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# Fabrication and evaluation of hyaluronic acid and calcium hydroxide and egg shell derived hydroxyapatite GTR membrane

**1 Ananya.R**
Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-77, Tamil Nadu, India.
**2 Balaji Ganesh.S**
Department of Periodontics, Saveetha Dental College and Hospitals,Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-77, Tamil Nadu, India.
**3 Rajalakshmanan Eswaramoorthy**
Department of Biomaterials, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077,Tamil Nadu, India.
**Corresponding Author:**
**Dr. Balaji Ganesh S**
Senior lecturerDepartment of PeriodonticsSaveetha Dental College and Hospitals,Saveetha Institute of Medical and Technical Sciences (SIMATS) Chennai-77, Tamil Nadu, India

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**KEYWORDS**
Eggshell-derived hydroxyapatite, GTR membrane, tissue and bone regeneration, periodontitis, bone graft.

ABSTRACT
INTRODUCTION: Guided tissue regeneration or GTR is a predictable and effective surgical treatment method for regeneration and treatment of periodontal defects. Hydroxyapatite is a calcium phosphate apatite that has been used as graft material with great success. Thus, a novel GTR membrane from HA, Gelatin and eggshell-derived hydroxyapatite could be beneficial in bone remodelling and in the treatment of periodontal defects among periodontally compromised patients whose bone formation levels are affected due to age or other factors.
MATERIALS AND METHOD: Eggshell-derived hydroxyapatite was fabricated by calcifying the eggshells at 900 degrees Celsius, adding 0.5M diammonium hydrogen phosphate solution and drying it at 100 degrees Celsius overnight. GTR membrane was prepared and the samples were then assessed for tensile strength, contact angle SEM and FTIR.
RESULTS: The average contact angle of the control sample is 70.01°. 0.5% EHA GTR membrane shows an average contact angle of 68.12° and 1% EHA GTR membrane shows an average value of 71.05°. In our study, it was seen that the fabricated samples showed similar contact angles to the control. All the peaks of EHA were assessed on the FTIR spectrum of EHA-HA gelatin membrane which indicates the successful mediation of EHA into the sample.
CONCLUSION: Eggshell-derived hydroxyapatite is a versatile and novel bone graft substitute that showed promising results within the study limitations. It is a safe synthetic graft substitute that exhibits good physical properties and is cost-effective and economical.

INTRODUCTION:
Periodontitis is one of the most commonly occurring diseases in the oral cavity that involves the periodontal tissues and its response to bacterial populations based on the immune response of the host[1]. It is the acute or chronic inflammation of the periodontium surrounding the tooth that leads to bone resorption, and infra bony defects and can eventually lead to tooth resorption if not treated. An effective management of the condition is required in order to improve the overall health and quality of life of the patient. The main aim of regenerative periodontal therapy is to regenerate the functional attachment of tissues that are destroyed by the disease, namely, the cementum, the alveolar bone and the periodontal ligament[3]. Guided tissue regeneration or GTR is a predictable and effective surgical treatment method for the regeneration and treatment of periodontal defects[4]. It aids in the creation of space around the diseased area by the placement of a non-resorbable or resorbable membrane that allows the alveolar bone and periodontal ligament cells to proliferate and cover the defects. It also aids in preventing the epithelial cells of the gingiva from migrating into the bony defect site[5]. There have been many innovations in the field of guided

bone and tissue regeneration including polytetrafluoroethylene (PTFE) which is a non resorbable membrane that is bioinert and non-reactive chemically. However, it is not cost-effective and due to its non-resorbable nature requires a second procedure to remove the membrane which can lead to an increased rate of failure of the procedure[6]. Resorbable membranes such as collagen membranes are widely used in periodontal bony repairs. However, collagen membranes can potentially cause an inflammatory response in the host tissue[7].

Hyaluronic acid (HA) is a nonsufated anionic glycosaminoglycan widely used in the medical field for faster wound healing and cosmetic procedures. It is also used in orthopedic and ophthalmic surgeries[8]. HA has a wide range of regenerative and bone healing properties and also facilitates the cell migration and differentiation in the stage of formation of tissues and their repair[9]. It has bone induction properties and increases bone formation by osteoblastic cells by increased differentiation and migration of mesenchymal cells. HA aids in both hard and soft tissue repair and decreases the possibility of bacterial contamination of surgical sites in guided tissue regeneration surgeries because of its bacteriostatic and bactericidal action[10]. HA has the ability to induce angiogenesis, promote cell migration, adhesion and proliferation which helps in regeneration of the defects[11]. Previous studies indicate the ability of HA to promote clinical attachment gain when used in treating infrabony defects of the periodontium as an adjuvant[12]. Calcium hydroxide is an odorless white powder that exhibits potent antibacterial properties by the damage of protein, cytoplasmic membranes and DNA via release of reactive hydroxyl ions in aqueous fluid. Several studies have shown the effects of calcium hydroxide in various types of bony defects[13]. It is also easily available and is proven to have antimicrobial and hard tissue formation properties. Calcium hydroxide tends to increase the grouping, migration and proliferation of stem cells of the periodontal ligament and also promotes cementogenesis and remineralisation of bone. However some studies contradict these observations due to the dose dependent effect of calcium hydroxide[14].

