RESEARCH PAPER

FABRICATION AND PHYSICOCHEMICAL CHARACTERIZATION OF CS/HAP/AgO BASED ON GBR MEMBRANE

Rithanya P[1], Subhashree R[2*], Sahana Selvaganesh[3], Thiyaneshwaran N[4]

[1]Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-77, Tamil Nadu, India. Email: 151701054.sdc@saveetha.com

[2*]Reader, Department of Implantology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai. Email: Subhashreer.sdc@saveetha.com (Corresponding Author). Phone number: +91-97907 41570

[3]Department of Implantology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai. Email: sahana.sdc@saveetha.com

[4]Professor and Head, Department of Implantology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai. Email: dr.thiyan@gmail.com

ABSTRACT

INTRODUCTION

Chondroitin sulfate is an important structural component of cartilage and provides much of its resistance to compression. It is necessary to prevent the invasion of soft tissue into bone defects for successful outcomes in guided bone regeneration (GBR). For this reason, many materials are used as protective barriers to bone defects. The aim of the study was to find out about the fabrication and physicochemical characterization of Chondroitin sulfate / Hydroxyapatite / silver oxide based on the GBR membrane.

MATERIALS AND METHODS

Fabrication of AgO, gelatin and Carrageenan and preparation of scaffold of control and test group. Micrographs of all scaffolds were taken at 100X. The expected pendant functionalities of scaffolds were confirmed by the FTIR spectrum. To determine the hydrophilicity of the scaffolds, the water contact angles of the scaffolds were measured by goniometer software. Three measurements at different positions of each scaffold were conducted. Swelling/shrinkage studies were performed to calculate the water content (%) of the scaffolds. After obtaining informed consent and ethical approval from the SIMATS ethics committee, the Dental Pulp stem cells were isolated from molars. The reaction product was transferred to a 96-well ELISA plate, and A590 was measured with an ELISA plate reader. All values are expressed as the mean ± standard error of the mean (SEM) of at least three independent experiments. A one-way ANOVA was followed by Scheffe's method. Statistical significance was set at $p < 0.05$.

RESULTS

The contact angle shows a chondroitin sulfate nature in addition to AgO nanoparticles, and also, the elevation of swelling indicates it can be used in the chemo static-based membrane or socket preservation procedures. The addition of AgO decrease in porosity is highly evident that AgO-based particles have higher cell adherence, increased cell migration, increased cell penetrating efficiency and a high flow of nutrition.

CONCLUSION

In this study, it has been concluded that a new material made by a chondroitin sulfate/Hydroxyapatite/AgO hybrid was fabricated and tested using Dental Pulp stem cells. We found that chondroitin sulfate with silver oxide exhibited a stronger osteoregenerative and better osteoconductive properties and significant intracellular responses for bone grafts, making it reasonable to suggest this chondroitin sulfate /AgO hybrid as a potential novel dental material for guided bone regeneration surgery.

KEYWORDS

Guided bone regeneration, silver oxide, Hydroxyapatite, chondroitin sulfate.

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INTRODUCTION

A typical oral condition is an alveolar bone defect. The medical application of the following implantation, orthodontic, periodontal, and functional restoration treatments has steadily become extremely difficult due to insufficient alveolar bone produced by trauma, tumour, periodontitis, and long-term tooth loss (1).

Natural teeth provide the alveolar bone with functional stimulation, which is lost when they are missing. This leads to gradual, cumulative, and irreversible bone resorption, and the alveolar bone is unable to maintain its shape or act as mucosal support (2). There are numerous techniques for enhancing the alveolar bone, including distraction osteogenesis, directed bone regeneration, alveolar crest cleavage, bone compression, maxillary sinus elevation, and autologous mass bone transplantation (3).

One of the most popular methods for maintaining or enhancing the alveolar ridge is guided bone regeneration (GBR), which is regarded as a conventional kind of therapy. By creating a barrier between soft tissue and the location of the bone defect, barrier membranes serve a crucial function in GBR by promoting osteoprogenitor cell proliferation and aiding the development of new bone tissues (4). A good material design of the "ideal" GBR membrane should consider the following qualities in addition to the function of space maintenance: cellular blockage limits the entrance of non-osteogenic cells into bone defect from the mucosa; biocompatibility: does not harm surrounding tissue and the healing process promotes easy handling not too rigid without sacrificing space maintenance function and bioactivation qualities stimulates tissue fusion and wound healing (5).

