PREPARATION OF BI- PHASIC SCAFFOLD WITH CHITOSAN,GELATIN & BMP-2 PROTEIN AND EVALUATION OF TENSILE STRENGTH ALONG WITH CHARACTERISATION USING SEM ANALYSIS.

Running title: preparation and characterisation of bi- phasic scaffold of Chitosan, gelatin and BMP-2 protein.

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Abstract:

Aim: The aim of the study was to develop a bi-phasic scaffold using the Chitosan, gelatin and BMP-2 protein and evaluation of the tensile strength and the scanning electron microscopic analysis of the scaffold.

Materials and methods:

The scaffold has been prepared by addition of 50 ml of acetic acid to 250 microlitre of De-ionized water to this solution 1 gram of Chitosan is added. The mixture of 5 grams of gelatin powder to 50 ml of deionized water and heating the solution in a 65 degree water bath for 30 minutes leading to dissolution of gelatin in the water. Then the solutions are mixed and then the gel is dried at 35 degrees for 24 hours for the scaffold formation. Then the scaffold is tested for the tea isle strength as well as the SEM analysis is done.

Discussion: Tensile strength test reveals that the Chitosan and gelatin concentration and composition can tolerate the strength above 1 MPa , hence the combination of Chitosan and gelatin scaffold has the stable tensile strength. The scanning electron microscopy test has revealed that spread well on both scaffolds, the fibers roughness is maintained and the spread is even in the SEM analysis.

Conclusion: The bi- phasic scaffold prepared by using Chitosan, gelatin and BMP-2 based scaffold shows good tensile strength and SEM analysis has even spread of the components.

Introduction:

The dental root cementum, the periodontal ligament (PDL), the gingiva with the dentogingival sulcus, and the alveolar bone make up the multi-tissue system known as the periodontal apparatus, which serves as an anchor for teeth to the jaw bones (19). Periodontal tissues are gradually disrupted by chronic periodontitis, which ultimately results in tooth loss. According to Richards (2014), severe periodontitis affects 11% of people worldwide and has a negative impact on public health.

In order to stop the inflammation, current therapies include local debridement using dental scaling and root planing. These procedures frequently cause the fibrin clot to become less adherent to the root surface and cause epithelial cells, which multiply more quickly than mesenchymal cells, to colonize the deficiency(Rödenbeck et al. 2023) (20). In order for the cementum or PDL fibers to form, a "long junctional epithelium" must first be created, which prevents multi-tissue periodontal repair (1).

The goal of regenerative periodontal treatment (RPT) is the morphological and functional regeneration of several tissues. RPT treatments include surgical approaches for wide defects, like open flap debridement paired with grafts or membranes (2). Infection and dehiscence at the membrane or graft site, as well as periodontal attachment, are the main challenges of RPT because it is difficult to promote perpendicular and oblique fiber insertion from the cementum into newly formed alveolar bone. Wound compartmentalization is the primary goal of RPT (22).(Mani et al. 2021)

A naturally occurring polysaccharide is chitosan. Its units are made up of a mixture of N-acetyl-D-glucosamine (acetylated unit) and -(1-4)-linked D-glucosamine (deacetylated unit) (3). Because of its superior biocompatibility, biodegradability, nontoxicity, bioactivity, and multifunctional groups, chitosan has been studied as a natural cationic biopolymer(Kumar et al. 2021) (4). Due to a variety of fantastic qualities it contains, it is also being thoroughly researched for food packaging film, bone substitutes, artificial skin, biomedical applications, and pH sensitive drug delivery, among other uses (15).

Natural collagen is partially hydrolyzed to form a mixture of peptides and proteins called gelatin (5). One of its remarkable characteristics is the carboxyl groups on its chain backbones, which give it the potential to create hydrogen bonds with chitosan for a well-mixed hybrid (17). Also, the fact that it is utilized in the production of cosmetics and pharmaceuticals, among other things, demonstrates its complete safety for human consumption(Jayan et al. 2020) (7). This explains why gelatin is preferred in research work because any adverse effects from substances created using it as a precursor can be minimized (16). In this study, to conduct hemostatic and cell barrier functions, we created multifunctional scaffolds with sandwich-like structures as GTR membranes (6).

