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# Fabrication and Characterization of PVA/Tricalcium Phosphate/Quercetin Doped Silver Membrane for Guided Bone Regeneration

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***Abstract***

*Periodontitis is a chronic inflammatory condition of the periodontal tissue caused by Aggregatibacter actinomycetemcomitans. Periodontitis eventually also leads to bone resorption. To treat it, biocompatible and wear resistant films/membranes have been introduced to enhance and boost the process of regeneration. Synthetic polymers are commonly used in tissue engineering as they have better mechanical characteristics. In this study, PVA nanofibers were incorporated with flavonoid doped silver oxide nanoparticles to facilitate bone regeneration and control infection and inflammation. The results indicated the successful formation of a membrane with nanofiber size of 100-150mm and pore size of 1-2 microns. The FTIR and XRD analysis proved the purity of the material. The contact angle test indicated the hydrophilicity of the membrane and the biocompatibility was found and confirmed by mtt assay and the osteoconductive ability of the membrane was observed. Hence this membrane proves to be successful in causing bone formation to a certain extent.*

***Keywords:*** *Bone Resorption, Membrane Synthesis, PVA, Quercetin Doped Silver Nanoparticle, TCP.*

## Introduction

Periodontal diseases are a serious health issue that have an adverse effect on many people's way of life [1]. A chronic inflammatory condition of the periodontal tissue called periodontitis is brought on by pathogenic bacteria and is characterised by the deterioration of the teeth' supporting components [2]. Aggregatibacter actinomycetemcomitans is the major bacterial group that causes rise in inflammatory response linked to onset of aggressive periodontitis. [3]. Early on, the gum inflammation is localised, but as it progresses, it penetrates deeper and forms sacks that anaerobic bacteria will colonise and destroy the teeth's supporting ligaments until they fall out [4]. Periodontitis develops through a 3 step procedure and eventually causes bone resorption.It begins with the formation of plaque on teeth which turns into tartar the combined effect of the two if left without cleaning for a long period of time results in an immune response that leads to inflammation [5]. At this stage it is called gingivitis.if gingivitis is left untreated for long then it turns into periodontitis which leads to development of periodontal pockets in the gums and thus creates a safe environment for bacterias to thrive [6]. The bacterias along with the chemicals released by bodies immune responses causes activation of osteoclasts [7]. Thus, periodontitis eventually causes bone resorption. In this situation, preventing periodontal disease becomes crucial, and the best course of action is to stop the causes—namely, the development of bacterial plaque—

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as soon as possible. A periodontitis therapy that works must reduce bacterial numbers in the afflicted areas in order to control the infection. [8]. In order to effectively treat periodontitis, the bacterial numbers in the infected periodontal pockets must be reduced along with inflammation. The basic treatment of periodontitis mainly includes multiple surgical procedures such as pocket reduction, gum grafting, bone grafting, scaling and root planing [9]. In daily practice, the possibility of early, unintentional removal from the site of action due to mechanical stress created during tooth brushing or chewing is one of the principal downsides of pharmaceutical formulations for local drug administration in the treatment of periodontitis. These treatments have a major drawback that is failure of the procedures ,recurrence of the disease and the pain due to treatment .hence it makes it essential to introduce a treatment plan that is less painful and has a long term effect. [11]. A perfect formulation would last for a long time at the application site and would release the therapeutic ingredients gradually. Films are favored over other formulations when creating local oral drug delivery systems because they are simple to make, frequently exhibit flexibility and mucoadhesivity, protect the inflamed surface region, and hasten the healing of periodontal wounds. [12]. Biopolymers, particularly polysaccharides and their derivatives, are ideally employed as part of the support matrix and in combination with the medicine in order to make films. However, natural polymers lack the necessary mechanical characteristics, while synthetic polymers suffer from a lack of biocompatibility.

