Development of Alpinia officinarum mediated strontium nanoparticles embedded membrane for guided tissue regeneration application

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ABSTRACT

Introduction:
Chitosan's inadequate mechanical strength during swelling is one of its limitations, which restricts its use in a number of industries. Several physicians are also concerned about this biomaterial's limited mechanical strength and quick resorption rate. The use of nanoparticles is constantly expanding in a wide range of disciplines, including biology and medicine, medication delivery, electronics, biosensors, catalysts, and industrial and agricultural research. Due to their distinct physical and chemical properties, metallic nanoparticles have attracted the most attention among all nanoparticles in recent years. Similar to calcium and magnesium, strontium is a metallic element in Group II of the periodic table. Sr is integrated in bone regeneration as Sr2+ (ions) and can linger in the body for a long period because of its similarities to calcium.

Materials and methods:
For preparation of strontium nanoparticles 1 g of alpinia officinarum powder was mixed with 100ml of distilled water, then the plant extract was boiled and filtered. For casting, pour 20 to 25 ml in a 90mm diameter petri dish kept in a hot air oven at 50 deg Celsius for 24 hours. Testing the material characteristics with SEM,FTIR,Contact angle, tensile strength, anti microbial activity (MIC assay).

Results:
In our study, the FTIR spectra showed peaks at 893.34, 599.61 and 519.27 cm-1 in pure SrNP, which shows the ν-Sr-O stretching frequency. Peak for the NH group and CH group of SrNP was observed at 3362.14 cm-1, 2919.68 cm-1 and C=O group at 1581.49 cm-1. The FTIR spectrum for SrNP exhibits almost all peaks of pure Strontium nanoparticles, which indicates that mediation of strontium into alpinia officinarum was successful. The wettability of the membrane, The average value of the control membrane was 77.63°, for the first membrane was 61.01° and for the second membrane was 54.24°. Wettability of a membrane increases if the contact angle decreases (acute angle). Hence, the second alpinia officinarum membrane has more wettability or hydrophilicity than the control chitosan membrane. The tensile strength for the control and the SrNP membranes were tested, in which the alpinia officinarum SrNP membrane (second membrane) had the highest tensile strength of 45.08 N than the control. The SEM pictures of the chitosan membrane were plain and smooth while the SrNP embedded membrane had a rough surface which would aid in the retention of the membrane. The antimicrobial activity of the membrane was analysed using MIC assay and the maximum effect was found in the 1x4 membrane for 4 hours.

Conclusion:
Alpinia officinarum Strontium nanoparticles embedded GTR membrane possesses good antibacterial properties, physical properties and can be used after GTR procedures after future investigations

Keywords: chitosan membrane, alpinia officinarum, strontium nanoparticles, guided tissue regeneration

INTRODUCTION

Guided tissue regeneration (GTR) is an efficient and reliable surgical method for treating periodontal intrabony defects. GTR entails the installation of either resorbable or non resorbable barrier membranes to enclose a space around the damaged root surface and permits cells from the periodontal ligament and alveolar bone to repopulate the defects [1,2]. Membranes for GTR therapy must be biocompatible, have an adequate degradation summary, have strong physical and mechanical qualities, and be capable of delivering enough constant power [3]. For proper nutrition absorption and cellular adaptation, GTR membranes should be permeable. Membranes are often divided into two classes: non-resorbable membranes and bio-resorbable membranes. Expanded polytetrafluoroethylene (e-PTFE), for example, is a non-resorbable membrane that needs to be surgically removed after implantation. However, since collagen-based bio-resorbable membranes shrink with time and do not require surgical removal, they do not necessarily need to be removed [4] As a second surgical procedure is required to remove implantations, non-biodegradable membranes composed of polytetrafluoroethylene (PTFE) and titanium appear to be at significant risk of postoperative complications. Therefore, using biodegradable materials might be quite effective in creating the GTR membrane [5].

Chitosan's inadequate mechanical strength during swelling is one of its limitations, which restricts its use in a number of industries [6]. Several physicians are also concerned about this biomaterial's limited mechanical strength and quick resorption rate. In addition, rapid collagen breakdown can produce collagenase through the enzymatic action of macrophages, polymorphonuclear leukocytes, and various microorganisms, which limits membrane resistance to collapse. This outcome enables undesirable cells to reach the site of the wound [7]. Additionally, the chitosan-based membrane's antibacterial effectiveness is worse at a neutral pH, which limits its application as an antibacterial. As collagen and chitosan based membranes have such limited capabilities, it is necessary to use new, high-quality materials.