Materials and methods:

Bi-phasic scaffold preparation: The scaffold preparation begins with the dissolution of 1 gramme of commercially available chitosan. The dissolving is carried out in a solution that is made by combining 250 microliters of deionized water with 50 milliliters of acetic acid. Then, 5 grams of commercial gelatin powder are added to 50 ml of deionized water separately. The mixture is then cooked in a 65 degree water bath for 30 minutes, causing the gelatin powder to thoroughly dissolve in the water.

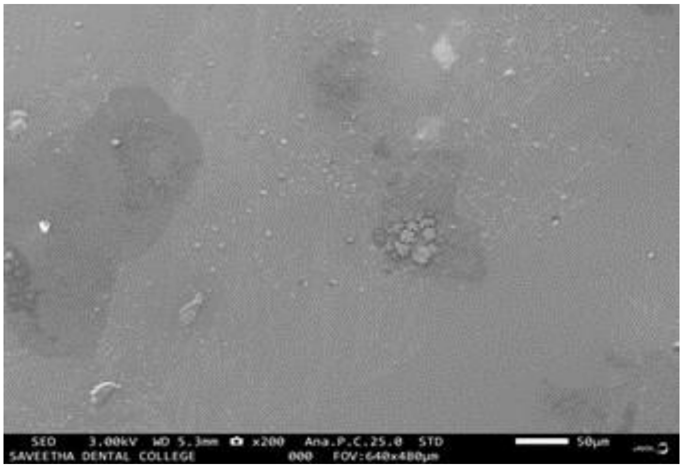
The two distinct solutions are combined, a gel is prepared, and the gel is then dried at 35 degrees for 24 hours in a hot air oven in order to build a scaffold.

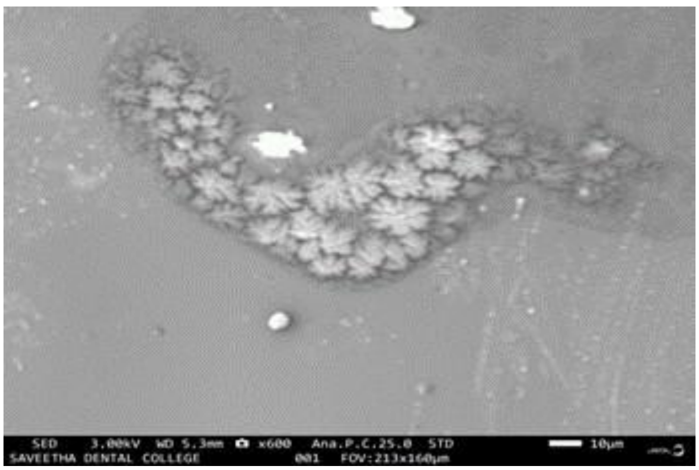
SEM analysis: The morphology of multilayer scaffolds was examined using a scanning electron microscope (SEM) (Quanta 200, FEI, US). Using ImageJ software v1.52e (NIH, Wisconsin, US) to scale SEM images, fiber diameters (n = 12) and pore sizes (n = 30) were measured. The thickness of multilayer scaffolds was determined using thickness gauge (n = 6).

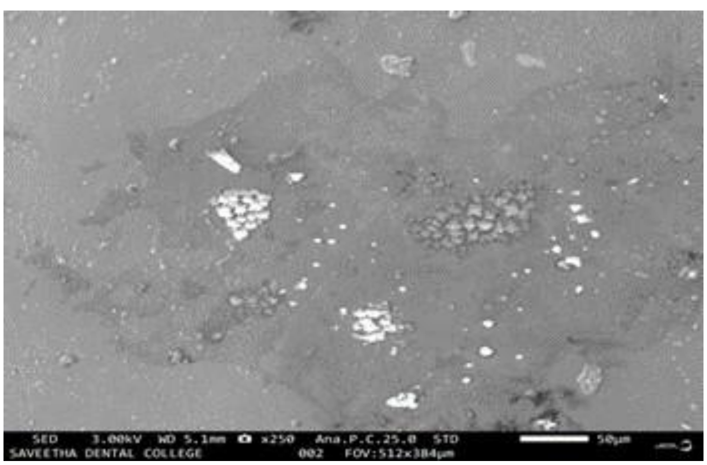
Tensile strength: The scaffolds' tensile mechanical qualities were evaluated in accordance with the previously described approach [25]. The samples were divided into pieces measuring 30 mm by 10 mm, placed in PBS, and tested using an Instron tensile testing machine (Norwood, Massachusetts, US) with a load of 100 N at a rate of 10 mm per second. In addition to reporting the stress-strain curve, the maximum stress and elongation at break were determined (n = 6).

