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ORIGINAL ARTICLE

Fabrication of hydroxyapatite reinforced polymeric hydrogel membrane for regeneration

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HAP membrane;
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Abstract *Background:* The regeneration of lost/damaged support tissue in the periodontium, including the alveolar bone, periodontal ligament, and cementum, is an ambitious purpose of periodontal regenerative therapy and might effectively reduce periodontitis-caused tooth loss. Guided tissue regeneration (GTR) is a technique currently used in dentistry for periodontal surgery, which allows osseous regeneration prior to soft tissue migration into the area of interest. Calcium phosphate-based bone grafts (mostly Tricalcium Phosphate or Hydroxyapatite) are bio ceramics that show the greatest similarity to the mineral found in the bone. Thereby, giving calcium-phosphate excellent biocompatibility, biodegradability and osteoconductivity. The aim of the study is to fabricate hydroxyapatite reinforced polymeric hydrogel membrane for regeneration.

Materials and Method: Pure alginate fabrication was done by cross linking sodium alginate with calcium chloride. Hydroxyapatite (HAP) alginate (Alg) was formulated by adding nanoparticles to the alginate mixture, which was then cross-linked with calcium chloride to formulate a HAP alginate polymeric membrane. The Fourier-transform infrared spectroscopy (FT-IR), Scanning Electron Microscope (SEM), and biocompatibility tests were performed to analyse the membrane characteristics.

Results: Fabricated Hydroxyapatite- alginate (Hap- Alg) membrane has longer durability, because of strong crystal structure which in turn might take a longer time to regenerate. The

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membrane was found to be biocompatible and HAp induces faster mineralisation which in turn will increase the tissue regeneration rate of the membrane.

Conclusion: The findings of our study suggests that the HAP-Alg hydro gel membrane is highly durable and hemocompatible and it has faster mineralisation capability thus making it superior from the clinically available membranes for GTR. Further analyses needs to be conducted to evaluate the potential of this membrane to be used for regeneration.

1. Introduction

The tissues that support teeth are called periodontium, and they are made up of alveolar bone, cementum, gingiva, and periodontal ligament (PDL)(Xiong et al., 2013). An inflammatory condition called periodontitis causes the periodontal tissues to deteriorate, resulting in tooth mobility and finally tooth loss(Roccuzzo et al., 2022). Scaling and root planing, as well as surgical procedures, are now the main clinical therapies for periodontitis that target removing plaque and reducing local inflammation (Duran-Pinedo et al., 2022, Rajesh S et al., 2011). Recent American Academy of Periodontology consensus report advised surgical surgery as the preferred course of treatment for intraosseous abnormalities(Tavelli L et al., 2023).

The restoration of missing tooth-supporting tissues, such as the periodontal ligament, alveolar bone, and cementum, has been the focus of numerous regenerative periodontal therapies over the past 20 years, including guided tissue regeneration(GTR), enamel matrix derivative (EMD), bone grafts, growth factor delivery, and the combination of cells and growth factors with matrix-based scaffolds (Tavelli L et al., 2023, Liang Y et al., 2020). GTR employs artificial membranes that are either resorbable or non-resorbable to prevent soft tissue from forming in these damaged areas (Rocuzzo M et al., 2018). This membrane is important because it prevents the slowly migrating osteoblastic cells from populating and developing at the spot while enabling the quicker migration of soft tissue cells to do so, thereby creating a barrier for the osteoblastic cells (Zhou T et al., 2017, Iviglia G et al., 2019).

Calcium apatite can be found naturally as the mineral hydroxyapatite (Hap)(De Ry SP et al., 2022). The mineral component of bones and other hard tissues seen in highly developed animals resembles HAp when it is synthesised (Shaheen MY, 2022). The tissues closest to and most compatible with HAp's chemical and physical characteristics are human bone and teeth tissues. Because it promotes bone ingrowth HAp is one of the most biocompatible, bioactive, and biodegradable materials for orthopaedic, dental, and maxillofacial applications (Mucalo M, 2015).