Hydroxyapatite is a calcium phosphate apatite that has been used as graft material with great success[15]. Hydroxyapatite tends to show high bioactivity levels and forms a fast bond with bone. Previous studies indicate that the bioactivity of hydroxyapatite is attributed to its ability to concentrate activated fibronectin on its surface[16]. Hydroxyapatite is generally available in a granular form or in a porous, dense block form. It is biocompatible and does not cause any foreign body reaction in the host tissues[17]. For a long time, eggshell formulations have been used as mineral-supplying agents and as trace elements[18]. Bone healing in rats has been observed on usage of eggshell powder. Previous studies show the efficacy of eggshell power that has been surface modified as having osteoconductive properties and indicate its utility as a bone-filling material with advantages in bone regeneration[19]. Literature shows that the mechanical and material properties of eggshell-derived hydroxyapatite (EHA) is better in comparison with graft materials that are commercially available[20]. Histological studies show the good new bone formation property of EHA[21]. In addition, the preparation of hydroxyapatite from eggshell waste is both cost-effective and economical[19]. Thus in this study, we are assessing the properties of GTR membrane fabricated with hyaluronic acid, calcium hydroxide and eggshell derived hydroxyapatite.

## MATERIALS AND METHOD:

### FABRICATION OF EGGSHELL DERIVED HYDROXYAPATITE:

Egg shells were collected and heated at 900-degree celsius in a box furnace to induce calcification and decompose the organic material in order for the conversion of eggshells into calcium hydroxide after exposure to air. The product was ground finely in a mortar and pestle. 0.3M suspension was formed with calcium hydroxide and distilled water. 0.5M diammonium hydrogen phosphate solution was added to the mixture. Irradiation of the mixture was done in a microwave oven and then repeatedly washed with distilled water for the removal of unwanted ions. It was dried in an oven at 100 degrees Celsius overnight to obtain EHA.

### GTR MEMBRANE FABRICATION:

The fabrication of the GTR membrane involved dissolution of 0.5g of hyaluronic acid and 2.5g of gelatin in 50ml of distilled water. Nitric acid was added drop by drop till the carbon sediments formed. The carbon sediments were the filtered using a filter paper. Phosphoric acid was added followed by ammonia until the pH of the solution turned 10. 250mg of 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 146mg N-Hydroxysuccinimide (NHS) were added to the mixture at the temperature of 40 degree celcius. The

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mixture was allowed to stir using a magnetic mixture until a homogeneous solution was obtained. The mixture was then divided into 3 parts labeled A, B and C and kept in separate beakers. 0.5% EHA was added to beaker A, 1% EHA was added to beaker B and beaker C was kept as a control. The solutions were poured into petri dishes of film thickness 2mm and incubated for 48 hours. The obtained membrane was then characterized.

CHARACTERIZATION OF GTR MEMBRANES:

The membranes were cut into 2x2cm and 2x4cm dimensions for testing. 2x2cm strips were used for checking the Contact angle using the contact angle goniometer and for Fourier transform infrared testing (FTIR). Tensile strength was assessed on the 2x4cm membranes using INSTRON Universal Testing Machine E-3000.

RESULTS:

1. CONTACT ANGLE:

0.5% EGG SHELL DERIVED HYDROXYAPATITE:

| FRAME NUMBER | TIME (s) | LEFT ANGLE (°) | RIGHT ANGLE (°) | AVERAGE ANGLE (°) | LEFT CONTACT POINT (Pixel) | PGHT CONTACT POINT (Pixel) | DROPLET WIDTH (Pixels) |
|---|---|---|---|---|---|---|---|
| 0.0 | 0.0 | 72.95 | 63.29 | 68.12 | 820.6 | 1210.0 | 389.4 |

Table1: Contact angle analysis of 0.5% EHA in HA Gelatin membrane


Figure1: Contact angle analysis of 0.5% EHA in HA Gelatin membrane

1% EGG SHELL DERIVED HYDROXYAPATITIE:

| FRAME NUMBER | TIME (s) | LEFT ANGLE (°) | RIGHT ANGLE (°) | AVERAGE ANGLE (°) | LEFT CONTACT POINT (Pixel) | PGHT CONTACT POINT (Pixel) | DROPLET WIDTH (Pixels) |
|---|---|---|---|---|---|---|---|
| 0.0 | 0.0 | 72.7 | 69.4 | 71.05 | 780.5 | 1151.7 | 371.2 |