The use of nanoparticles is constantly expanding in a wide range of disciplines, including biology and medicine, medication delivery, electronics, biosensors, catalysts, and industrial and agricultural research. Due to their distinct physical and chemical properties, metallic nanoparticles have attracted the most attention among all nanoparticles in recent years. Similar to calcium and magnesium, strontium is a metallic element in Group II of the periodic table [8]. Strontium is integrated in bone regeneration as Sr2+ ions and can linger in the body for a long period because of its similarities to calcium [9]. Even though the effects are less, the signaling theory and metabolic pathways respond to calcium in a manner similar to that of strontium. Strontium contributes to the formation of bones by enhancing osteoclast activity during bone production whereas it suppresses

osteoclast activity during bone breakdown. Strontium acts on the calcium sensing receptor (CaSR) of bone forming cells to activate the signaling pathways linked to calcium-regulated bone metabolism. With a rise in β-catenin expression and a decrease in Wnt pathway inhibitors, Sr may boost the transcription of osteogenic factors and prostaglandins [10]. When muscles tense and blood clots, Sr might take the role of Ca. By incorporating Sr coated halloysite nanotubes (SrHNTs) into bone structure, osteoblasts are stimulated to produce more bone. Strontium is often utilized as a bone regenerator, growth promoter, and calcium signalling stimulant.

Due to its beneficial effects on orthopedics, strontium has drawn interest in this respect. In terms of physiology, Sr is a trace element that may build up in human hard tissues like bone and replace calcium in the apatite phase of bone mineral. Additionally, Sr was linked to an increase in bone compressive strength, and its lack has been proven to have negative effects on hard tissues. Sr ions are utilized as a pharmacological agent to treat osteoporosis because they can increase bone growth and decrease bone resorption both in vitro and in vivo [11]. Due to their similarities to calcium in terms of characteristics, strontium based nanoparticles have since attracted interest in the fields of medicine and dentistry. Additionally, strontium conjugated nanoparticles have antibacterial properties and are effective in removing hazardous pollutants from industrial effluent. Due to their ability to trigger a sustained immune response and usage in targeted medication administration, strontium nanoparticles are effective immunotherapeutic agents. Strontium nanoparticles have been used in diabetic patients, where they can manage the pathophysiology of diabetes by controlling insulin release [12].

Most dental caries and other dental issues are concentrated in the pits and fissures, or grooves and microcracks, of the posterior teeth. Plaque formation is enabled by the presence of bacteria in these areas. By creating protective sealant materials for these locations, which have been demonstrated to be beneficial in preventing tooth decay, caries production can be avoided. A substance that might prevent secondary caries at the interaction face of the tooth and the restorative chemicals is still under investigation, despite the fact that several materials are employed as sealants. Nanomaterials' vast surface area makes it possible to use them to create treatments and restorative procedures [13]. It has been discovered that strontium fluoride (SrF2) nanoparticles operate as a barrier against oral germs. Due to the prolonged release of fluoride and to stop secondary caries, tooth deterioration can also be avoided. In a study, a sealant was created by combining Bis GMA ,TEG-DMA, UDMA mixed monomers, SrF2NP, yttria stabilised zirconia, and poly-ε-L-lysine. This sealant gives the sealant physicomechanical and antibacterial properties. It was shown that SrF2 nanoparticles and ε-PL interact favourably, increasing the sealant's capacity to kill bacteria. By including the right amounts of Yttria Stabilized Zirconia nanoparticles (YSZ NPs) into the sealants, the mechanical limits may be compensated [14].
Dental resins with HApNP fillers have completely changed the way that restorative dentistry is practised by offering benefits including increased polymerization and remineralization. The poor radiopacity in these materials, however, prevents the identification of subsequent caries. The incorporation of innovative SrHApNP with better radiopacity, simple tracking of real remineralization, and the ability to identify recurrent caries led to the construction of an unique radiopaque dental adhesives.