Due to its advantageous qualities, such as biocompatibility and ease of gelation, alginate is a biomaterial that has been used extensively in biomedical research and engineering (Cardoso DA et al., 2014). Since these gels maintain a structural resemblance to the extracellular matrix in tissues and may be modified to perform a number of essential roles, alginate hydrogels have shown to be particularly appealing in wound healing, drug delivery, and tissue engineering applications to date (Kloukos Det al., 2022). Hydrogels are networks of hydrophilic polymers with a high water content that are cross-linked in three dimensions (Sahoo DR et al., 2021).

The aim of the study is to fabricate HAp reinforced polymeric hydrogel membrane for periodontal regeneration.

2. Materials and methods

2.1. Preparation of HAp- alginate polymeric membrane

3% (w/v) Alginate (Chromalgin, Piscum health science, Mumbai, Maharashtra)® was prepared by dissolving alginate and Hydroxyapatite in double distilled water. The research was done in the White lab of Saveetha Dental college and hospitals. This dissolved polymeric solution crosslinked with 6% calcium chloride solution to create a solid membrane (Allcock HR, Kwon S., 1989, Huang RYM et al., 1999). The final hydrogels had weight ratios of alginate to hydroxyapatite of 3:1, which are referred to as 2.5HAP-ALG. Alginate hydrogel, ALG-HAp hydrogel, and ALG-HAp hydrogel were thus created, with the alginate membrane serving as the control group.

2.2. FTIR analysis

A Fourier transform infrared (FTIR) spectrometer (BRUKER)® was used to evaluate the cross linking (Romanova V et al., 2002). Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra were recorded using dried films. Casting the corresponding hydrogels into a polystyrene Petri dish and allowing them to dry for three days at room temperature gave alginate, gelatin, and substance films. By examining the polymeric bonding of the membrane, the FTIR spectrometer may reveal information on the crystal structure of the membranes (by seeing the bond between amide and carboxylic group).

2.3. Morphology of membrane

Morphological analysis was done using a scanning electron microscope (SEM) (JEOL-JSM-IT800 Electron Microscopy) ® at 1 kV. With the help of a critical point dryer (Leica EM CPD300)® the samples were dried up before SEM analysis.

2.4. Biocompatibility assessment

The solution was exposed to the three membranes, and the rates of hemolysis were assessed using a positive and negative control. The RBCs in the control sample exhibit total rupture in the positive control and complete protection in the negative control. In this experiment, 950ul of double-distilled water was combined with 50ul of blood as a positive control. As a negative control, 950ul of phosphate buffer saline (PBS) and 50ul of blood were combined. Positive controls result in full RBC rupture and haemoglobin release, which turns the fluid completely crimson. Only mechanical injury, caused by PBS in the negative control, results in limited hemolysis (Jaganathan H, Godin B., 2012).

3. Results

3.1. Morphological analysis by FE-SEM

The SEM (Fig. 1) shows that there is increased folds on the membrane. The alginate membrane was shown to be a smooth membrane with few imperfections and irregularities. The HAp-Alg membrane displayed folding, which increased the scaffold's surface area and roughness. The results show that Hap- Alg has longer durability but due to strong crystal structure it might take a longer time to regenerate. HAp induces faster mineralisation which in turn increases the tissue regeneration rate of the membrane, thereby increasing the chance of periodontal regeneration.

3.2. FT-IR

Fig. 2 shows the graphical representation of the FTIR analysis. The FTIR spectrometer is useful in analysing the polymeric bonding of the membrane thereby providing information regarding the crystals structure. This is possible by the presence of phosphate and carboxyl groups. A wide range of phosphate stitching was noted for these membranes along with oxygen as displayed in the graph. Only by the presence of phosphate bond it is noted that there was a cross-linking of alginate with hydroxyapatite leading to the fabrication of membrane. Calcium phosphate bond ratio in the HAp-Alg membrane was 1.67 with the external structure, making its disintegration difficult. The benefits of hydroxyapatite and alginate are combined in HAp-Alg nanoparticles, where hydroxyapatite offers pH-responsive degradability and alginate offers high biocompatibility and COO functionality.