The effective insertion of dental implants or the regeneration of bone in a variety of dental and periodontal abnormalities can be facilitated by the use of guided bone regeneration, a beneficial dental treatment. The goal of tissue engineering (TE) is to replace lost tissue and organ functions as a result of injury, aging, or disease. By restoring or mending the tissue's structural integrity after an acute injury, a procedure known as tissue regeneration can be carried out. The focus of TE has been on developing structures that facilitate and promote the regeneration of numerous types of tissues, including skin, cartilage, bone, tendon, and cardiac tissues. For body parts that are deeply buried and cannot be repaired by standard surgical procedures, bone regeneration is used. For the purpose of mending fractured bone, bone grafts were employed. Artificial biomaterials are employed to replace bone grafts on the basis of their biomechanical characteristics, these biomaterials were chosen for structural rehabilitation, to promote tissue growth, to be bioactive or bioresorbable. At present scaffolds are made to promote vascularization, prevent infection, reduce inflammation and promote bone growth [11]. The materials often used in scaffolds are proteins (collagen, silk fibroin) and polysaccharides (chitosan, alginate, hyaluronic acid, and cellulose).

Poly vinyl alcohol (PVA) has excellent water absorption capacity, chemical resistance, considerable biocompatibility and biodegradability hence it is the most widely used polymer. PVA membrane has proven to be useful for controlled drug delivery, (12) for local applications in periodontal pockets and has good flexibility [13]. Silver is one of the most used metallic nano particle due to its inherent antibacterial [14]. activity against oral pathogens as well [15]. Silver nanoparticles have shown to be useful in prevention and disinfection of diseases in oral cavity as well as prophylactic application. The incorporation of such medications into a polymeric matrix can boost the effectiveness of wound healing due to their enhanced retention at the site of action, sustained release.and greater interaction with the wound environment [16]. In this study quercetin doped silver oxide nanoparticles are added as Quercetin is known for its anti-inflammatory, antioxidant and bone forming properties. This would aid in the prevention of

infection and inflammation. The aim of the current study is to fabricate and synthesize a biocompatible membrane using PVA/B-TCP and quercetin doped silver nanoparticles that will aid effective long term bone regeneration as a treatment to multitudes of dental problems.

## Materials and Methods

### Fabrication of Membrane

A 10% w/v solution of PVA was blended with 0.5% of B-TCP and 5mg/ml of quercetin doped silver oxide (Q-AgO). The solution was homogeneously stirred for 24h. The polymer solution was loaded in a 5ml syringe and extruded through a 22 G blunt end needle charged at 10 kV [17]. Continuous fibers were collected at the flow rate 0.9ml/h onto the collector plate positioned at 10cm apart from the center of the tip of the needle. The fabricated fibers were further analyzed.

### Fourier Transform Infrared (FTIR) Analysis

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) which is a powerful method to determine chemical interactions was used to check the functional group changes in the material upon addition of nanoparticles. The Alpha II Bruker type spectrometer used wave numbers between 4000 and 500 cm-1 to analyse the changes in functional groups [18]. The expected pendant functionalities of the scaffolds were confirmed using the FT-IR spectrum.

### Scanning Electron Microscope (SEM) Analysis

The morphological characteristics of the fabricated GBR membranes were observed using Scanning electron microscopy. JSM-IT800 NANO SEM, a Field Emission Scanning Electron Microscope with a JEOL Energy Dispersive X-ray Spectrometer (EDS) model was used to conduct this analysis [19]. The fibre diameter and the porosity of the electrospun samples were analysed using SEM.8mm discs were punched from the electrospinned sheets using a biopsy punch. The samples were coated with platinum via sputter-coater at ambient temperature. The micrographs were obtained at 2.50kX.The average diameter and porosity of the fibres were determined from the SEM micrographs using ImageJ software.

### X-Ray Diffraction Analysis

The crystallinity of the nanoparticles was studied using X-Ray diffraction analysis at a voltage of 40 kV and a current of 30 mA using (Cu Ka = 1.5406 A) radiation as an X-ray source. Its scanning rate was performed at 2° $min^{-1}$ in the 20 range 10° to 80°.

### Contact Angle

The hydrophilicity of the membrane was determined by checking the water contact angle using goniometer software. Specimen of 1cm x 1cm square was taken and placed on a testing plate on which 50uL of distilled water was dropped. The photos of the membrane were taken immediately upon dropping of liquid to measure the contact angles between water droplet and membrane. The measurement of the GBR membrane was conducted at different positions three times.

### Human Dental Pulp Stem Cell (DPSC) Culture

The Dental Pulp stem cells (DPSC) were extracted from molars after the SIMATS ethics committee granted informed consent and ethical approval [20]. The cells were cultured in Dulbecco's Modified Eagle Medium F12 (DMEM F12) supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin. For further assays, 10,000 cells were seeded into 48-well plates after two passages.