The Zingiberaceae family includes Chitharathai, commonly known as Galangal (Alpinia officinarum and Alpinia galangal). This perennial plant grows on the plains of West Bengal,

Assam, and the Eastern Himalayas in India and is native to southeast China and Indonesia. It is both a food and a medicinal plant. For hundreds of years, people have relied on its dried rhizome to treat symptoms including stomach aches, colds, ulcers, and diarrhea. The chitharathai seed is employed as a laxative, digestive aid, and mouth refresher. The fragile stalks and blooms are used as a vegetable or spice. A source of essential oil and a spice, the root or rhizome is utilized (like ginger). Recent pharmacological research revealed that it possesses properties that are anti-inflammatory, anti-oxidative, antidiabetic, anti-ulcer, anti-diarrhea, antiemetic, analgesic, anticoagulant, anticancer, and anti-vitiligo [15]. The flavonoid galangin, which is the primary active ingredient in Alpinia officinarum, has been shown to have potent antioxidant and free radical scavenging properties. The preparation and evaluation of the GTR membrane, which combines the effects of alpinia officinarum and strontium nanoparticles with the conventional chitosan membrane, are the aim of this study.

MATERIALS AND METHODS

For preparation of the extract 1 gram of alpinia officinarum powder was mixed with 100 ml of distilled water, then the plant extract was boiled and filtered. For the preparation of strontium nanoparticle, 50 ml of distilled water was mixed with 0.573 g of strontium nitrate. 10 ml of plant extract is mixed with 50 ml of the ZnNP and left for 24 hours for synthesis of Nanoparticles. Synthesized extract with nanoparticles was centrifuged and the pellet was dried, grinded and stored. 0.3 ml of acetic acid along with 29.7 ml of H20 and 0.012 g of synthesized Alpinia officinarum strontium nanoparticle and 0.588 g of chitosan, then the mixture was heated for 1 hour, to which 0.15 ml of glycerol (plasticiser) was added. The mixture was poured in a 90mm diameter petri dish kept in a hot air oven at 50 deg Celsius for 24 hrs. After 24 hours, the membrane was neutralised, by placing the membrane in NaOH solution for 2 hrs (2g of Na in 50ml of H2O) followed by keeping the membrane in distilled water for 1 hour then drying the membrane . then the membrane was tested for material characteristics such as scanning electron microscopy (SEM),Fourier transmission infrared spectroscopy (FTIR),Contact angle and tensile strength, anti microbial activity (MIC assay)

RESULTS

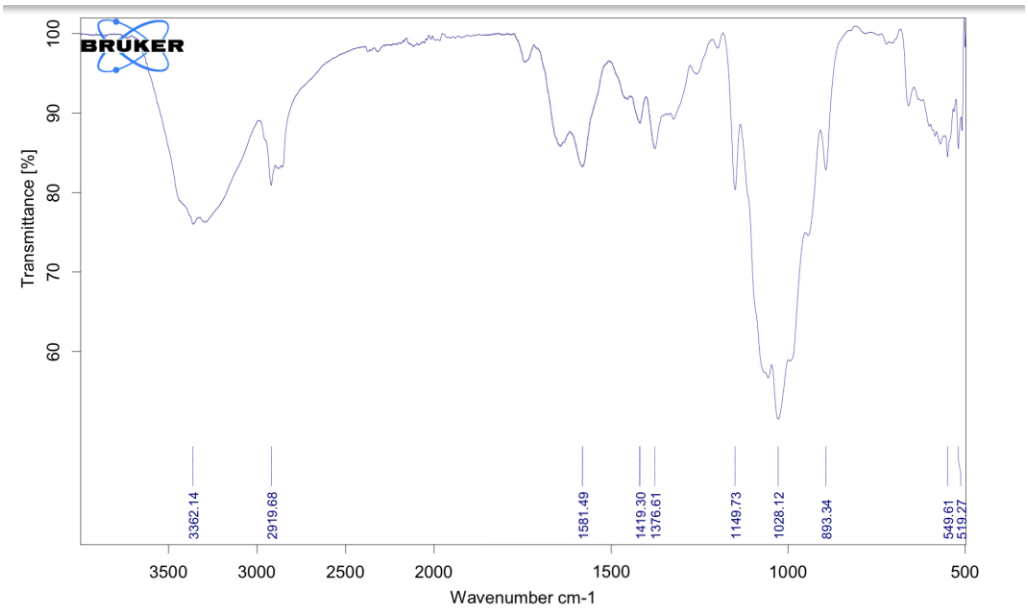
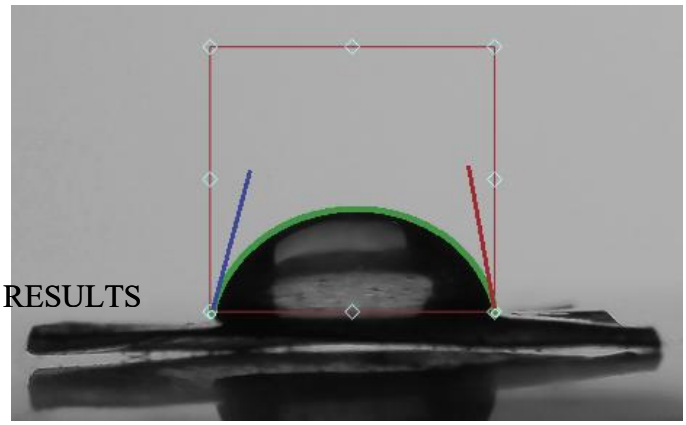


