CHITOSAN AND ZINC OXIDE NANOPARTICLE BASED GUIDED TISSUE REGENERATION MEMBRANE PREPARATION AND ITS CHARACTERISATION

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ABSTRACT

Introduction-
A regeneration membrane is surgically implanted as part of the GTR treatment for periodontitis with the express purpose of restoring the periodontal tissues supporting the teeth. The membrane serves as a barrier to stop epithelial and gingival connective cells from developing into periodontal lesions and as a favourable niche to maximise the migration and proliferation of periodontal ligament (PDL) cells, cement oblasts, and osteoblasts within the periodontal lesions to promote the reconstruction of the supporting tissue. Due to its antibacterial properties, the FDA typically recognises zinc oxide nanoparticles as safe.

Materials & Methods-

To the plant extract of *Aloe barbadensis*, acetic acid, water, ZnO nanoparticles and chitosan were added. They were heated for an hour and then left to mix for 6 hours. To this solution, 0.15ml of glycerol was added and mixed. The solution was transferred in a petri dish and kept in the hot air oven for 24 hours at 50 degrees to form the membrane. Following membrane formation, Fourier Transform InfraRed Spectroscopy (FTIR), Tensile testing, antimicrobial activity and contact angle was compared between the chitosan control group membrane and the nanoparticle incorporated membrane.

Results -
The tensile strength of the test membrane was better than the control membrane. The contact angle of the control membrane was 77.63, whereas for the test membrane it was 72.75. The test membrane had better wettability, tensile strength and anti microbial activity as compared to the control group

Conclusion-
Zinc oxide nanoparticles embedded in the chitosan membrane for GTR purpose possess good antibacterial and physical properties. It should be used after a GTR procedure after necessary future investigations.

Keywords: chitosan membrane, zinc oxide nano particles, guided tissue regeneration

INTRODUCTION

Clinicians continue to face difficulties in the regeneration of periodontal tissues as a result of severely destructive periodontal disease, mainly periodontitis. To replace the missing cementum, alveolar bone, and periodontal ligaments (PDL), periodontists employ a variety of procedures. The outcomes, however, can vary and are unexpected. Using polymers and cells in tissue engineering (TE), novel methods for periodontal regeneration may be possible.

The most often used regeneration technique is the application of a guided tissue regeneration membrane. In order to encourage the regeneration of periodontal tissues, GTR includes placing a barrier between the connective tissues and the gingival epithelium. There are a few crucial conditions that must be met for a material to perform as an excellent GTR membrane.

The membrane must first effectively separate the periodontal tissues from the epithelium. Second, it ought to encourage vascularization and shield the underlying clot from connective tissue and epithelial growth. Finally, it must disintegrate over a predetermined length of time without producing any hazardous byproducts in order to facilitate the regeneration of periodontal tissue.

The goal of GTR is to specifically infiltrate reparative periodontal cells into the area of the lesion. The regeneration potential of the available GTR membranes in the present scenario, however, is greatly hampered by the fact that currently employed GTR membranes fail to meet all of the desired characteristics, such as bioactivity and unfavorable degradation rates.

Currently, using antibacterial nanoparticles (NPs) is a potential tactic to combat the rising prevalence of germs that are resistant to antibiotics. (1) In particular, zinc oxide nanoparticles (ZnO-NPs) have shown antibacterial action against a range of bacteria, including those that are resistant to antibiotics, and are "generally regarded as safe" by the FDA. By using the electrospinning technique, NPs can be embedded into polymeric matrices, preventing the unintended burst release of NPs into the organism and utilising the naturally high surface-area-to-volume ratio of electrospun fibrillar membranes to maintain a high surface exposure of the NPs and a high antibacterial activity.