Results:

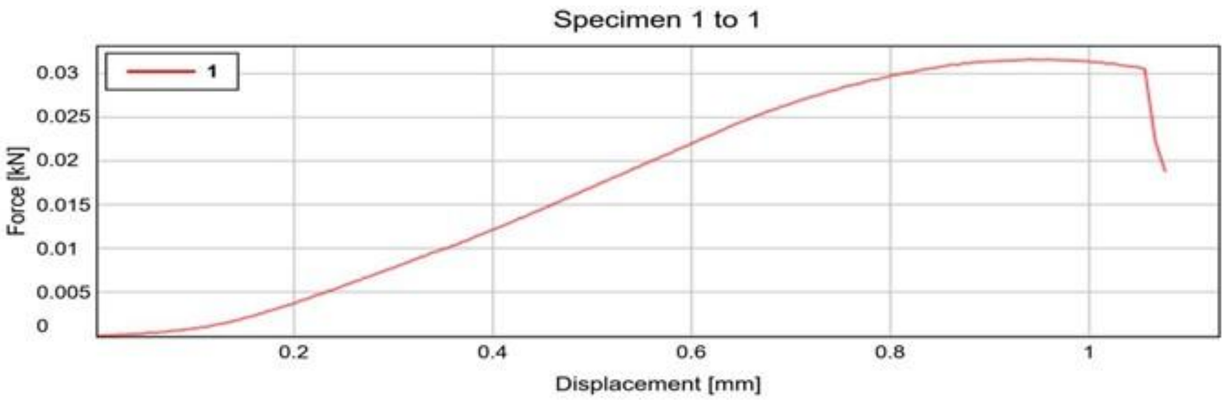
An analysis of the biphasic scaffold's characteristics:
SEM was used to examine the topography of multilayer scaffolds, as illustrated. All fiber ratios were uniformly smooth, dense, and randomly oriented (9). Bi-phasic scaffolds were around 1 mm thick (8). Cross-sectional views showed that the bi-phasic scaffold was thicker. Gelatin and freeze-dried chitosan were used to create a porous connection between two layers. The mixture of multilayers displayed a good sandwich-like structure since it was homogeneous and well packed (10).







Tensile strength/mechanical characteristics: The scaffold's ability to be stretched to between 70% and 90% of its original length demonstrated that two-layer scaffolds superimposed on top of one another had better mechanical qualities than single-layer scaffolds alone (12). The results of the stress and strain testing are displayed. The Chitosan and gelatin concentrated bi-phasic scaffold can bear the strength above 1 MPa, according to a tensile strength test (15). Hence, the bi-phasic scaffold made of chitosan and gelatin exhibits a consistent tensile strength (11).



	Maximum Force [N]	Tensile stress at Tensile strength [MPa]	Tensile strain (Displacement) at Break (Standard) [%]
1	31.60	15.80	3.02

	Specimen label	Tensile stress at Break (Standard) [MPa]
1	1	15.24

Discussion:

People's quality of life is significantly impacted by periodontal disease. The use of GTR materials in clinical periodontal surgery is common (18). By promoting cell adhesion, proliferation, and differentiation, GTR techniques have been used to promote periodontal tissue regeneration [23]. Because of their good biological activity, hemostasis, and barrier function, the multifunctional Chitosan gelatin and BMP 2 integrated scaffolds were created in an effort to improve current commercial materials.

Chitosan, gelatin, and BMP 2 protein were all used in this investigation. Gelatin and chitosan fibers both displayed random fiber dispersion. When the chitosan was submerged in gelatin solution, it was able to completely saturate the space between the fibers. Chitosan and gelatin could bind closely and create a stable structure after being lyophilized. In order to resist masticatory pressures and prevent membrane collapse, GTR's mechanical qualities are crucial [25, 28]. The various groups' Young's moduli exceeded 0.25 MPa, which was considered to be appropriate for periodontal regeneration [25].