Figure 1: FTIR graph showing the functional groups present in the alpinia officinarum SrNp membrane at different wavelength, confirming the activation of the functional groups
893.34, 599.61 and 519.27 cm-1 peaks - Strontium nanoparticles
NH group of chitosan = 3362.14 cm-1
CH group of chitosan = 2919.68 cm-1
C=O :1581.49 cm-1



RESULTS

Figure 2 : Contact angle of the control membrane =77.63°

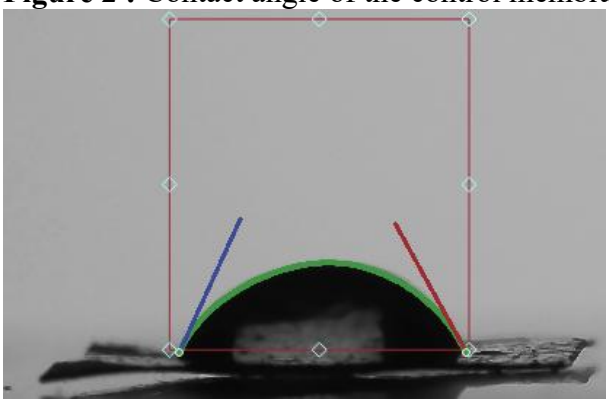


Figure 3: Contact angle of the 1 st test membrane=61.01°

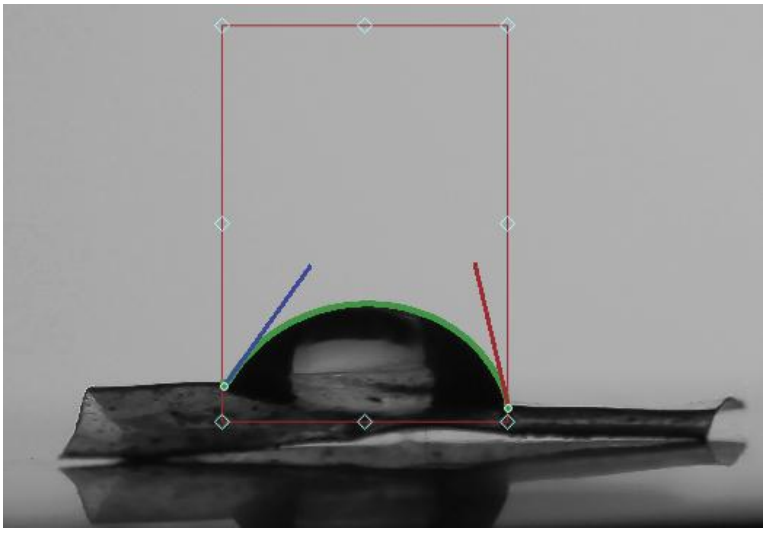


Figure 4 : Contact angle of the 2 nd test membrane=54.24°

	Maximum force (N)	Tensile stress at tensile strength
Control	14.72	23.36
1st membrane	18.90	30.00
2nd membrane	28.45	45.08