### Cell Viability/MTT Assay

1 mg/ml of PVA/B-TCP/Quercetin doped with silver nanoparticle membrane sample was prepared. The prepared sample was immersed in DMEM F12 media formulated with 10 % FBS and 1% Penicillin/streptomycin [21]. The

media were collected after 24 hours of immersion and treated with cells to test compatibility. After 24hrs of culture, 10 uL/100 mL of MTT reagent 5 mg/mL stock) was added to cultured cells and then incubated for 4 h to allow formation of the formazan dye at 37°C. The medium is exchanged to DMSO (200 uL) and stand for 10 min. The reaction product was transferred to a 96 well ELISA plate and A570 was measured with ELISA plate reader.

**Bone Formation Assay**

The MG63 cells, osteoblast cells were cultured for 14 days in a differentiation medium with DMEMF12 and 10mM B-glycerophosphate,0.05mM Ascorbic acid and Silver oxide nanoparticle. Alizarin red staining was performed to determine calcium deposition [22]. After 2 weeks the cells were stained with 2% Alizarin red solution again for 10 mins.The cells were then washed twice with 1X PBS. For quantitative analysis 200ul of DMSO was added to each well and incubated for 1h. The quantity of Alizarin was measured in a spectrophotometer at 405 nm.

## Statistical Analysis

The mean+- standard error of the mean (SEM) of at least three independent experiments is used to express all values. The significant differences were tested using one-way ANOVA (analysis of variance). Multiple comparisons were performed using Scheffe's method. Statistical significance was set at $p<0.05$.

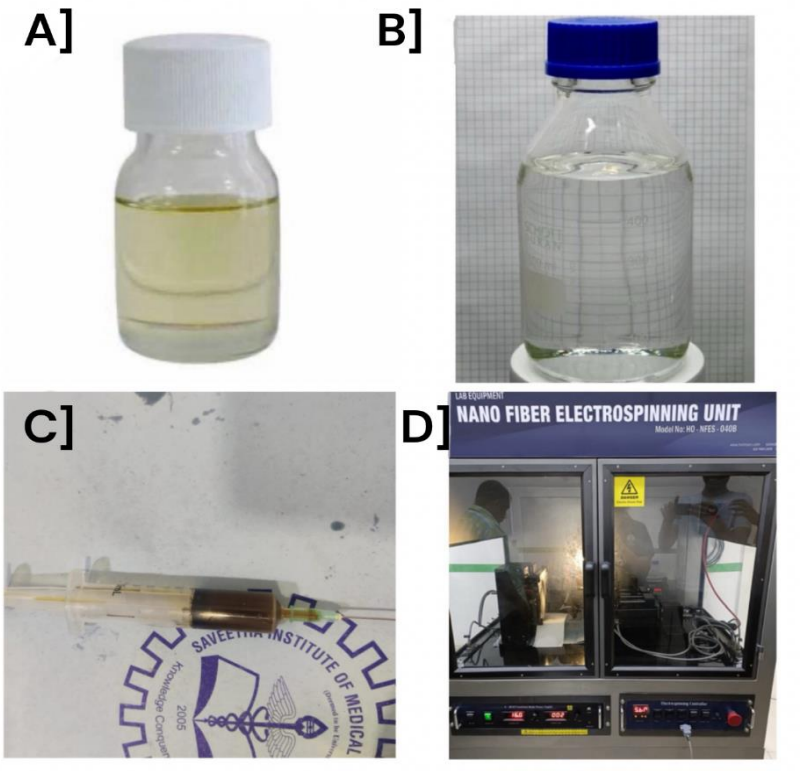


**Fig. 1.** Materials Used to Synthesize the GBR membrane A) PVA solution; B) B-TCP solution; C) PVA, B-TCP, Q-AgO mixture; D) Electrospinning machine.

## Results

The test membrane (PVA/B TCP/Q-AgO) (Fig. 1) nanofibers was characterised using FTIR (Fig. 2). The peaks help to identify the various functional groups of the substances present in the membrane. The peaks observed at 3278.69cm$^{-1}$(O–H stretching), 2909.40 cm$^{-1}$ (asymmetric stretching of $CH_2$), 1419.55 cm$^{-1}$(bending of CH2), 1333.74cm$^{-1}$ (deformation of CH), 1089.66cm$^{-1}$ (stretching of C—O) corresponds to PVA which is similar to that of

previous report (20). The incorporation of Q-AgO was confirmed by the presence of peak at 606.35 cm$^{-}$for AgO (21).