Porosity and swelling capacity play crucial roles in the flow of oxygen and nutrients inside the scaffolds. They also have significant impacts on cell migration and infiltration [23], which are helpful for the preservation of blood and growth factors. Pore sizes of the various PG fiber ratios were less than 10 m, which could create a thick barrier to thwart cell invasion. This is a perfect characteristic for periodontal GTR membranes because it promotes bone development beneath the membrane and is necessary for them to prevent soft tissue from penetrating the faulty area

[27]. The sandwich-like scaffold's porous structure might yet allow for improved swelling properties. Hydrophilic substrates were found to have an impact on cell adhesion, proliferation, and wound healing in earlier studies [29]. An essential quality is optimal hydrophilicity for the GTR membranes.

Moreover, cells (like macrophages) that build up near implant sites may provide a walling effect that encourages the breakdown of scaffolds. For instance, the body's enzymes, acidic byproducts, and free radicals may accelerate the process of breakdown [29]. The creation of new tissue can be delayed by scaffolds with slow disintegration rates because they can set off a persistent inflammatory response. To synchronize the regeneration of periodontal tissue with the rate of membrane resorption, the membranes normally need to be functional for at least 4-6 weeks (27).

Bi-phasic scaffolds containing chitosan, gelatin, and BMP-2 showed extensive characteristics overall (2). The studies support the scaffold's modification to increase its functionality and widen its range of potential applications (5). For instance, the introduction of BMP enables us to encourage the repair of alveolar bone and soft tissue. Clearly, animal models are also necessary to demonstrate the effects of our multilayer scaffolds on periodontal regeneration, and a thorough comparative research with commercial products would be appropriate in this case (9). Overall, our bi-phasic scaffold with chitosan gelatin and BMP 2 has excellent potential for further development for use in periodontal tissue regeneration (12).

Conclusion: In our study, better GTR membranes were created utilizing a bi-phasic scaffold made of chitosan and gelatin. The findings of the experiments showed that the bi-phasic scaffolds had favorable bioactivity and readily controllable characteristics by varying the proportion of chitosan and gelatin. The scaffold made of Chitosan and gelatin has a higher tensile strength. Gelatin and chitosan scaffolds with filaments and small pores may function as efficient barriers to stop cell infiltration. Animal models will be required in future experimental studies to examine the tissue regeneration capabilities of scaffolds in vivo. In conclusion, our bi-phasic scaffold made of chitosan and gelatin might offer adequate qualities for periodontal regeneration.

References:

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2. D.F. Kinane, P.G. Stathopoulou, P.N. Papapanou, Periodontal diseases, Nat. Rev. Dis. Primers.3 (2017) 17038, , https://doi.org/10.1038/nrdp.2017.38.
3. I. Gotsman, C. Lotan, W.A. Soskolne, S. Rassovsky, T. Pugatsch, L. Lapidus, et al., Periodontal destruction is associated with coronary artery disease and periodontal infection with acute coronary syndrome, J. Periodontol. 78 (2007) 849–858, https://doi.org/10.1902/jop.2007.060301.