Alveolar bone loss is a common problem that affects dental implant placement (6)(7,8). A barrier between the bone substitute and gingiva that can prevent fibrous-tissue ingrowth and bacterial infection and induce bone formation is a key factor in improving the success of alveolar ridge reconstruction (9). Chondroitin sulfate is a sulfated glycosaminoglycan (GAG) composed of a chain of alternating sugars (N-acetylgalactosamine and glucuronic acid). It is usually found attached to proteins as part of a proteoglycan (6)(7,8). A chondroitin chain can have over 100 individual sugars, each of which can be sulfated in variable positions and quantities (10).

Chondroitin sulfate is an important structural component of cartilage and provides much of its resistance to compression (11). It is necessary to prevent the invasion of soft tissue into bone defects for successful outcomes in guided bone regeneration (GBR). For this reason, many materials are used as protective barriers to bone defects. The aim of the study was to find about the fabrication and Physicochemical characterization of Chondroitin sulfate / Hydroxyapatite/ silver oxide based on GBR membrane (12).

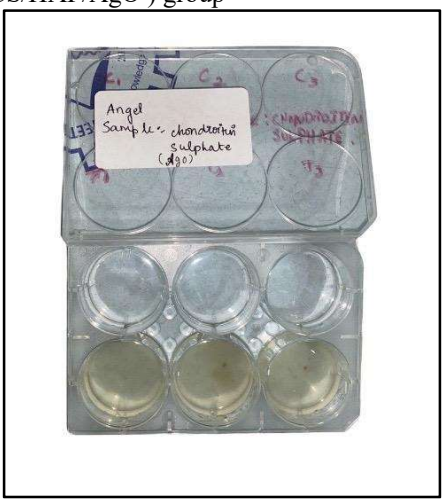
MATERIALS AND METHODS

Fabrication of Scaffolds

The stock solution of (1% chondroitin sulfate), 0.5% carrageenan and 1% gelatin were prepared. To fabricate the scaffold the materials were blended in the ratio 6:1:3 respectively. Then, 5/5mg of (HAP and AgO nano particles) was added to the solution for the test group. 3ml of the homogeneous mixture was transferred to six well plates. 100 ul of the crosslinking agents TPP (15%) was added to each well. The plates were stored in -20 C for 12 hrs and followed by -80 C overnight. The samples were then lyophilized for 24hrs and stored in dry condition.

FIGURE:1: This Picture depicts the Fabrication of the material of both Control(CS + HAP) and Test(CS + HAP + AgO) group

FIGURE 2: This picture depicts the Scaffold preparation of Control (CS + HAP) and Test(CS/HAP/AgO) group



SEM Analysis:

The morphological characteristics of scaffolds were observed using scanning electron microscopy (SEM, JEOL, Tokyo, Japan) after freeze drying. The cross-

sections of freeze-dried samples were coated with platinum via a sputter-coater at ambient temperature. Micrographs of all scaffolds were taken at 100X.

Fourier transform infrared (FT-IR) analysis
Attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR) is a powerful technique to determine any possible chemical interaction ATR-FTIR spectroscopic analysis was performed using Bruker ATR infrared spectrometer model. The expected pendant functionalities of scaffolds were confirmed by the FT-IR spectrum.

Contact Angle
To determine the hydrophilicity of the scaffolds, water contact angles of the scaffolds were measured by goniometer software. During the measurements, the scaffolds were cut into square specimens with the size of 1 cm × 1 cm, and further they were placed on the testing plate. Subsequently, 50 μL distilled water was carefully dropped onto the prepared specimens. The contact angles between water droplets and the scaffolds were measured by taking photos immediately (within 2s) when the droplets touched the surface of the scaffolds. Three measurements at different positions of each scaffold were conducted.

Swelling ratio (%) of scaffolds
Swelling/shrinkage studies were performed to calculate the water content (%) of the scaffolds, wherein 10 mg of freeze-dried scaffolds were placed in 500 μl of PBS at 37 °C. After 24 hours, these scaffolds were removed from the PBS, dabbed with a Kimwipe to remove any excess water on the surface, weighed and placed back into the buffer. The swelling ratio and shrinkage ratio (%) were calculated using the following equations. All experiments were performed 6 times.

Swelling ratio (SR)= ((Ww−W0)/W0)×100%
W0 and Ww are the initial dry weight and the wet weight, respectively.