The SEM analysis helps to analyse the surface structure and variations between the control group and the test group. It was clearly visible by the SEM image (Fig 3), with the addition of Q-AgO nanoparticles in the test group a reduced porosity was observed than the control group. In the nanoparticle incorporated membrane the diameter of the fibres also reduced to a great extent. Pore size was found to be 1-2 microns and nanofiber size was found to be 100-150 nm. This indicates that the membrane is compatible for cell attachment and nutrition flow.

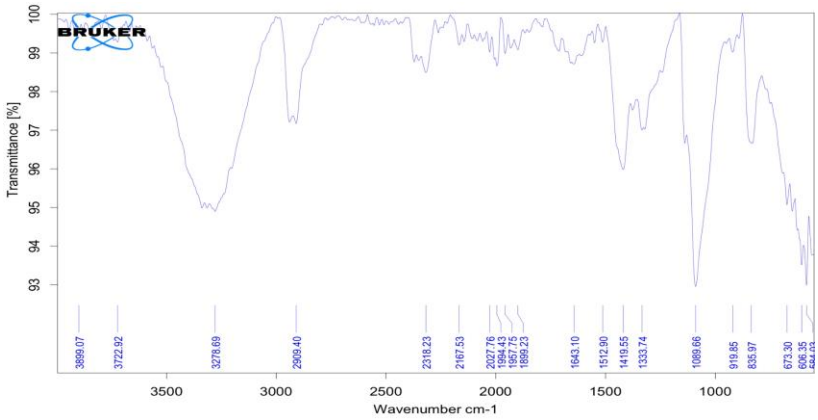


**Fig. 2.** FTIR Spectrum of Test Membrane

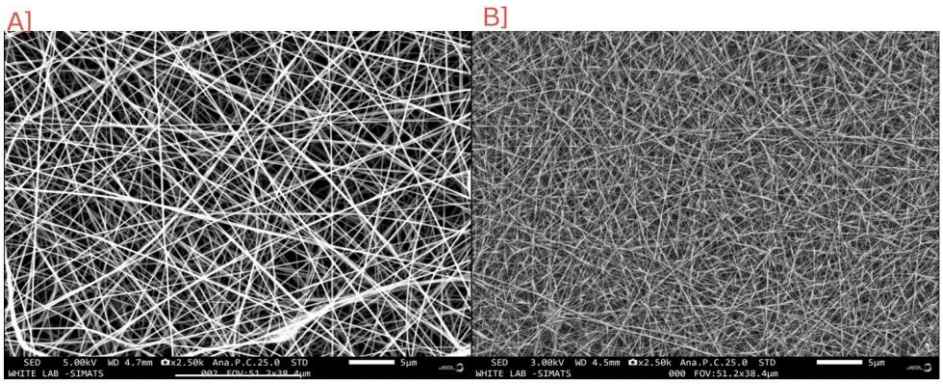


**Fig. 3**. SEM Characterization of A] Control; B] Test

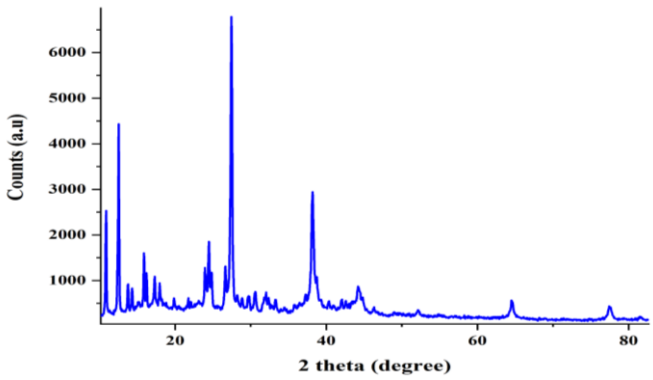


**Fig. 4**. X-Ray diffraction pattern of quercetin doped AgO nanoparticle

The XRD pattern pattern of the nanoparticle incorporated in the membrane was analysed. The peaks obserevd at 38.19°, 64.74°, 77.46° corresponds to the AgO (Fig 4).