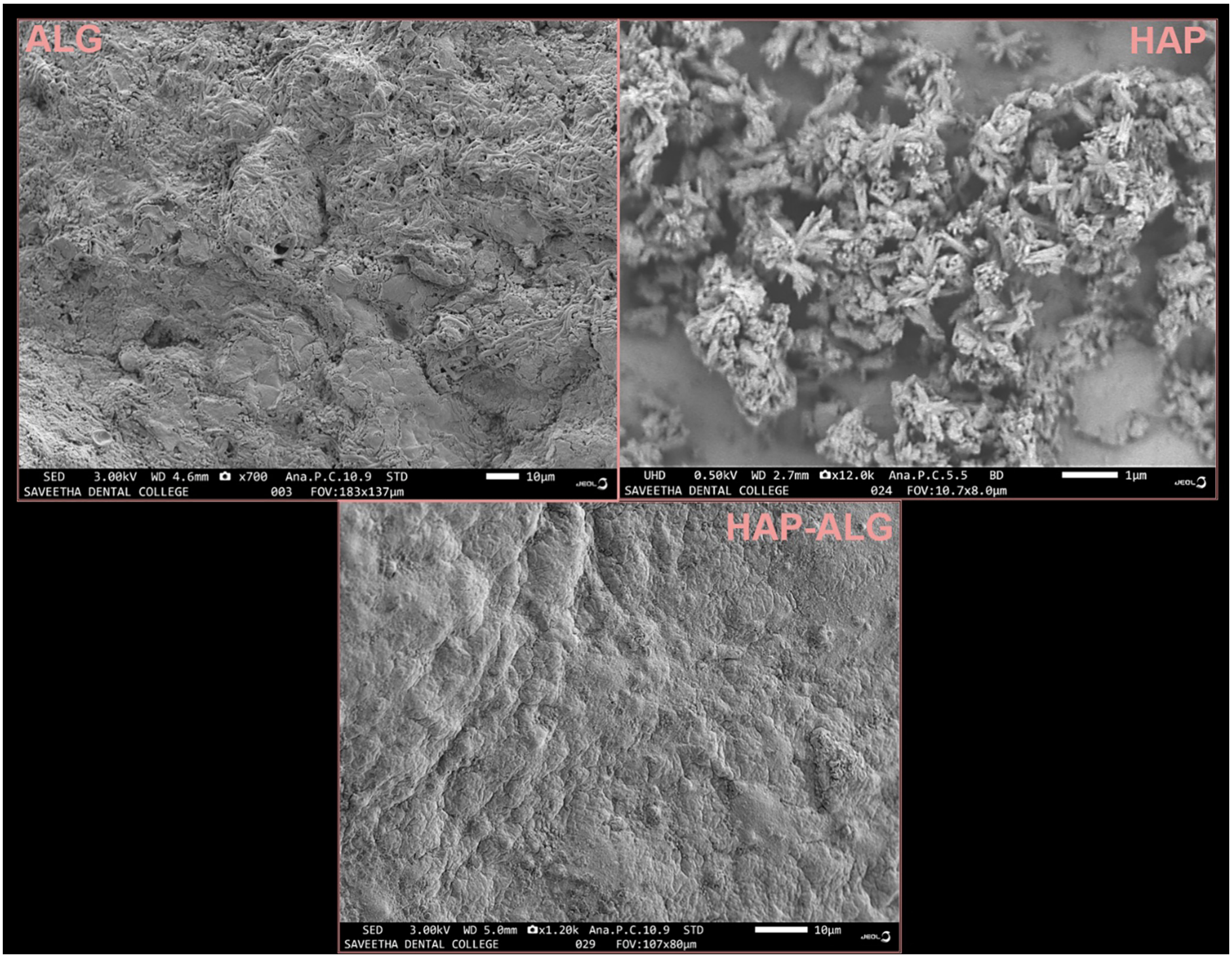

Fig. 1

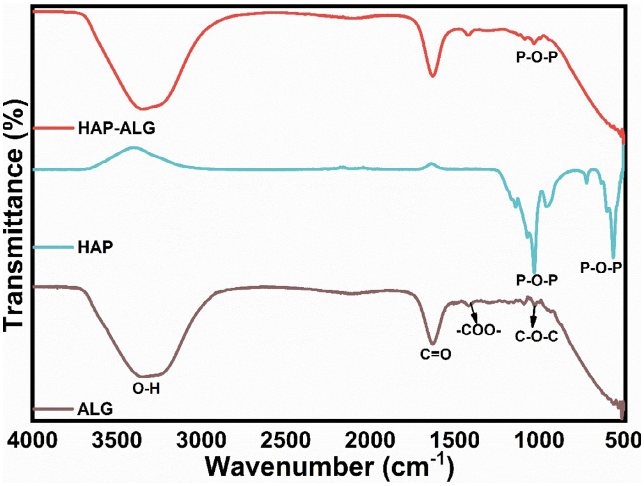

Fig. 2

3.3. *Blood compatibility*

The biocompatibility was assessed for the membranes (Fig. 3). 50μl of blood was mixed with 950 μl of double-distilled water to create the positive control. The negative control sample contained 950μl of phosphate buffer saline and 50μl of blood (PBS). In the case of a successful control, every single RBC entirely burst and released its haemoglobin, colouring the solution crimson in the process. Limited hemolysis only produced by mechanical injury brought on by PBS in the negative control. Both solutions were exposed to the membranes, and a UV spectrophotometer was used to measure the rate of lysis. In order for the membrane to be biocompatible and suitable for use in biomedical applications, the lysis rate should be below 5%, according to the American Standard for testing materials. All three membranes in this investigation showed hemolysis rates that were under 5%. The most biocompatible membrane of the three was found to be HAp-Alg, in both concentrations (15 mg and 30 mg).

4. Discussion

The GTR technique involves the application of a physical barrier to stop epithelial downgrowth on the exposed root surface, which is known to impede the development of new attachment components thereby preventing inflammation and bacterial infection. In addition to all of these benefits, GTR has the ability to serve as drug carriers to transport medications into the oral cavity over an extended period of time with minimal adverse effects(Mondal S et al., 2019, Pretzl B et al., 2009, Sethiya KR et al., 2022).

In a study, polycaprolactone (PCL), polyethylene glycol (PEG), and bioactive glass nanopowders (BGs) were used to modify nano composite membranes for periodontal regeneration (Dehnavi SS et al., 2018). This membrane had a more moist surface, high levels of adipose-derived stem cell proliferation, better alkaline phosphatase activity, and a strong ability to mineralize bone, which was validated by an Alizarin red assay. Another research examined the characteristics of three varieties of electrospun composite fibrous membranes consisting of nano-apatite (nAp) and polycaprolactone. The results of this investigation showed that nAp-incorporated membranes are physiologically viable and increase bioactivity (Karahaliloglu Z et al., 2022). Deep three-wall defects saw greater levels of CAL gain following GTR therapy than two- or one-wall defects, thereby supporting the theory that intrabony defect structure affects the outcomes. According to a comprehensive review, using a grafting material in addition to barrier membranes alone produced better histology findings for bone repair in two-wall intrabony defect models of periodontal regeneration (Sculean A et al., 2008). A wide range of applications for three-dimensional (3D) printing are now being thoroughly researched, including the creation of bioengineered tissues, as well as the manufacturing of useful