In addition, ZnO NPs have demonstrated remarkable promise in biological applications owing to their high stability, strong biocompatibility, low cost, and less toxicity. Due to their outstanding antibacterial, antifungal, electrical, chemical, and optical capabilities, zinc oxide nanoparticles (ZnO NPS) have received a lot of attention during the past 20 years. ZnO NPs may be produced simply using a variety of methods. Recent research has shown that while ZnO NPs have non-toxic effects on human cells at the same concentration, they have cytotoxic effects on bactericidal cells. Due to their capacity for ion-shedding, ZnO NPs displayed more toxic effects on the cell of the pathogen than other metal oxide NPs such as TiO2.(2)

Despite the relatively considerable clinical success brought about by the use\age of membranes in GTR techniques, tissue regeneration is highly dependent on the lack of infection and, consequently, the correct upkeep of a bacterial-free environment (3). In order to do this, a number of controlled-release technologies, including PerioChip® and Atridox® (both doxycycline and chlorhexidine based), have been effectively employed to locally administer antimicrobial drugs to the periodontal pocket (4,5). GTR/GBR membranes have recently acquired antibacterial activity thanks to the incorporation of antimicrobial compounds(6). Despite this, it is generally known that antibiotics can have some serious side effects, particularly those that are connected to bacterial strain resistance, a problem that is currently affecting the entire world as bacteria are developing resistance to various antibiotic treatments(3,7). The preparation and evaluation of the GTR membrane, which combines the effects of zinc oxide nanoparticles with the conventional chitosan membrane, are the aim of this study.

MATERIALS AND METHODS

1.1 PREPARATION OF NANOPARTICLES:

20 mg of Azadirachta indica (Neem) plant extract was dissolved in 100 ml of distilled water and placed in the magnetic stirrer for 1 hour (as seen in Figure 1). After this it was kept in the centrifuge for 1 cycle of 10 minutes at 10,000 rpm. The resultant supernatant was taken. 20 ml of the extract was taken in a beaker to which 5.6 ml titanium isopropoxide was added. This mixture was centrifuged for 3 cycles which were of 10 minutes each at 10,000 rpm. The mixture was emptied into a glass petri plate and placed in the hot air oven at 50 degree celsius for 2 days. The resultant was ground to a fine powder using mortar pestle and stored in tubes. Zinc Oxide nanoparticles were prepared from the plant extract.

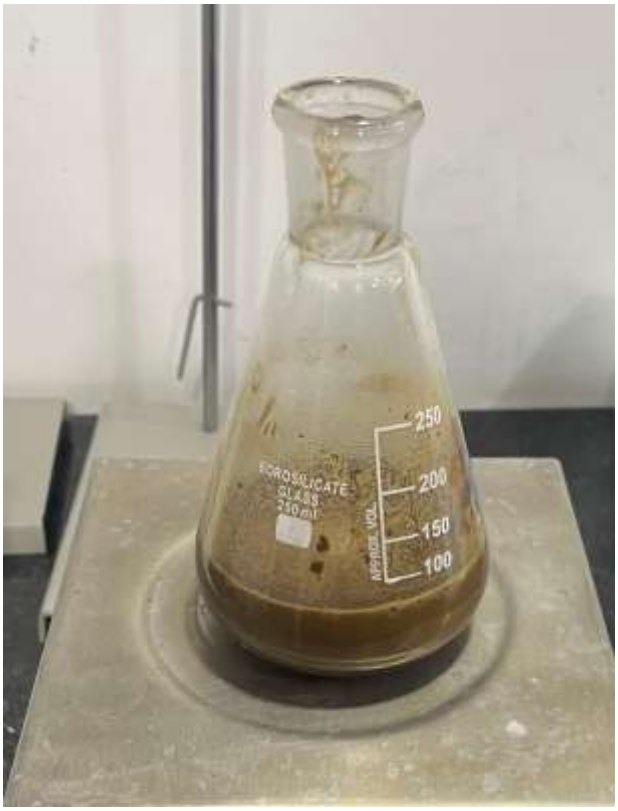


Figure 1: Depicting the preparation of the Nanoparticles from the plant extract placed on the magnetic stirrer.