Contact angle analysis of the membrane helps to analyse the membrane's hydrophilicity. The contact angle of PVA is found to be 20°. The contact angle of the synthesized membrane was found to be 35.80°. With the addition of b-TCP and AgO nanoparticles the hydrophilicity of the PVA was reduced (Fig 5). A contact angle below 90° indicates the hydrophilic nature of the substance hence proving the hydrophilic and binding nature of the membrane obtained.

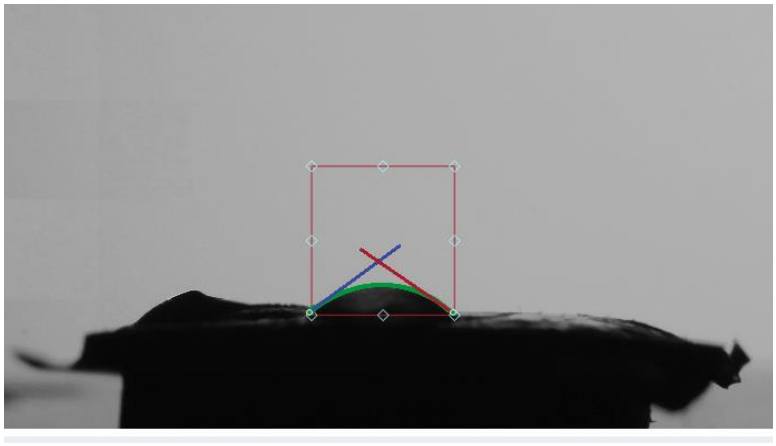

**Fig. 5.** Contact angle

The compatibility of the prepared membrane was studied by treating the membrane against DPSCs. The prepared membranes have shown more than 80% cell viability when compared to the untreated group suggesting the membranes are non-toxic and compatible (Fig 6).