Table 2: Contact angle analysis of 1% EHA in HA Gelatin membrane

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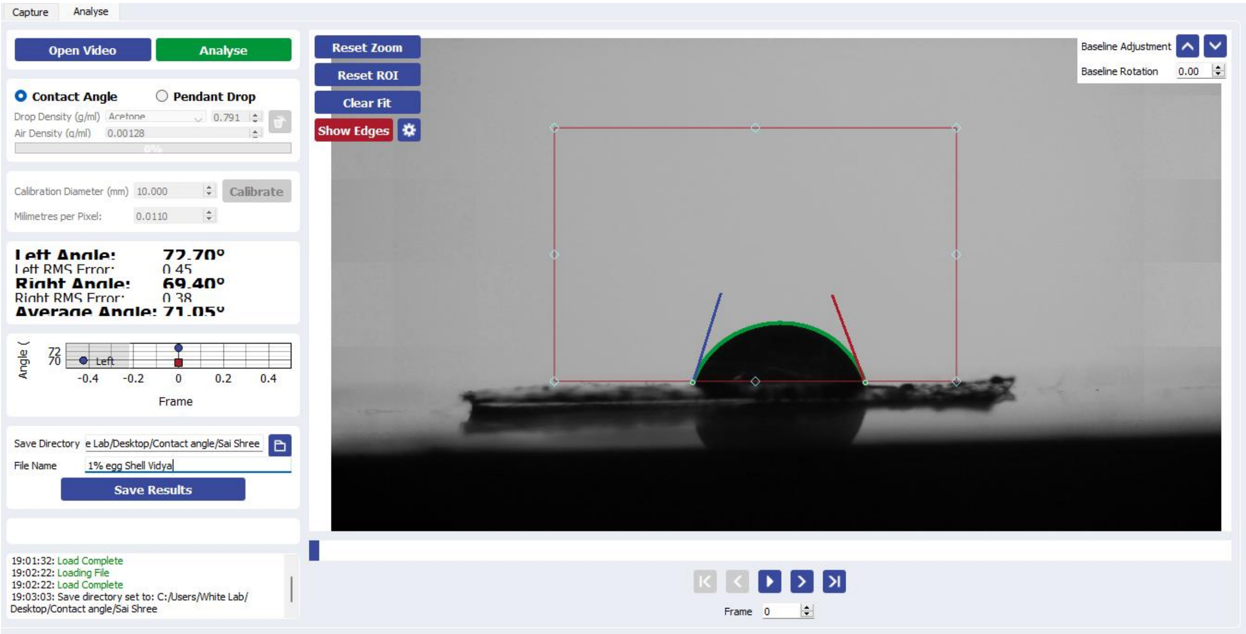


Figure 32: Contact angle analysis of 1% EHA in HA Gelatin membrane

CONTROL:

| FRAME NUMBER | TIME (s) | LEFT ANGLE (°) | RIGHT ANGLE (°) | AVERAGE ANGLE (°) | LEFT CONTACT POINT (Pixel) | PGHT CONTACT POINT (Pixel) | DROPLET WIDTH (Pixels) |
|---|---|---|---|---|---|---|---|
| 0.0 | 0.0 | 68.74 | 71.28 | 70.01 | 699.0 | 1040.7 | 371.7 |

Table 3: Contact angle Analysis of HA Gelatin control membrane

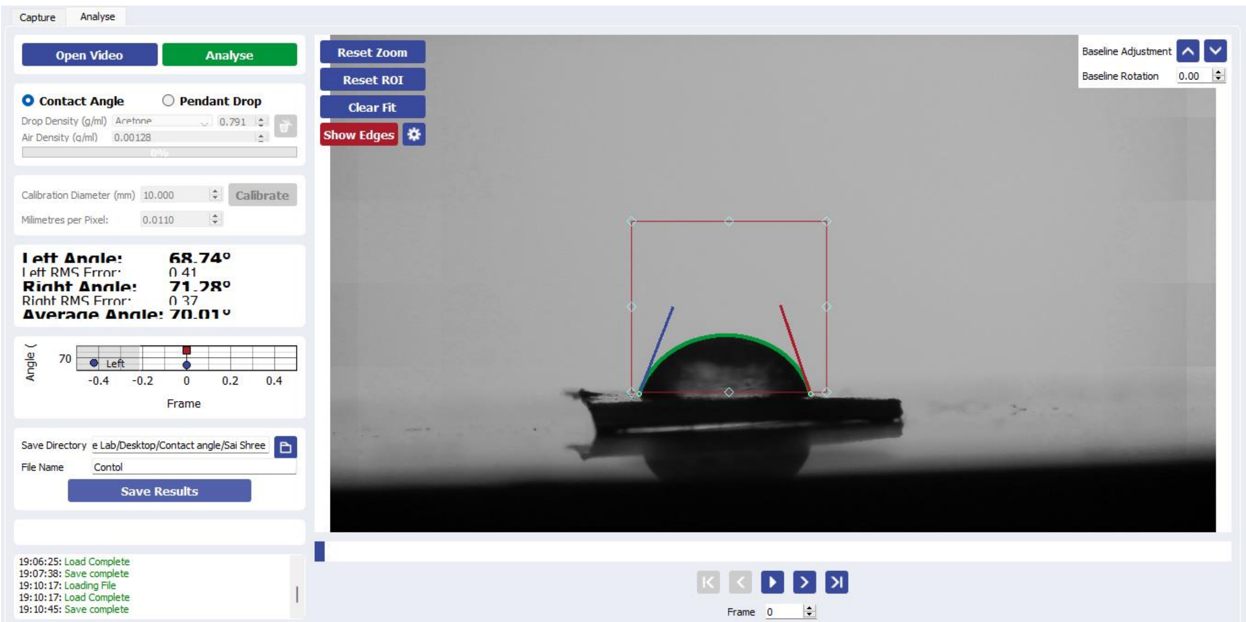


Figure 3: Contact angle Analysis of HA Gelatin control membrane

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2. FTIR:

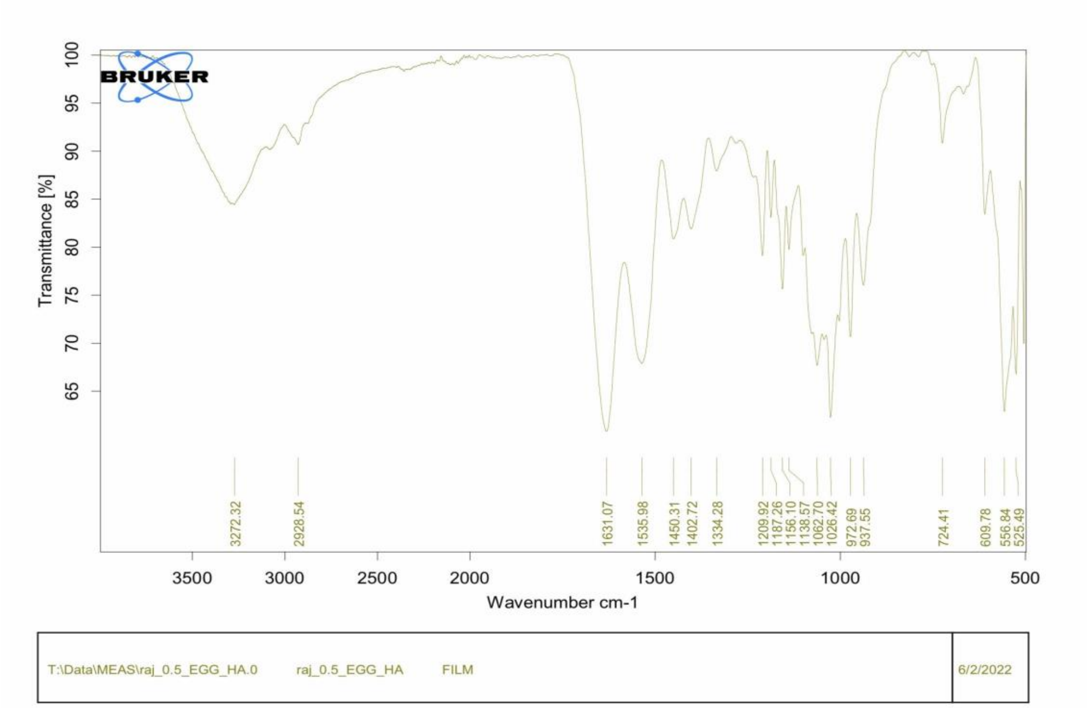


Figure 4: FTIR analysis of the 0.5% EHA-HA Gelatin membrane

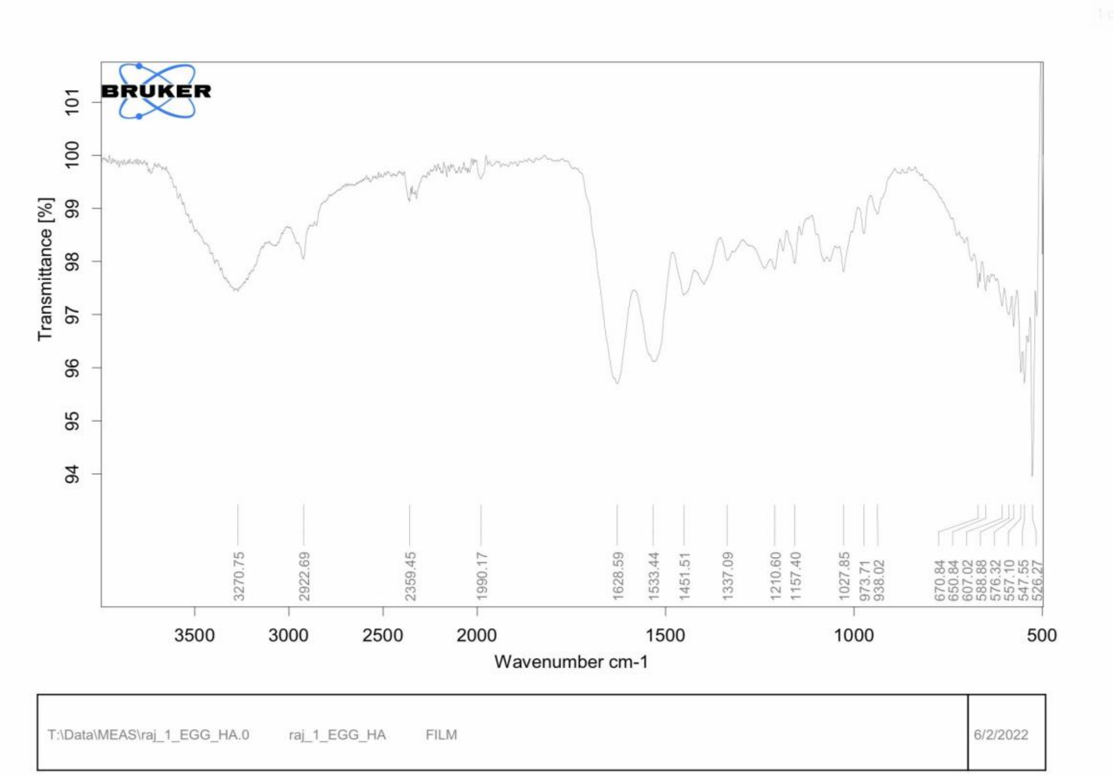


Figure 5: FTIR analysis of the 1.0% EHA-HA Gelatin membrane

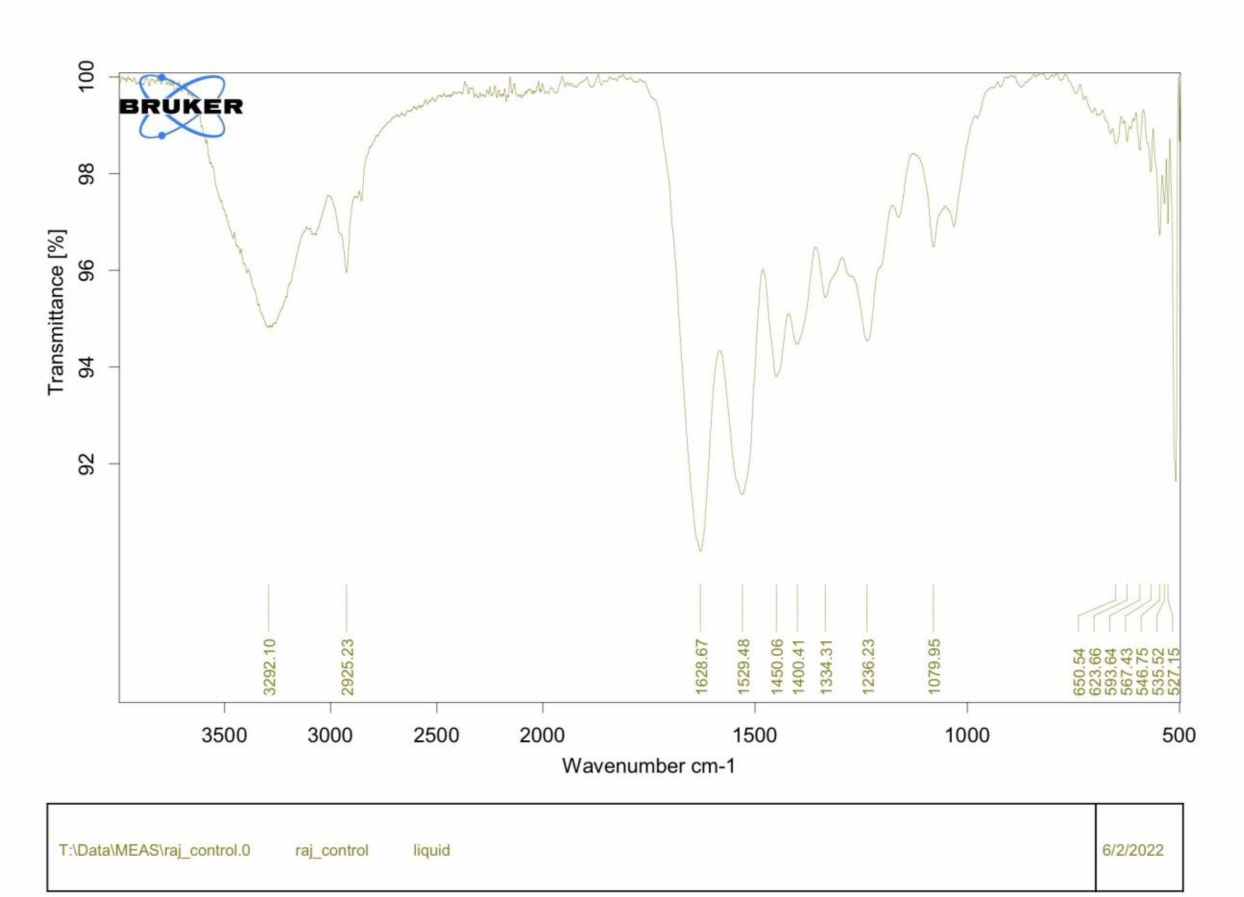


Figure 6: FTIR analysis of the HA Gelatin control

3. TENSILE STRENGTH:

| SPECIMEN LABEL | TENSILE STRENGTH AT BREAK (STANDARD) (MPa) |
|---|---|
| 1% Egg shell | 41.07 |
| 0.5% Egg shell | 77.29 |
| Control | 48.60 |