4. E.M. Varoni, S. Vijayakumar, E. Canciani, A. Cochis, L. De Nardo, G. Lodi, et al., Chitosan-based trilayer scaffold for multitissue periodontal regeneration, J. Dent. Res. 97 (2018) 303–311, https://doi.org/10.1177/0022034517736255.
5. B.H. Porras-Reyes, T.A. Mustoe, Platelet-activating factor: improvement in wound healing by a chemotactic factor, Surgery 111 (1992) 416–423.
6. M.C. Bottino, V. Thomas, G. Schmidt, Y.K. Vohra, T.M. Chu, M.J. Kowolik, et al., Recent advances in the development of GTR/GBR membranes for periodontal re- generation—a materials perspective, Dent. Mater. 28 (2012) 703–721, https://doi. org/10.1016/j.dental.2012.04.022.
7. Y.D.Rakhmatia,Y.Ayukawa,A.Furuhashi,K.Koyano,Current Barrier Membranes: titanium mesh and other membranes for guided bone regeneration in dental applications, J. Prosthodont. Res. 57 (2013) 3–14, https://doi.org/10.1016/j.jpor.2012.12.001.
8. B.L. Pihlstrom, R.B. McHugh, T.H. Oliphant, C. Ortiz-Campos, Comparison of surgical and nonsurgical treatment of periodontal disease. A review of current studies and additional results after 61/2 years, J. Clin. Periodontol. 10 (1983) 524–541.
9. M. Masoudi Rad, S. Nouri Khorasani, L. Ghasemi-Mobarakeh, M.P. Prabhakaran,M.R. Foroughi, M. Kharaziha, et al., Fabrication and characterization of two-layered nanofibrous membrane for guided bone and tissue regeneration application, Mater. Sci. Eng. C Mater. Biol. Appl. 80 (2017) 75–87, https://doi.org/10.1016/j.msec. 2017.05.125.
10. E. Zhang, C. Zhu, J. Yang, H. Sun, X. Zhang, S. Li, et al., Electrospun PDLLA/PLGA composite membranes for potential application in guided tissue regeneration, Mater. Sci. Eng. C Mater. Biol. Appl. 58 (2016) 278–285, https://doi.org/10.1016/j. msec.2015.08.032.
11. J. Lu, C. Cheng, Y.S. He, C. Lyu, Y. Wang, J. Yu, et al., Multilayered graphene hydrogel membranes for guided bone regeneration, Adv. Mater. 28 (2016) 4025–4031, https://doi.org/10.1002/adma.201505375.
12. J.M. Silva, A.R. Duarte, S.G. Caridade, C. Picart, R.L. Reis, J.F. Mano, Tailored freestanding multilayered membranes based on chitosan and alginate, Biomacromolecules 15 (2014) 3817–3826, https://doi.org/10.1021/bm501156v.
13. M.C. Bottino, V. Thomas, G.M. Janowski, A novel spatially designed and func- tionally graded electrospun membrane for periodontal regeneration, Acta Biomater. 7 (2011) 216–224, https://doi.org/10.1016/j.actbio.2010.08.019.
14. A. Farooq, M. Yar, A.S. Khan, L. Shahzadi, S.A. Siddiqi, N. Mahmood, et al., Synthesis of piroxicam loaded novel electrospun biodegradable nanocomposite scaffolds for periodontal regeneration, Mater. Sci. Eng. C Mater. 56 (2015) 104–113, https://doi.org/10.1016/j.msec.2015.06.006.
15. A. Hamlekhan, F. Moztarzadeh, M. Mozafari, M. Azami, N. Nezafati, Preparation of laminated poly(epsilon-caprolactone)-gelatin-hydroxyapatite nanocomposite scaf- fold bioengineered via compound techniques for bone substitution, Biomatter 1 (2011) 91–101, https://doi.org/10.4161/biom.1.1.17445.
16. M. Labet, W. Thielemans, Synthesis of polycaprolactone: a review, Chem. Soc. Rev. 38 (2009) 3484–3504, https://doi.org/10.1039/b820162p.