1.2 PREPARATION OF CHITOSAN MEMBRANE (CONTROL GROUP)

First, Acetic acid solution was prepared containing 0.3ml of acetic acid and 29.7ml of distilled water. 0.6g of chitosan was added to the acetic acid solution and this mixture was stirred for 6 hours. After 6 hours, 0.15 ml of glycerol was added to the solution so as to obtain a clear solution and this was placed on the magnetic stirrer for 1 hour. The mixture was filtered through a muslin cloth and placed in a small glass petri dish. The plate was placed inside the hot air oven and stored

overnight at 50 degree celsius. Membrane was obtained the following day by peeling off from the glass plate.

1.3 PREPARATION OF NANOPARTICLE INCORPORATED MEMBRANE:

Similar to the chitosan membrane, Acetic Acid solution was prepared using 0.3ml of acetic acid and 29.7ml of distilled water. To this, 0.012g of nanoparticles were added and the solution was placed on a magnetic stirrer for 3 hours. After stirring, 0.588g of chitosan was added to the solution and it was stirred for 6 hours. 0.15ml of glycerol was added to the solution and stirred for one hour. The mixture was poured into a glass petri dish and placed in the hot air oven for 1 day at 50 degree celsius. Membrane incorporated with nanoparticles was obtained. (As seen in Figure 2 and Figure 3).

Figure 2: Preparation of Zinc Oxide nanoparticle membrane mixture placed on magnetic stirrer.

Figure 3: Showing the Zinc Oxide nanoparticle membrane after complete drying

1.4 NEUTRALIZATION OF MEMBRANES:

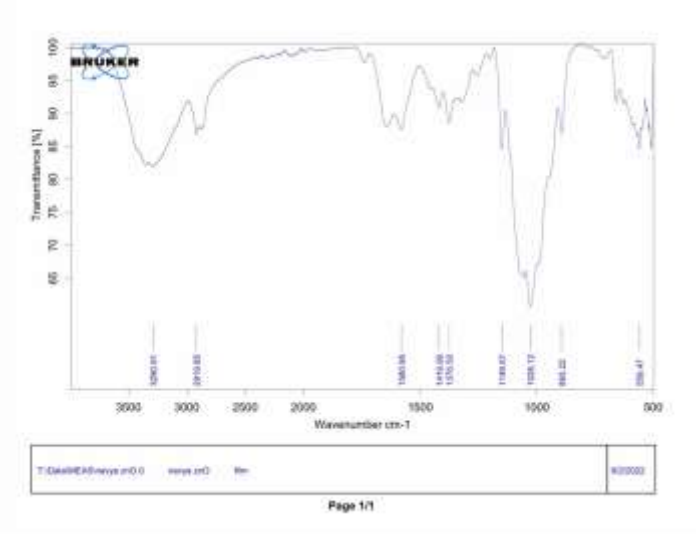
Since the membranes are present in an acidic environment, to neutralize this environment, it is placed in 50 ml of 1M NaOH solution for 2 hours. The membrane is then rinsed with tap water and kept in distilled water for 1 hour to remove any impurities. It can then be wiped with cotton and stored for further study.

1.5 CHARACTERISATION OF THE MEMBRANES:

The membranes were cut into smaller samples and tested using different parameters. FTIR (Fourier Transform InfraRed Spectroscopy), Tensile testing, antimicrobial activity and contact angle was compared between the chitosan control group membrane and the nanoparticle incorporated membrane.

RESULTS AND DISCUSSION

FTIR spectroscopy analysis was performed to analyze the molecular composition and structure of the sample and recognise the biomolecules responsible for capping of ZnO NPs synthesized using plant extract. Absorption peaks were located at 559.47 (Zn–O bond),1028.62 (C–N aliphatic amines),1580.95 (C–O stretching vibrations in alcoholic groups), 3290.91 (N–H bond) and 3360.58 (O–H stretching due to alcoholic group) as seen in Graph 1.