Dental Pulp stem cells (hDPSC) Cell Culture
After obtaining informed consent and ethical approval from SIMATS ethics committee, the Dental Pulp stem cells were isolated from molars. The cells were cultured in DMEM low glucose/10% FBS/1%Penicillin;streptomycin. After two passages, 10000 cells per well were seeded in 48 well plates for cell viability and compatibility assays.

Biocompatibility Analysis (MTT Assay)
100 mg of 5 mm cylindrical blocks were prepared. The prepared blocks were immersed in DMEM- low glucose media formulated with 10 % FBS and 1% Penicillin/streptomycin. The media were collected after 24 hrs and 7 days of immersion and treated with cells to test the compatibility. After 24hrs of culture, add the 10uL/100mL of MTT reagent (5 mg/mL stock) to cultured cells and then incubate for 4 h to allow formation of the formazan dye at 37℃. The medium is exchanged to DMSO (200 μL) and stands for 10min. The reaction product was transferred to a 96 well ELISA plate and A590 was measured with ELISA plate reader.

Statistical analysis
All values are expressed as the mean ± standard error of the mean (SEM) of at least three independent experiments. A one-way ANOVA (analysis of variance) was used to test for significant differences, and multiple comparisons were performed using Scheffe's method. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

FIGURE 3 : The Picture depicts the SEM analysis of Control(CS + HAP) Group

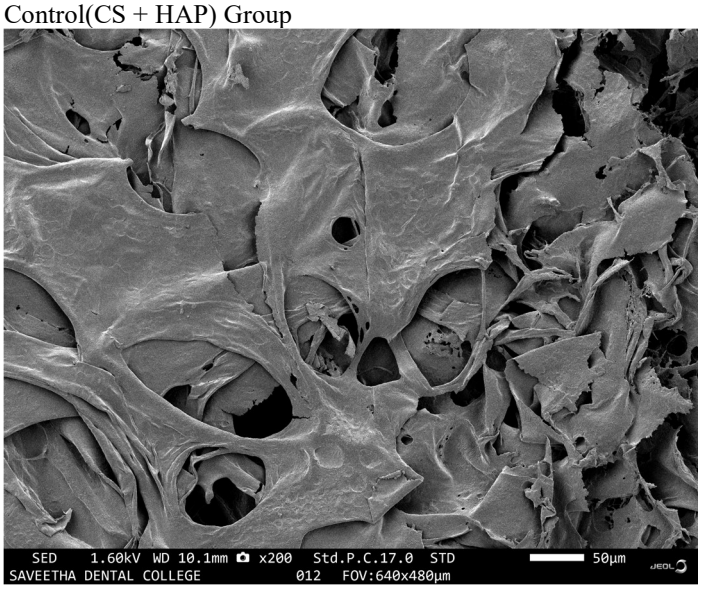


FIGURE 4: The Picture depicts the SEM analysis of Test(CS + HAP + AgO) Group

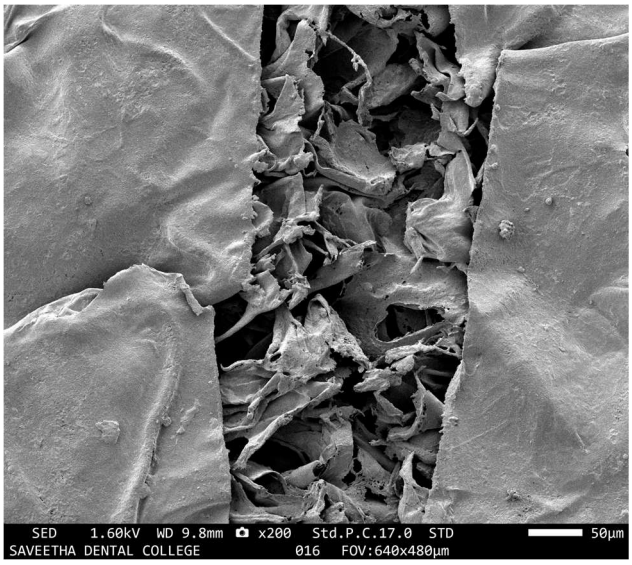


FIGURE 5: The Graph represents the FTIR of Control(CS + HAP) group

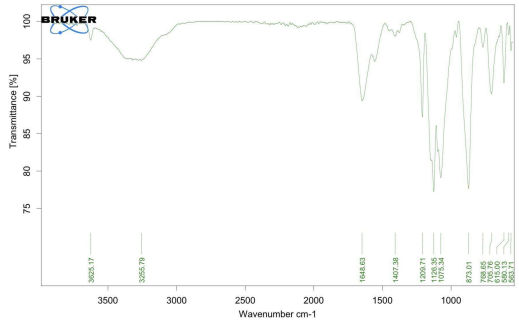


FIGURE 6: The Graph represents the FTIR of Test(CS + HAP + AgO) group

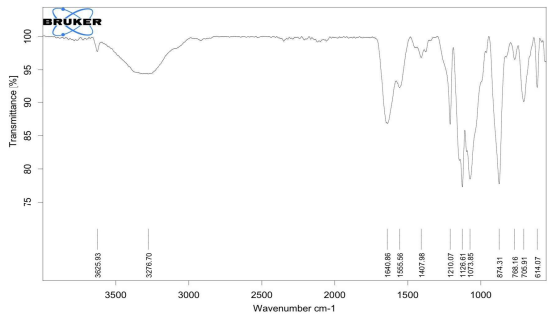


FIGURE 7: The picture depicts the Contact angle of Control(CS + HAP) group

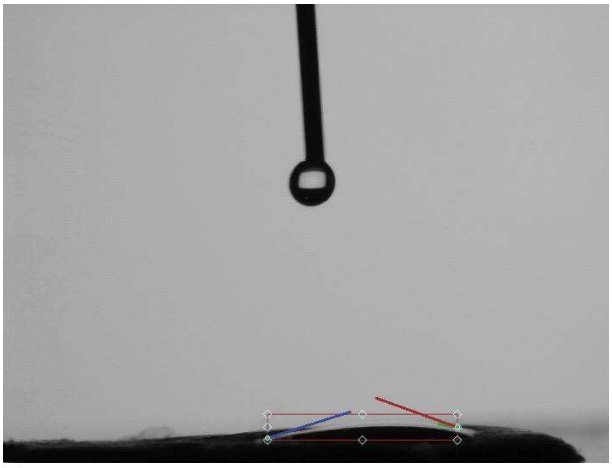


FIGURE 8: The picture depicts the Contact angle of Test(CS + HAP + AgO) group

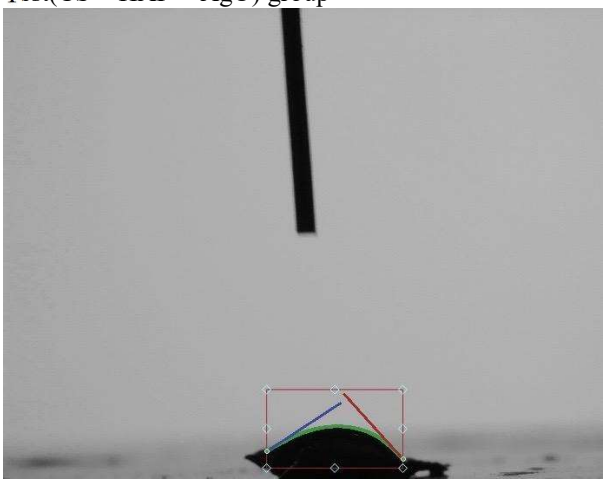
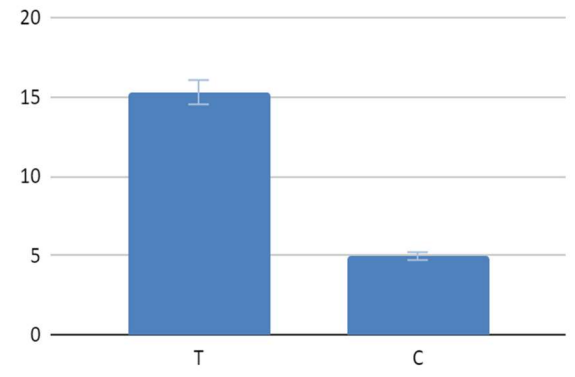


TABLE 1: The Table depicts the Contact angle of both Control(CS + HAP) and Test(CS + HAP + AgO) group