Table 1 : Tensile strength of control and the Alpinia officinarum SrNP membranes

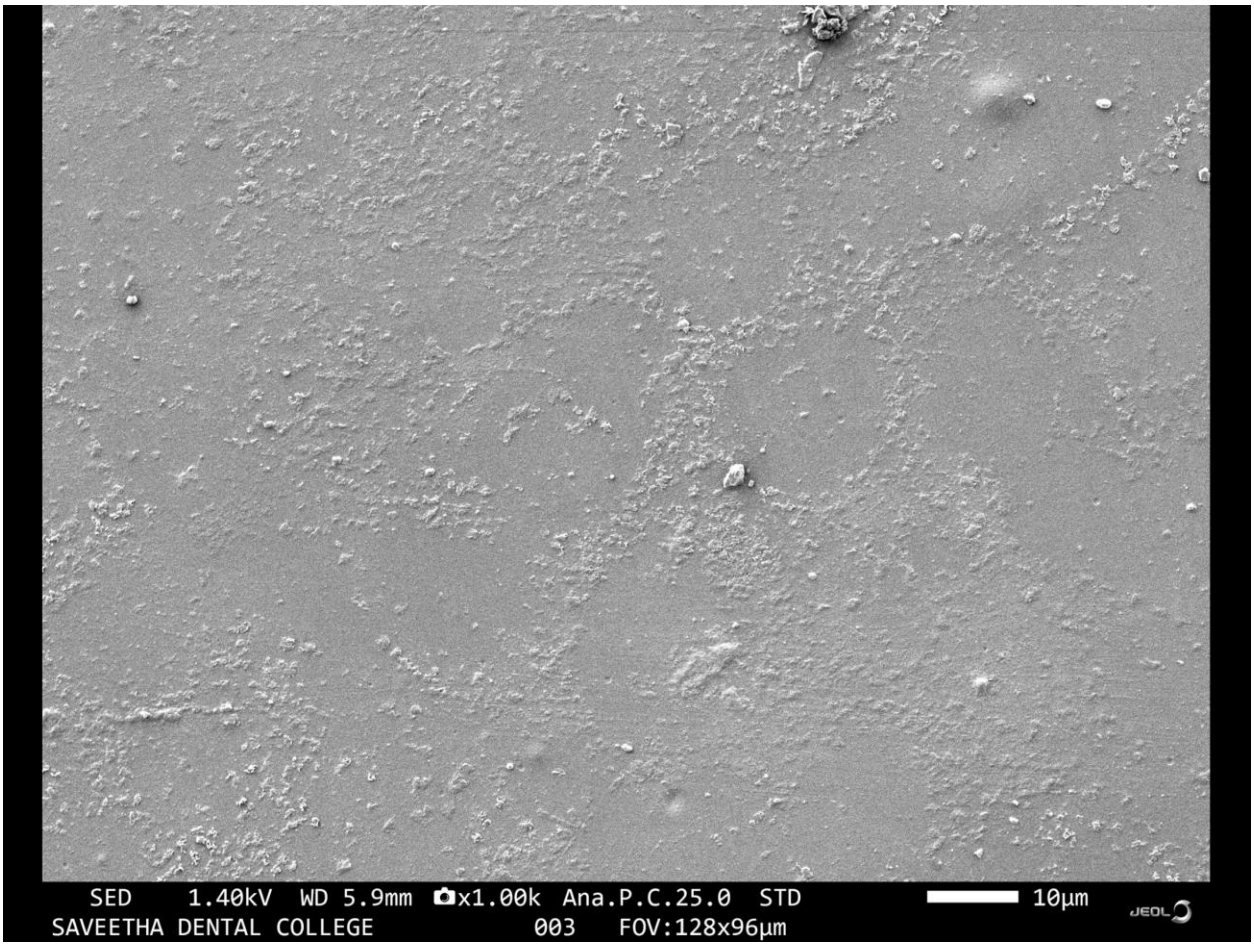


Figure 5: SEM of Control membrane

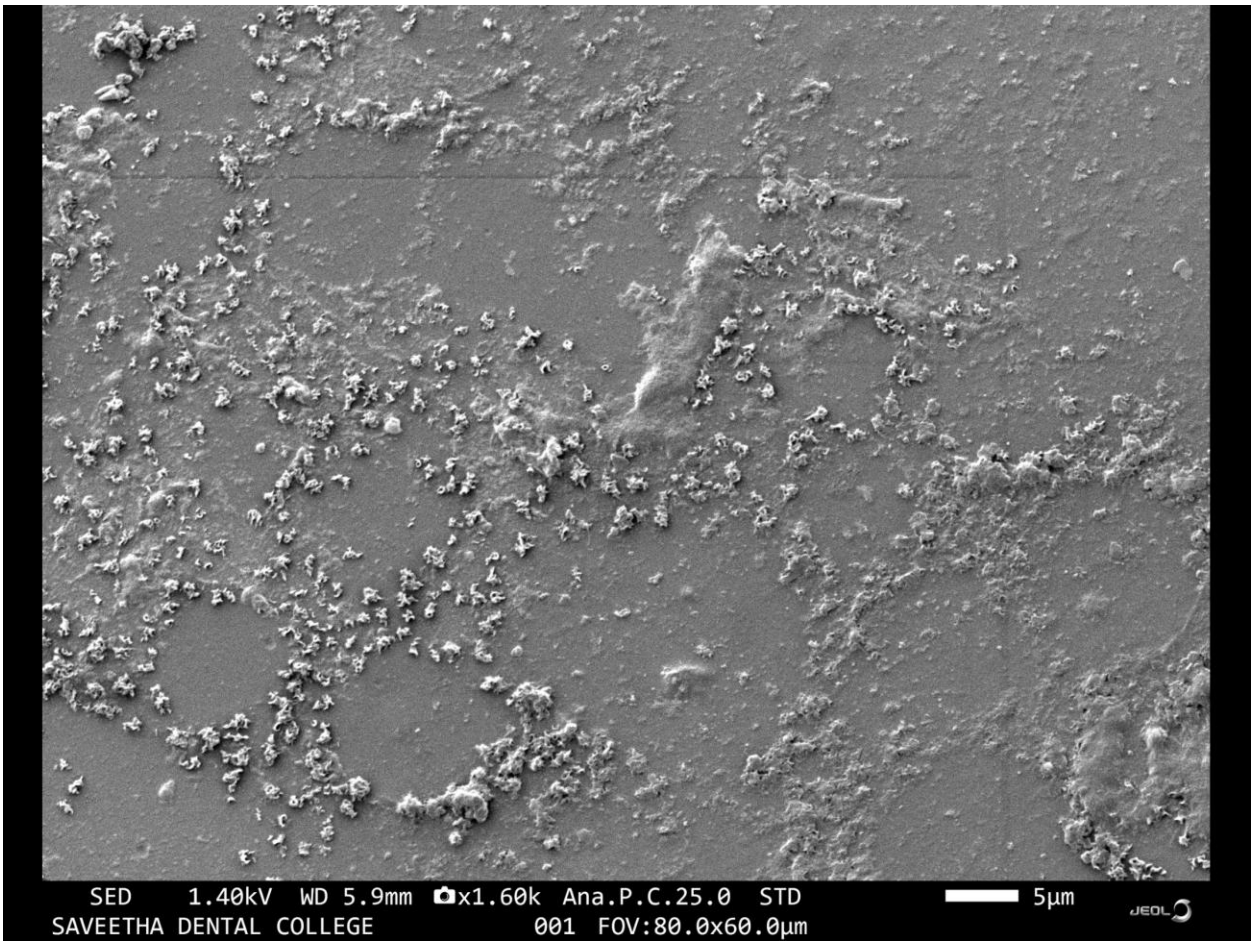


Figure 6 : SEM of Alpinia officinarum SrNP membrane

	1 hour	2 hours	3 hours	4 hours
1x1	0.411	0.386	0.354	0.326
1x2	0.406	0.374	0.322	0.305
1x3	0.401	0.350	0.310	0.287
1x4	0.386	0.336	0.280	0.258
Positive control	0.437	0.447	0.464	0.482

