, *39*, 113-120. https://pubs.acs.org/doi/10.1021/am403966x

33) Leung, E. L.-H., Huang, J., Zhang, J., Zhang, J., Wang, M., Zhu, Y., . . . Ma, L. (2023). Novel Anticancer Strategy by Targeting the Gut Microbial Neurotransmitter Signaling to Overcome Immunotherapy Resistance. *Antioxidants & Redox Signaling*, *38*(4), 298-315. https://pubmed.ncbi.nlm.nih.gov/10392631/

34) Luo, Y., & Roth, G. S. (2000). The roles of dopamine oxidative stress and dopamine receptor signaling in aging and age-related neurodegeneration. *Antioxidants and Redox Signaling*, *2*(3), 449-460. https://pubmed.ncbi.nlm.nih.gov/11229358/

35) Murray, M. T., & Pizzorno, J. (2010). *The encyclopedia of healing foods*. Simon and Schuster. https://books.google.co.in/books/about/The_Encyclopedia_of_Healing_Foods.html?id=UKtAMVUT57EC&redir_esc=y

36) Padmapriya, A., Preetha, S., Selvaraj, J., & Sridevi, G. (2022). Effect of Carica papaya seed extract on IL-6 and TNF-α in human lung cancer cell lines-an In vitro study. *Research Journal of Pharmacy and Technology*, *15*(12), 5478-5482. https://www.semanticscholar.org/paper/Effect-of-Carica-papaya-seed-extract-on-IL-6-and-in-P.-P./38de57e0cd65f15736e93664c186c2349d8200ab

37) Prathap, L., & Jayaraman, S. (2022). Anti Proliferative Effect of Endogenous Dopamine Replica in Human Lung Cancer Cells (A549) Via Pi3k and Akt Signalling Molecules. *Journal of Pharmaceutical Negative Results*, 1380-1386. https://www.pnrjournal.com/index.php/home/article/view/1228

38) Prathap, L., & Lakshmanan, G. (2022). Evaluation of Incidence of Various Types of Coronoid Process in South Indian Population. *Journal of Pharmaceutical Negative Results*, 1387-1390. https://www.pnrjournal.com/index.php/home/article/view/1230