Table 4: Tensile strength analysis of control and samples

4. SEM ANALYSIS:

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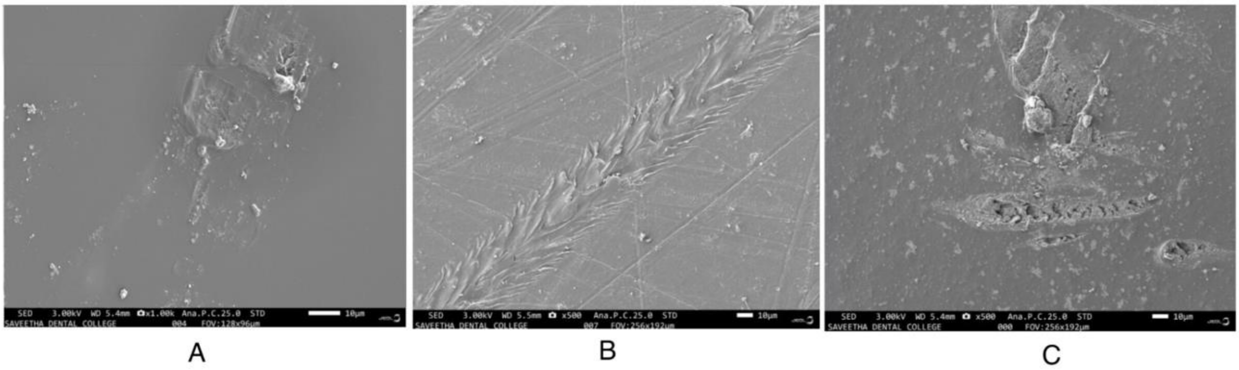

Figure 7: SEM analysis images of A: HA Gelatin control membrane; B: 0.5% EHA-HA Gelatin control membrane; C: 1.0% EHA-HA Gelatin control membrane

DISCUSSION:

The above study shows the fabrication of hyaluronic acid, calcium hydride and eggshell derived GTR membrane and characterisation of its physical properties including contact angle, tensile strength, FTIR and scanning electron microscopy analyses. Table 1, 2 and 3 show the contact angle of the samples. The average contact angle of the control sample is 70.01°. 0.5% EHA GTR membrane shows an average contact angle of 68.12° and 1% EHA GTR membrane shows an average value of 71.05°. In previous invitro and invivo studies,, it was found that hydrophilic surfaces promote osteoblast cell adhesion, differentiation and bone formation in the early stage[22]. Both epithelial and fibroblastic movements should be inhibited by GTR membranes. GTR membranes should also promote migration of osteoblasts. Invivo studies on rats show that increased hydrophilicity of GTR membranes tends to increase the fibroblast attachment on the membrane[23]. Table 4 shows the tensile strength of the sample. Contact angle is also inversely proportional to wettability[24]. Wettability is required for a certain amount in order to transport nutrients to the areas requiring regeneration. However, high levels of wettability can compromise the stiffness of the membrane and can cause easier tearing of the membrane. In our study it was seen that the fabricated samples showed similar contact angles to the control.

FTIR analysis was used to assess the functional groups present in the sample. Figure 5 shows the FTIR spectra of 0.5% EHA-HA gelatin membrane where the hydroxyapatite peaks were found to be around 1026.42 cm-1, the peak for NH group of hydroxyapatite was around 3272.32 cm-1, for CH group at 2928.54 cm-1 and C=O at 1631.07 cm-1. For the 1% EHA-HA gelatin sample the hydroxyapatite peaks were around 1027.85 cm-1, NH group peak was around 3270.75 cm-1, CH group peak was around 2922.68 cm-1 and C=O peak was seen to be around 1628.59 cm-1 (Figure 6). All the peaks of EHA were assessed on the FTIR spectrum of EHA-HA gelatin membrane which indicates the successful mediation of EHA into the sample. Based on the tensile strength, 0.5% EHA membrane shows significantly higher tensile strength (77.29 MPa) when compared to the control (48.60) and 1% EHA sample (41.07) (Table 4). SEM analysis (Figure 7) shows the electron microscopy pictures of the samples. A is seen to have a smooth surface with minimal surface irregularities under 10k x magnifications (scale bar= 10µm). B for 0.5% EHA-HA gelatin membrane showed embedded amorphous particles in the membrane and C with 1% EHA showed tightly packed amorphous particles in the membrane.