17. K. Van de Velde, P. Kiekens, Biopolymers: overview of several properties and consequences on their applications, Polym. Test. 21 (2002) 433–442, https://doi.org/10.1016/S0142-9418(01)00107-6 (doi:Pii S0142-9418(01)00107-6).
18. R.A. Gross, B. Kalra, Biodegradable polymers for the environment, Science 297 (2002) 803–807, https://doi.org/10.1126/science.297.5582.803.
19. G.A. Ward, The Science and Technology of Gelatin, Academic Press, 1977.
20. H. Jiankang, L. Dichen, L. Yaxiong, Y. Bo, Z. Hanxiang, L. Qin, et al., Preparation of chitosan-gelatin hybrid scaffolds with well-organized microstructures for hepatic tissue engineering, Acta Biomater. 5 (2009) 453–461, https://doi.org/10.1016/j.actbio.2008.07.002.
21. R.A.A. Muzzarelli, Chitins and chitosans for the repair of wounded skin, nerve,cartilage and bone, Carbohydr. Polym. 76 (2009) 167–182, https://doi.org/10.1016/j.carbpol.2008.11.002.
22. A. Di Martino, M. Sittinger, M.V. Risbud, Chitosan: a versatile biopolymer for orthopedic tissue-engineering, Biomaterials 26 (2005) 5983–5990,https://doi.org/10.1016/j.biomaterials.2005.03.016.
23. E.I. Rabea, M.E.T. Badawy, C.V. Stevens, G. Smagghe, W. Steurbaut, Chitosan as antimicrobial agent: applications and mode of action, Biomacromolecules 4 (2003)1457–1465, https://doi.org/10.1021/bm034130m.
24. I.A. Sogias, A.C. Williams, V.V. Khutoryanskiy, Why is chitosan mucoadhesive?Biomacromolecules 9 (2008) 1837–1842.
25. V.T. Tchemtchoua, G. Atanasova, A. Aqil, P. Filee, N. Garbacki, O. Vanhooteghem,et al., Development of a chitosan nanofibrillar scaffold for skin repair and re-generation, Biomacromolecules 12 (2011) 3194–3204.
26. M.S. Lord, B. Cheng, S.J. McCarthy, M. Jung, J.M. Whitelock, The modulation of
27. platelet adhesion and activation by chitosan through plasma and extracellular matrix proteins, Biomaterials 32 (2011) 6655–6662, https://doi.org/10.1016/j.biomaterials.2011.05.062.
28. W.G. Cui, Y. Zhou, J. Chang, Electrospun nanofibrous materials for tissue engineering and drug delivery, Sci. Technol. Adv. Mater. 11 (2010), https://doi.org/10.1088/1468-6996/11/1/014108 (Artn 014108).
29. W.J. Li, C.T. Laurencin, E.J. Caterson, R.S. Tuan, F.K. Ko, Electrospun nanofibrous structure: a novel scaffold for tissue engineering, J. Biomed. Mater. Res. 60 (2002) 613–621, https://doi.org/10.1002/jbm.10167.
30. S.B. Qasim, S. Najeeb, R.M. Delaine-Smith, A. Rawlinson, I.U. Rehman, Potential of electrospun chitosan fibers as a surface layer in functionally graded GTR membrane for periodontal regeneration, Dent. Mater. 33 (2017) 71–83, https://doi.org/10.1016/j.dental.2016.10.003.
31. K. Garg, N.A. Pullen, C.A. Oskeritzian, J.J. Ryan, G.L. Bowlin, Macrophage functional polarization (M1/M2) in response to varying fiber and pore dimensions of electrospun scaffolds, Biomaterials 34 (2013) 4439–4451, https://doi.org/10.1016/j.biomaterials.2013.02.065.

32. Jayan, Lekshmy, N. Priyadharsini, R. Ramya, and K. Rajkumar. 2020. “Evaluation of Antifungal Activity of Mint, Pomegranate and Coriander on Fluconazole-Resistant.” *Journal of Oral and Maxillofacial Pathology: JOMFP* 24 (3): 517–22.
33. Kumar, Balu Santhosh, Sridevi Krishnamoorthy, Sandhya Shanmugam, and Angambakkam Rajasekharan PradeepKumar. 2021. “The Time Taken for Retrieval of Separated Instrument and the Change in Root Canal Volume after Two Different Techniques Using CBCT: An Study.” *Indian Journal of Dental Research: Official Publication of Indian Society for Dental Research* 32 (4): 489–94.
34. Mani, Vadivel, Ramya Badrachalam, Sharon Nallathannikulam Shanmugam, Manikandan Balraj, Revathi Kasthuri, Anandhi Danavel, and Shyamaladevi Babu. 2021. “Effect of β-Caryophyllene on Insulin Resistance in Skeletal Muscle of High Fat Diet and Fructose-Induced Type-2 Diabetic Rats.” *Bioinformation* 17 (8): 741–47.
35. Rödenbeck, Maria, Olaniyi Ayobami, Tim Eckmanns, Mathias W. Pletz, Jutta Bleidorn, and Robby Markwart. 2023. “Clinical Epidemiology and Case Fatality due to Antimicrobial Resistance in Germany: A Systematic Review and Meta-Analysis, 1 January 2010 to 31 December 2021.” *Euro Surveillance: Bulletin Europeen Sur Les Maladies Transmissibles = European Communicable Disease Bulletin* 28 (20). https://doi.org/10.2807/1560-7917.ES.2023.28.20.2200672.