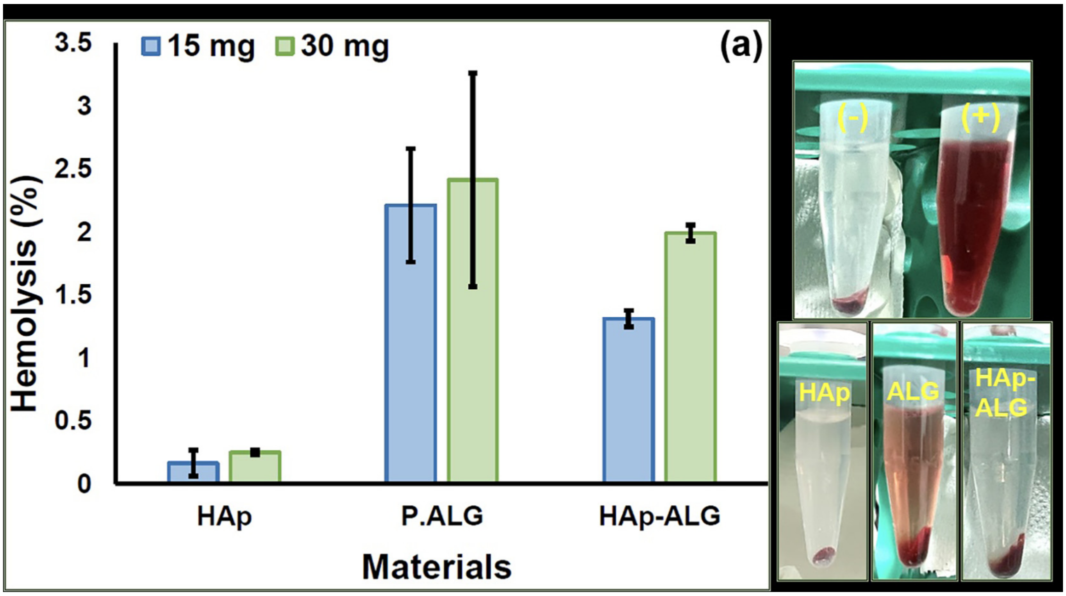

Fig. 3

biomedical materials and devices for dentistry and orthopaedic purposes. A recently done study showed 3D printing which manufactured a soft membrane made of gelatin, elastin, and sodium hyaluronate (Tayebi L et al., 2018). The technique resulted in a soft membrane with distinct and diverse pore configurations at various layers, thereby making it appropriate for directed tissue regeneration. When compared to our study with hydroxyapatite and alginate showed better biocompatibility and longer durability but due to strong crystal structure it takes a longer time to regenerate (Lim Jl et al., 2022, Miguel Oliveira J et al., 2018). The gelatin, elastin, and sodium hyaluronate from the previous study when compared with HAP will increase the mineralization activity thereby better periodontal regeneration would be appreciated.

One of the studies showed the use of EMD is superior to control treatments but equally effective as resorbable membranes in the treatment of intrabony defects. When combined with a coronally advanced flap for recession coverage, EMD offers results that are superior to those of a control while still being as efficient as a connective tissue transplant. In comparison to resorbable membranes, the use of EMD in furcations will result in a greater reduction in the depth of the horizontal furcation defect (Allcock HR, Kwon S., 1989).

The membranes' capacity to renew periodontal tissues has to be investigated further as part of this investigation. The biocompatibility of the membranes should be demonstrated by animal testing, followed by human studies, allowing for the product's commercialization. Additionally, recent research showed the importance of paraprobiotics (Butera A et al., 2022), lysates (Shanbhag S et al., 2021, Shanbhag S et al., 2021) and postbiotics (Butera A et al., 2022) in clinical dentistry. Further research is needed explore the wound healing and true regenerative capacities of HAp-Alg membrane.

5. Conclusion

Our study is in fact an attempt to fabricate an GTR membrane for periodontal regeneration. From the preliminary analyses our HAp-Alg membrane is highly durable and biocompatible and has a faster mineralisation capability because of HAp and can be considered as a potential regenerative material.

Ethical approval

The authors declare that the present study has been approved by the Ethical Review Board of Saveetha Dental College and Hospitals, Chennai.

CRediT authorship contribution statement

Anju Cecil: Conceptualization, Methodology, Formal analysis, Supervision, Validation, Visualization, Writing – review & editing. **S Chitra:** Conceptualization, Methodology, Formal analysis, Investigation, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sdentj.2023.05.021.

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