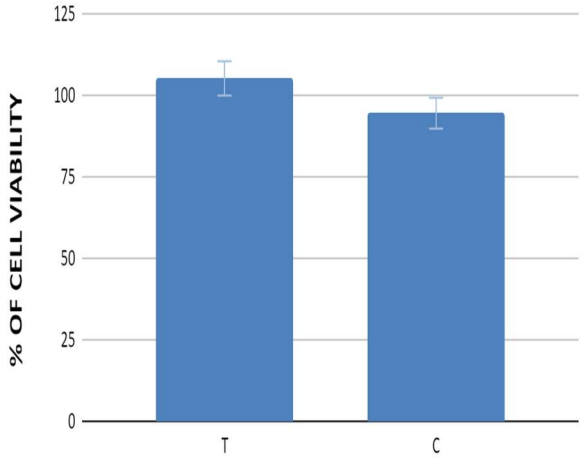
GROUP	CONTACT ANGLE	
Test	CS + HAP + AgO	40.57
Control	CS + HAP	18.66

FIGURE 9: The Graph represents the Comparison of Swelling between Control(CS + HAP) and Test(CS + HAP + AgO) group



SWELLING

FIGURE 10: The Graph represents the Comparison of Compatibility between Control(CS +HAP) and Test(CS + HAP + AgO) group



COMPATIBILITY

Alveolar bone loss is a common problem that affects dental implant placement (12). A barrier between the bone substitute and gingiva that can prevent fibrous-tissue ingrowth, bacterial infection and induce bone formation is a key factor in improving the success of alveolar ridge reconstruction (13). Chondroitin sulfate is a sulfated glycosaminoglycan (GAG) composed of a chain of alternating sugars (N-acetylgalactosamine and glucuronic acid) (14). It is usually found attached to proteins as part of a proteoglycan (15). A chondroitin chain can have over 100 individual sugars, each of which can be sulfated in variable positions and quantities (16). Chondroitin sulfate is an important structural component of cartilage, and provides much of its resistance to compression (17). It is necessary to prevent the invasion of soft tissue into bone defects for successful outcomes in guided bone regeneration (GBR) (18).

Figure 3 and Figure 4 illustrate the results of SEM analysis of the control group and the test group respectively. It is seen that the test group containing AgO based groups has comparatively less porosity and in an evenly distributed manner whereas the control group has more porosity and in a scattered manner. Additionally, in AgO decrease in porosity is highly evident that AgO based particles have higher cell adherence, increased cell migration, increased cell penetrating efficiency and a high flow of nutrition. Figure 5 and Figure 6 depicts the peak values of the control group and test group of AgO particles, which shows the characteristics of amine peak was noticed at 852, The spectral peak at 1103 was observed in the Silver oxide group which reveals the presence of AgO nanoparticle in the membrane.

From figure 7, the contact angle of the chondroitin sulfate and Hydroxyapatite is found to be 18.66 degrees, According to figure 8, the contact angle of the AgO particle with chondroitin sulfate is valued to be 40.57 degrees. Chondroitin sulfate, hydroxyapatite and this infusion of AgO nanoparticles has led to increase in the contact angle which further lead the substance to be hydrophilic which in terms produces a greater efficiency as a guided tissue regeneration membrane. Figure 9 explains about the swelling test between the two groups in which the control group has swelling of about 5% and addition of silver oxide elevated the swelling upto 16% swelling . This elevation indicates it can be used in the chemo static based membrane or socket preservation procedures. Figure 10 speaks about the compatibility between the control group and test group where the control group values around 98% and in AgO based particles shows around 112% which indicates this AgO based membrane is highly biocompatible and can be used in oral treatments.

Similarly, the study evaluated two silver coating methods and found controllable and precise coating achieved by sonication compared with sputtering (19). The optimized AgNP-coated collagen membrane exhibited excellent anti-bacterial effects against Staphylococcus aureus (S. aureus) and Pseudomonas aeruginosa (P. aeruginosa) with limited cellular toxicity (20). It also displayed effective anti-inflammatory effects by reducing the expression and release of inflammatory cytokines including IL-6 and TNF-alpha. Additionally, AgNP-coated collagen membranes were able to induce osteogenic differentiation of mesenchymal stem cells that guide bone regeneration. These findings demonstrate the potential application of AgNP-coated collagen

membranes to prevent infection after bone graft introduction in alveolar ridge reconstruction.

CONCLUSION

In this study a new material made by a chondroitin sulfate / Hydroxyapatite/silver oxide particle was fabricated and tested using Dental Pulp stem cells. In this study in that all the experiments were performed using a Dental Pulp stem cells were isolated from molars fully represent conditions in the human oral cavity, our data show that the CS/HAP/AgO hybrid fabricated in this study provides better osteoconductive properties and significant intracellular responses for bone grafts, making it reasonable to suggest this CS/HAP/AgO hybrid as a potential novel dental material for guided bone regeneration surgery.

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