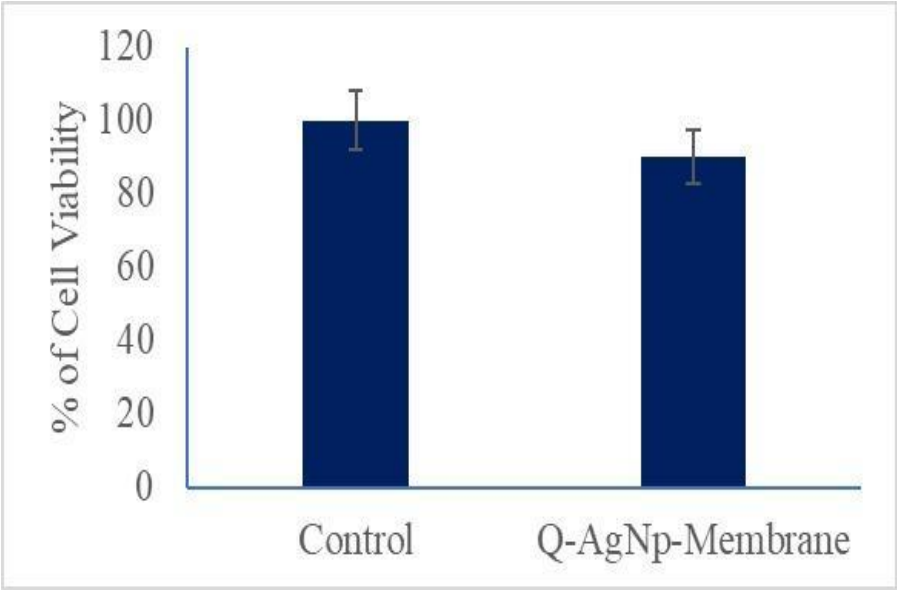


**Fig. 6.** Biocompatibility of the fabricated membrane

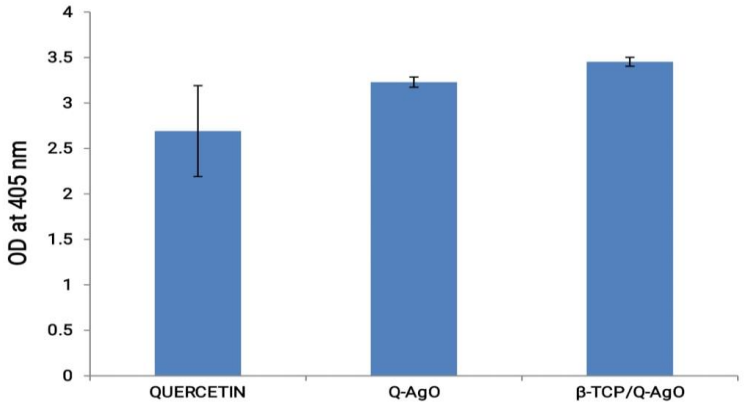


**Fig. 7.** Bone formation assay. OD observed at 405nm

Alizarin red staining shows the calcium-based matrix mineralisation during then osteogenesis process. Quercetin acts as positive control as previous studies proven to be osteoconductive. The test group Q-AgO nanoparticle and the Q-AgO/B-TCP have shown a non-significant increase in matrix mineralisation than the positive control. Although previous studies have reported that AgO nanoparticles have little effect on bone formation, an increased bone formation was observed in our study (Fig 7). This can be due to the effect of quercetin coated on the surface of the nanoparticles. As the nanoparticles can be internalized by the cell than the bulk quercetin, there can be an enhanced matrix mineralisation.

## Discussion

Periodontal diseases have proven to be one of the leading causes for bone resorption. For effective treatment of the disease the bacterial number in the infected pocket and the inflammation must be reduced. The local drug delivery technique is not as effective in the treatment of periodontitis as during daily practice unintentional removal of the drug might take place due to mechanical stresses exerted by chewing or brushing. In order to formulate a long term and effective treatment for the disease, bone regenerative membrane such as PVA membrane, Chitosan membrane and doped membranes. Membranes prove to be more effective as they exhibit flexibility, mucoadhesive, aid in protecting the inflamed surface region and hasten the process of healing [22].

Bone resorption is the major issue in periodontitis. Microbial invasion induces osteoclastogenesis directly or indirectly through the host immune system. The current treatment includes use of antibiotics, scaling and root planning and pocket reduction surgery are being performed. These treatment measures can stop the progression of the disease but cannot reverse the damage of the tissue [23]. To replace or regenerate the lost tissue where studies are in progress. Bone grafts have long been used in the treatment of periodontal disease [24]. They can be allografts, xenografts, alloplastic, and autograft materials. These grafts facilitate the natural osseous repair, but they are associated with drawbacks such as donor site morbidity, infection, immune response. Guided bone regeneration membrane aims in the development of new bone by preventing fiber tissue intrusion at the defective site. The ideal GBR membrane must be able to exclude unwanted cells or tissue, have rigid framework, porous structure that facilitate the cell migration and proliferation, biodegradable by the native enzymes and biocompatible [25].

Synthetic materials are commonly used as their physiochemical and mechanical properties closely resemble the native tissue. In this study, PVA based electrospinned membrane incorporated with B-TCP and quercetin doped silver oxide nanoparticles. PVA is commonly

used in the field of tissue engineering as they have better mechanical properties and are biocompatible [26]. They act as better carriers for the release of drugs or small molecules. B-TCP is added to the fabricated membrane as they are proven osteoconductive material. Quercetin doped AgO nanoparticles were also incorporated to prevent infection and inflammation in the disease condition [27]. The mineralization of artificial substitutes such as calcium carbonate, PRF, and nano-hydroxyapatite has demonstrated clinical benefits in various fields [28-30]. Also, various studies have proven the effect of quercetin on the differentiation of mesenchymal stem cells towards osteogenic differentiation. This was observed in our study, where the Alizarin red assay showed that the test group (B-TCP/Q-AgO) has better matrix mineralization than the other groups.

## Conclusion

The synthesised GBR membrane, upon analysis, proved to be biocompatible and hydrophilic, thus confirming its effectiveness in initiating bone formation. The utilisation of PVA is advantageous due to its biocompatibility, biodegradability, and localised drug delivery properties. The use of B-TCP is advantageous due to its osteogenic properties, and silver, due to its antimicrobial properties, aids in providing a safe environment for bone regeneration. Further research is still required to improve hydrophilicity and biocompatibility, as the synthetic membranes, even though they have mechanical strength, have considerably less biocompatibility.

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