Egg shell derived hydroxyapatite has been a subject of interest in the research field due to its economical nature, cost efficiency and ease of access. Previous literature indicates the use of egg shell derived hydroxyapatite in the field of regenerative periodontics. EHA is seen as a viable regenerative material due to its biocompatibility, ease of use and lack of disease transfer risks. EHA added at the right amount is hydrophilic in nature and is absorbed by the blood and other body fluids thus increasing the ease of handling. In a study by Kattimani et al., it was seen that EHA showed a higher bone density in osseous defect sites when compared to bovine derived hydroxyapatite[19,25]. Further research also showed that EHA showed high bone regeneration rates comparable to that of synthetic hydroxyapatite[21]. In a similar study by Wardhana et al., it was seen that EHA promoted complete bone regeneration in periodontal defects and also resulted in increased bone density similar to that of synthetic hydroxyapatite in the grafted area[26]. When EHA was placed in extraction sites, increased density of bone with a trabecular bone pattern was observed within 3 months. There was reduction in the bone depth during the follow up period and the area showed better bone regeneration without any infections or inflammatory reactions[27]. Research by Kavarthapu et al. with EHA as a graft and membrane material showed it to be a good interposition material which discouraged epithelial migration and aided in neovascularization and osteoblastic proliferation[28]. Thus, from this study it can be inferred that egg shell derived hydroxyapatite apatite is an effective material for regeneration therapy and showed better physical properties when compared with the control sample.

CONCLUSION:

Guided tissue regeneration has been a subject of interest in the field of periodontal therapy for the past years. There have been various alterations of similar HA gelatin membranes with the addition of different materials. Eggshell derived hydroxyapatite is a versatile and novel bone graft substitute that showed promising results within the study limitations. It is a safe synthetic graft substitute that exhibits good physical properties and is cost effective and economic. Bone formation assays can be further done to assess the osteogenic and osteoconductive properties of EHA. Further research on this material can aid in development of newer and superior graft materials which can help in the formulation of better treatment modalities.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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REFERENCES:

1. Periodontitis [Internet]. 2020. Available from: http://dx.doi.org/10.3390/books978-3-03943-508-1
2. Bontemps L. Diagnostic difficulty between severe chronic periodontitis and generalized aggressive periodontitis among adults [Internet]. Available from: http://dx.doi.org/10.26226/morressier.5ac3831f2afeeb00097a4135
3. Susin C, Wikesjö UME. Regenerative periodontal therapy: 30 years of lessons learned and unlearned [Internet]. Vol. 62, Periodontology 2000. 2013. p. 232–42. Available from: http://dx.doi.org/10.1111/prd.12003
5. Cortellini P, Tonetti MS. Focus on intrabony defects: guided tissue regeneration [Internet]. Vol. 22, Periodontology 2000. 2000. p. 104–32. Available from: http://dx.doi.org/10.1034/j.1600-0757.2000.2220108.x
6. Dibart S. Guided Tissue Regeneration [Internet]. Practical Periodontal Plastic Surgery. p. 45–8. Available from: http://dx.doi.org/10.1002/9780470344637.ch9
7. Mussel-inspired antimicrobial coating on PTFE barrier membranes for guided tissue regeneration [Internet]. Available from: http://dx.doi.org/10.37473/dac/10.1088/1748-605x/abf27e
8. Wang HL, Modarressi M, Fu JH. Utilizing collagen membranes for guided tissue regeneration-based root coverage [Internet]. Vol. 59, Periodontology 2000. 2012. p. 140–57. Available from: http://dx.doi.org/10.1111/j.1600-0757.2011.00438.x
9. Gielen AMC, Ankone M, Grijpma DW, Poot AA. Hybrid Networks of Hyaluronic Acid and Poly(trimethylene carbonate) for Tissue Regeneration. Biomacromolecules [Internet]. 2022 Nov 23; Available from: http://dx.doi.org/10.1021/acs.biomac.2c00861
10. Kaya ÖA, Muğlali M. THE USE OF HYALURONIC ACID HYDROGELS FOR TISSUE REGENERATION IN ORAL SURGERY: A REVIEW [Internet]. Vol. 26, Atatürk Üniversitesi Diş Hekimliği Fakültesi Dergisi. 2016. Available from: http://dx.doi.org/10.17567/dfd.02364
11. Lai S, Novillo F, Cárdenas G, Verdugo F, Rada G. Hyaluronic acid for tissue and bone regeneration

after tooth extraction. Systematic review and meta-analysis [Internet]. Available from: http://dx.doi.org/10.1101/2020.06.02.20120733