Table 2 : Antimicrobial activity with MIC assay against streptococcus mutans

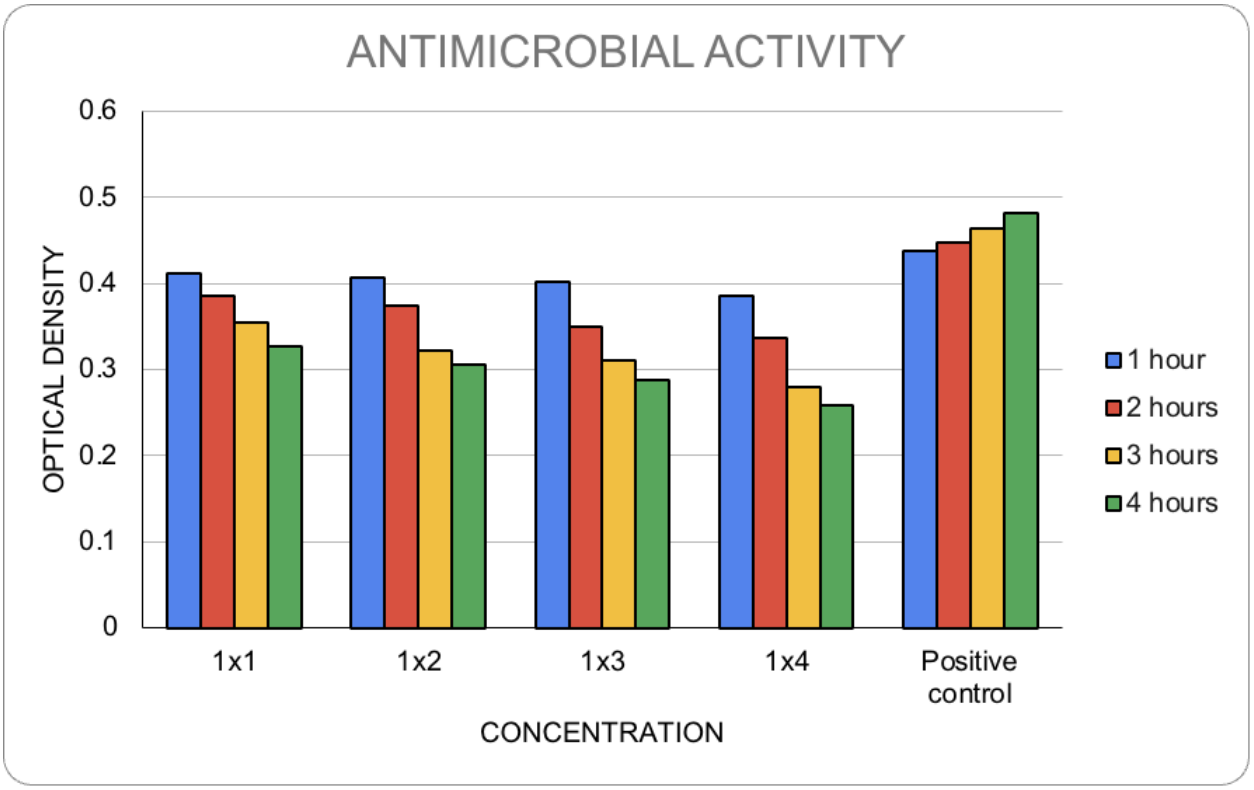


Figure 7: This represents the anti-microbial activity with MIC assay

DISCUSSION

Chitosan can be broken down using either chemical or enzyme catalysis. Chitosan degradation is influenced by the level of deacetylation and the accessibility of amino groups. Additionally, the US-FDA and EU have authorised chitosan as safe for use in foods and as a wound dressing. However, when the degree of deacetylation and charge density increase, chitosan's toxicity rises. As of this writing, we have not discovered any published data questioning the safety of chitosan for human use or demonstrating human toxicity of chitosan-based formulations. However, a number of studies on animal toxicity have found that they are both in vivo and in vitro safe [16]. Strontium nanoparticles, which have been found to have strong osteogenic properties, were added to speed up osteogenesis.

Sr can both strengthen bones and defend against tooth decay. The primary mineral component of teeth is thought to be hydroxyapatite (HAp), and synthetic HAp exhibits outstanding bioactive properties including osteoconductivity and biocompatibility since it most closely resembles the mineral makeup of bone tissue. According to reports, substituting Sr for HAp can enhance the biomaterial's crystalline structure and rate of physiological disintegration. Sr substituted HAp (SrHAp) has excellent solubility and osteoinductive characteristics. Sr is useful for long-term usage because it has favourable and inductive effects on cell development and differentiation when it is included in the HAp structure. An essential enzyme involved in bone mineralization, alkaline phosphatase (ALP), serves as a helpful biochemical indicator of bone development [17]. Additionally, it was discovered that SrHAp nanoparticles boosted the ALP activity, which is linked to the cloning of hard tissues, and enhanced the process of tooth remineralization.