Graph 1: FTIR graph showing the functional groups present in the alpinia officinarum ZnONp membrane at different wavelength, confirming the activation of the functional groups

559.47 cm-1 peak - Zinc nanoparticles

NH group of chitosan = 3290.91 cm-1

CH group of chitosan = 2919.85 cm-1

C=O =1580.95 cm-1

Tensile strength was measured in MPa using INSTRON 3000. The control group has a tensile strength of 18.32 MPa. On the other hand, the membrane incorporated with nanoparticles had a tensile strength value of 22.55 MPa. This shows an enhanced property of tensile strength in the membrane that was incorporated with zinc oxide nanoparticles. This is shown in Graph 2.

Graph 2: Tensile strength of control and ZnONP membranes

Contact Angle was measured. Control chitosan membrane had a contact angle of 77.6 degrees. This implies higher wettability. Whereas the Nanoparticle incorporated membrane had a contact angle of 80.46 degrees. This suggests lower wettability of the membrane. Shown in figure 4,5.

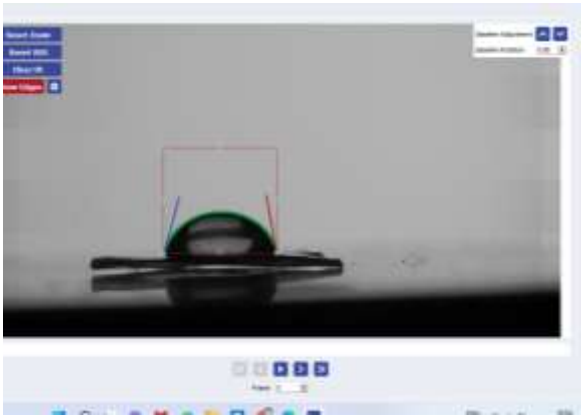


Figure 4: Contact angle of the control membrane =77.63°

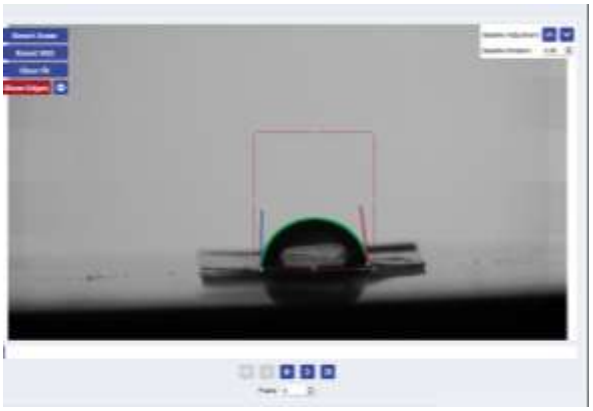
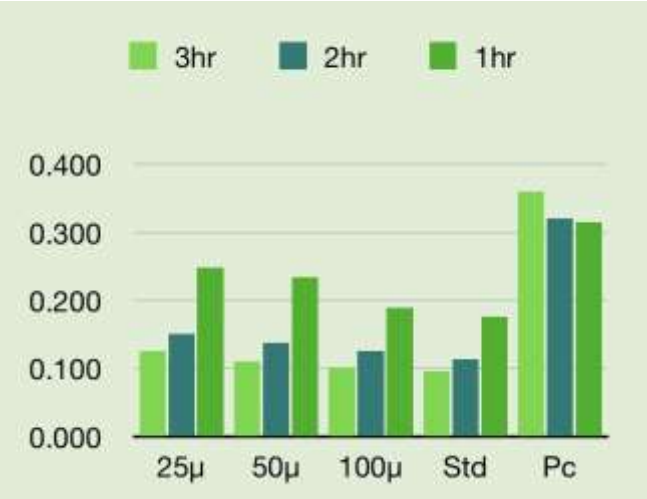