12. Fallacara A, Durini E, Vertuani S, Manfredini S. Hyaluronic Acid Fillers in Soft Tissue Regeneration [Internet]. Vol. 33, Facial Plastic Surgery. 2017. p. 087–96. Available from: http://dx.doi.org/10.1055/s-0036-1597685
13. Yang R, Tan L, Cen L, Zhang Z. An injectable scaffold based on crosslinked hyaluronic acid gel for tissue regeneration [Internet]. Vol. 6, RSC Advances. 2016. p. 16838–50. Available from: http://dx.doi.org/10.1039/c5ra27870h
14. Yang L, He X, Jing G, Wang H, Niu J, Qian Y, et al. Layered Double Hydroxide Nanoparticles with Osteogenic Effects as miRNA Carriers to Synergistically Promote Osteogenesis of MSCs. ACS Appl Mater Interfaces [Internet]. 2021 Oct 20;13(41):48386–402. Available from: http://dx.doi.org/10.1021/acsami.1c14382
15. Fang CH, Sun CK, Lin YW, Hung MC, Lin HY, Li CH, et al. Metformin-Incorporated Gelatin/Nano-Hydroxyapatite Scaffolds Promotes Bone Regeneration in Critical Size Rat Alveolar Bone Defect Model. Int J Mol Sci [Internet]. 2022 Jan 5;23(1). Available from: http://dx.doi.org/10.3390/ijms23010558
16. Elkayar A, Elshazly Y, Assaad M. Properties of Hydroxyapatite from Bovine Teeth [Internet]. Vol. 2, Bone and Tissue Regeneration Insights. 2009. p. BTRI.S3728. Available from: http://dx.doi.org/10.4137/btri.s3728
17. Asefnejad A, Behnamghader A, Khorasani MT. Preparation of Polyurethane / Hydroxyapatite Composite Scaffold for Tissue Regeneration [Internet]. Vol. 7, Mechatronic Systems and Control. 2010. Available from: http://dx.doi.org/10.2316/j.2010.216.680-0114
18. Chalisserry EP, Nam SY, Anil S. Effect of Silicone-Doped Hydroxyapatite on Bone Regeneration and Osseointegration: A Systematic Review [Internet]. Vol. 7, Journal of Biomaterials and Tissue Engineering. 2017. p. 1209–18. Available from: http://dx.doi.org/10.1166/jbt.2017.1689
19. Asadipour K, Nezafati N, Nourbakhsh MS, Hafezi-Ardakani M, Bohlooli S. Characterization and biological properties of a novel synthesized silicon-substituted hydroxyapatite derived from eggshell. Int J Artif Organs [Internet]. 2019 Feb;42(2):95–108. Available from: http://dx.doi.org/10.1177/0391398818806159
20. Vivekanand K, Krishna Prasad L, Chakravarthi P, Anil M, Hemal K, Sampathkumar T, et al. Egg shell derived hydroxyapatite as bone graft material in maxillofacial skeleton � A randomized blind multi-center clinical trial [Internet]. Vol. 4, Frontiers in Bioengineering and Biotechnology. 2016. Available from: http://dx.doi.org/10.3389/conf.fbioe.2016.01.01893
21. E N, Niranjana E, Arun S, Ganesh S, Venkatesh N, Mohammed Abbas Sherif J. Enhanced Mechanical Strength Of Egg Shell Based Hydroxyapatite (Ha) In Polymer Matrix As Potential Tissue Scaffold Application [Internet]. Vol. 7, International Journal of Applied Chemistry. 2020. p. 15–7. Available from: http://dx.doi.org/10.14445/23939133/ijac-v7i1p104
22. Kattimani V. Comparative histomorphometric evaluation of synthetic and egg-shell derived hydroxyapatite for bone regeneration in Wistar rats [Internet]. Vol. 51, International Journal of Oral and Maxillofacial Surgery. 2022. p. e14. Available from: http://dx.doi.org/10.1016/j.ijom.2022.03.049
23. Chan M, Ng S. Effect of membrane properties on contact angle [Internet]. AIP Conference Proceedings. 2018. Available from: http://dx.doi.org/10.1063/1.5055437
24. Unsal B, Kurtiş B, Ozcan G, Ozdemir A, Karaöz E. An investigation of resorption and tissue reaction after subcutaneous implantation of collagen based membrane materials in rats. J Marmara Univ Dent Fac [Internet]. 1997 Sep;2(4):609–15. Available from: https://www.ncbi.nlm.nih.gov/pubmed/9569786
25. Hebbar RS, Isloor AM, Ismail AF. Contact Angle Measurements [Internet]. Membrane Characterization. 2017. p. 219–55. Available from: http://dx.doi.org/10.1016/b978-0-444-63776-5.00012-7
26. Kattimani V, Lingamaneni KP, Chakravarthi PS, Sampath Kumar TS, Siddharthan A. Eggshell-Derived Hydroxyapatite [Internet]. Vol. 27, Journal of Craniofacial Surgery. 2016. p. 112–7. Available from: http://dx.doi.org/10.1097/scs.0000000000002288
27. Wardhana HA, Idulhaq M, Saputra RD, Ermawan R, Roshada MF. Potential Use of Eggshell as Bone

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www.jchr.org
JCHR (2024) 14(2), 1573-1582 | ISSN:2251-6727


Graft Compared with Bovine for Bone Defect: A Systematic Review Study [Internet]. Vol. 9, Open Access Macedonian Journal of Medical Sciences. 2021. p. 470–3. Available from: http://dx.doi.org/10.3889/oamjms.2021.7103

28. Kattimani V, Lingamaneni KP, Yalamanchili S, Mupparapu M. Use of eggshell-derived nano-hydroxyapatite as novel bone graft substitute-A randomized controlled clinical study. J Biomater Appl [Internet]. 2019 Oct;34(4):597–614. Available from: http://dx.doi.org/10.1177/0885328219863311
29. Kavarthapu A, Malaiappan S. Comparative evaluation of demineralized bone matrix and type II collagen membrane versus eggshell powder as a graft material and membrane in rat model [Internet]. Vol. 30, Indian Journal of Dental Research. 2019. p. 877. Available from: http://dx.doi.org/10.4103/ijdr.ijdr_489_17