Although titanium (Ti) dental implants are often utilized in clinical settings, there are issues with the possibility of prosthesis loosening and post-operative infection. To get around these restrictions, a number of surface changes have been employed. Numerous studies have been done on titanium oxide nanotubes (NT-TiO2) because of their tubular form and easily changeable diameter. Sr modifies the surface of NT-TiO2 to enable targeted distribution [18]. Sr is a perfect clinical interest factor because of its benefits for improved bone growth and preventative bone resorption. Through a process that is dependent on the calcium-sensing receptor, it promotes osteoblast replication, differentiation, and mineralization of the bone matrix (CaR). In a Sr(OH)2 solution, Sr-loaded nanotubular structures (NT-Sr) underwent hydrothermal treatment to become NT-TiO2. These structures can deliver Sr2+ in a regulated, long-term manner, resulting in favourable osteogenic properties. Angiogenesis aids in bone regeneration and repair to reestablish an efficient blood supply. Better tissue absorption at the implant site results from improved angiogenesis, which also limits infection by activating the host immune system. Sr2+ can also cause osteoblast cells to release VEGF, which has angiogenic and osteogenic properties. The hydrothermal treatment does not alter the NT shape but converts the amorphous TiO2 found in NT-Ag to cuboidal SrTiO3, which has no impact on the nanotubular architecture. NT-Sr-Ag created hydrothermally can sustainably discharge Sr2+ and Ag+. With hydrothermal treatment duration, the tube diameter did, however, slightly decrease, which could have an impact on Sr2+ release [19].

In our study, FTIR spectra were used to identify the presence of different functional groups in the tested samples. FTIR spectra of Alpinia officinarum SrNP membrane is shown in figure 1. The pure SrNP spectrum peaks at 893.34, 599.61 and 519.27 cm-1, which shows the ν-Sr-O stretching frequency [20]. Peak for the NH group of chitosan was observed at 3362.14 cm-1, CH group of chitosan = 2919.68 cm-1 and C=O group at 1581.49 cm-1. The FTIR spectrum for SrNP at chitosan exhibits almost all peaks of pure Sr nanoparticles, which indicates that mediation of strontium into chitosan is successfully done.

Contact angle data for the three test membranes are plotted in Figure 2, 3 and 4. The average value of the control membrane was 77.63°, for the first membrane was 61.01° and for the second membrane was 54.24°. Wettability of a membrane increases if the contact angle decreases (acute angle). Hence, the second alpinia officinarum membrane has more wettability or hydrophilicity than the control chitosan membrane. Table 1 shows the tensile strength for the control and the SrNP membranes, in which the alpinia officinarum SrNP membrane (second membrane) had the highest tensile strength of 45.08 N than the control. The SEM pictures of the chitosan membrane were plain and smooth while the SrNP embedded membrane had a rough surface which would aid in the retention of the membrane (figure 5 & 6). The antimicrobial activity of the membrane was analysed using MIC assay and the maximum effect was found in the 1x4 membrane for 4 hours. The contact angle with water is frequently used to establish the wettability criterion. A system's wettability is another term for a fluid's ability to spread out across a solid surface. The wettability of a constructed scaffold is important for cell adherence in the formation of scaffolds. Wettability has a considerable impact on how the solid surface is fluidized as well as how the fluid is first dispersed. Scaffold hydrophilicity is a helpful parameter for biological metabolism. It is necessary to increase the composite scaffold's hydrophilicity in order to maximize cell proliferation and growth [21]. This improves cell adherence to a scaffold and protein adsorption. In this study only material characterisation is done, in future, in-vitro and in-vivo studies can be done.

CONCLUSION

In this study, it was found that the alpinia officinarum strontium nanoparticles embedded membrane had good wettability, tensile strength and even exhibited better antimicrobial properties than the chitosan membrane. In addition, strontium nanoparticles have been proven to promote osteogenesis and can provide a better regenerative property than the normal chitosan membrane.

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CONFLICT OF INTEREST

The authors have none to declare.

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