Figure 5: Contact angle of the test membrane =80.46°

The nanoparticle membrane showed a good antimicrobial activity against the tested bacteria (Streptococcus mutans). Zone of inhibition was found to be an average of 0.358, 0.312, 0.264 at different concentrations of 25µl, 50µl and 100µl; compared to the average value of 0.192 of the control group. (Figure 6) This shows an enhanced antimicrobial activity that can be attributed to the nanoparticles in the membrane. (Graph 3)

Figure 6: The anti-microbial activity membrane



Graph 3: The anti-microbial activity with MIC assay

Chemical or enzyme catalysis can be used to break down chitosan. The degree of deacetylation and the accessibility of amino groups affect how quickly chitosan breaks down. Additionally, chitosan has been approved as safe for use in foods and as a wound dressing by the US-FDA and EU. However, chitosan's toxicity increases as both charge density and the degree of deacetylation rise. As of this writing, there are no published studies indicating the toxicity of chitosan-based formulations to humans or casting doubt on the safety of chitosan for human usage. However, several research on the toxicity to animals have discovered that they are secure both in vivo and in vitro. nanoparticles of zinc, which have been found to have strong osteogenic properties, were added to speed up osteogenesis.

Our team has extensive knowledge and research experience that has translated into high quality publications. (8–17) As was previously mentioned, GTR membranes should exhibit good mechanical characteristics in addition to sufficient morphology, microstructure, and wettability to prevent membrane collapse in the periodontal defect due to the severe compressive forces of the soft tissues and mastication. (7) In order to resist cell attachment and growth, GTR membranes must also exhibit the proper stiffness; otherwise, the membranes may not be therapeutically relevant (3). A recent study showed that 3T3 fibroblasts cultured on stiffer membranes had an increased cell viability and proliferation than on softer ones (3)

It has been reported that ZnO nanoparticles can produce reactive oxygen species (ROS), such as hydroxyl radicals, super oxides, and hydrogen peroxide, which may penetrate the cell wall and affect bacteria integrity (18–20), despite the fact that the mechanism of action of ZnO is still poorly understood. Since the 30 weight percent concentration produced larger inhibition zones when compared to the less concentrated groups, the antibacterial activity demonstrated by the ZnO-incorporated membranes in the current investigation appeared to be dose-dependent (Graph 3). In

fact, it is predicted that the more nanoparticles that are introduced, the easier it will be for the active ingredient to permeate into the agar medium and successfully stop bacterial growth(18).

The wettability criteria is usually established using the contact angle with water. Another name for a fluid's capacity to spread out on a solid surface is wettability. For cells to attach during scaffold creation, a created scaffold's wettability is crucial. The fluidization of the solid surface and the initial dispersion of the fluid are both significantly influenced by wettability. Hydrophilicity of the scaffold is a useful metric for biological metabolism. To maximise cell proliferation and growth, the hydrophilicity of the composite scaffold must be increased. This enhances protein adsorption and cell adherence to a scaffold. Only the material characterisation is carried out in this study; future in vitro and in vivo studies are possible.

CONCLUSION

The following study showed that zinc Oxide incorporated membranes showed a higher tensile strength and good mechanical properties. Good antimicrobial properties and enhanced wettability were observed as well. The membrane can serve multipurpose applications and can be incorporated in various periodontal procedures and regeneration. The antimicrobial biocompatibility and cytotoxicity of the membrane will be tested in the future. Regenerative potential of the material will have to be tested in animal models. Zinc oxide nanoparticles incorporated guided tissue regeneration membranes showed promising results.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Periodontology, Saveetha Dental College, Blue Lab, Saveetha Dental College and White Lab Material Research Center for providing a platform for research and development and enhancing our knowledge.

CONFLICT OF INTEREST

The authors have none to declare.

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