

SYNTHESIS AND CHARACTERIZATION OF A NOVEL BONE GRAFT MATERIAL CONTAINING BIPHASIC CALCIUM PHOSPHATE AND CHITOSAN FORTIFIED WITH *ALOE VERA*

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RESEARCH ARTICLE

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ABSTRACT

The bone grafts were prepared using nanohydroxyapatite (nanoHAP) with the extracts of AV and mixed with beta tricalcium phosphate (β TCP) in a ratio of 60:40. The formed BCP was then conjugated with CH to form the bone graft. The Phytochemicals of AV imparts an anti-inflammatory and wound healing property to the bonegraft in addition to its osteoconductivity.

The bone graft was subjected to various conventional characterization techniques like FTIR, XRD, SEM, UV spectrophotometric analysis, compression and tensile strength to reveal its chemical composition, surface morphology and mechanical strength. The UV spectrophotometric analysis and SEM images revealed the mineral deposition on the bone graft after immersing in SBF, thereby increasing its ossification property. FTIR and XRD revealed the presence of BCP with nano HAP crystals and Chitosan in the graft. Compression and tensile strength of the graft showed that the graft can be used in non-load bearing areas.

In this study, a bone graft was synthesized using a novel technique of introducing the extracts of AV in the graft along with BCP and CH. The results revealed that the bone graft can be used as an Osteoconductive material for various biomedical applications.

Keywords: *Aloe vera*; Biphasic calcium phosphate; Chitosan; Nanohydroxyapatite; β -tricalcium phosphate; Bone graft.

INTRODUCTION

Calcium phosphate based bioceramics particularly hydroxyapatite (HAP) ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is widely used as a bone graft material due to its similarity with the inorganic phase of bone. (1) HAP shows the property of biocompatibility, osteoconductivity and non-antigenic response *in vivo*. (2, 3) Currently researchers are showing more interest in mimicking the natural nano composites system for various applications. Though nanoHAP is desirable to prepare bone/dental implants, due to its brittleness and poor mechanical properties biphasic calcium phosphate (BCP) is used in the current scenario. (4) BCP is more advantageous than HAP and β TCP due to its controlled

resorbability and rapid bone formation around the implant site. The resorbability can be strengthened by adjusting HAP/ β TCP ratio. The ratio of 60:40 is found to be ideal for new bone formation and osteoconduction. (5) This ratio shows a high uptake of calcium when compared with other combinations of HAP/ β TCP. BCP and biodegradable polymers such as Starch, Collagen, Chitosan (CH), casein, etc are the most suitable candidates for preparing biomaterials for use in bone tissue regeneration and fracture healing.

In this study, we have used Chitosan, a product obtained by the removal of acetyl group from chitin along with BCP. Chitosan is used in various biomedical applications due to its significant antibacterial activity, biodegradability, non toxicity, and

biocompatibility and beyond this it has positive charge which acts as a binding site for other functional groups. (6,7) Thereby, when chitosan is conjugated with BCP it improves the mechanical strength of the bone graft. The novelty of this paper lies in introducing the phytochemicals of *Aloe vera* while preparing nanoHAP. Extracts of AV is traditionally used as a medicine for rheumatoid arthritis and it imparts anti-inflammatory and wound healing property to the bone graft. (8-10) In addition to these properties it also provides mineral supplements for the surrounding tissues. This paper focuses on preparing a novel bone graft material using the phytochemicals of *Aloe vera*, BCP and CH. The prepared graft was characterized by FTIR, XRD and SEM to confirm their chemical composition and surface morphology. Moreover, mechanical strength of the bone graft was also tested to check its efficacy for use in non-load bearing areas.

MATERIALS AND METHODS

Preparation of β -TCP

An aqueous solution of $(\text{NH}_4)_2\text{HPO}_4$ (Sigma Aldrich) (325mL) was added to an aqueous solution of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (Sigma Aldrich) (500mL) under stirring. To this, 5mL of ammonia solution was added and stirred for 2 h. The mixture was filtered and dried in the oven at 60°C for 24 h. The flakes were then powdered and calcinated in the furnace at 850°C for 12 h followed by cooling to obtain single phase β -TCP.

Preparation of phytonano HAP

The whole plant of *Aloe vera* was collected and washed with distilled water. 2g of the whole AV was boiled in 50 mL of distilled water for 15 min and cooled to room temperature. 30 mL of the AV filtrate was added to 350mL of 0.4M alkaline calcium nitrate tetra hydrate solution, mixed thoroughly and incubated for 24 h at 30°C. The resulting solution was added to 400mL of 0.156M alkaline diammonium hydrogen phosphate salt solution, stirred and aged for 1 week at an ambient temperature of 30°C. Then the precipitate was washed several

times with deionized water and oven dried at 80°C.

Preparation of BCP

BCP was prepared by mixing phytonano HAP and β -TCP in the ratio of 60:40.

Preparation of BCP-AV-CH bone graft

0.5g of Chitosan (CH) (Sigma Aldrich) was dissolved in 3.0 mL of distilled water. This CH solution was added to 5 g of BCP and mixed thoroughly to make dough. This dough was extruded through a glass tube (1cm diameter) and dried. These implants were dried initially at room temperature (30°C) for 2 h and later at 100°C for 5 h, later cooled and stored.

Preparation of Simulated Body Fluid (SBF)

SBF was prepared by dissolving reagent grade NaCl (11.994 g), NaHCO_3 (0.525) g, KCl (0.336) g, $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ (0.342g), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (0.4575g), CaCl_2 (0.417 g) and Na_2SO_4 (0.1065 g) in Deionized water. (11,12) The solution was buffered at pH 7.4 with tris (hydroxyl methyl) Amino methane $(\text{CH}_2\text{OH})_3\text{CNH}_2$ and 1M Hydrochloric acid at $36.5 \pm 1^\circ\text{C}$. (13)

In vitro biomineralization test:

The biomineralization of the specimen were evaluated by their apatite forming abilities in SBF which was similar to human blood plasma. The prepared cylindrical implant BCP-AV-CH was soaked in SBF for 21 days. The immersed specimens were removed from the SBF, then abundantly rinsed using de-ionized water and dried for SEM investigations to show the formation of bone-like apatite layer on their composite surfaces.

Characterization

UV spectrophotometric analysis was performed for the SBF supernatant at 210 nm at an interval of 3 days and it was plotted as graph. The IR spectra of the prepared samples were read at wave length range of $4000\text{--}400\text{ cm}^{-1}$ using Nicolet Impact 400 FTIR spectrophotometer using KBr pellet containing 1–2 mg of the sample. XRD analysis of

prepared sample was conducted using an analytical X'Pert PRO alpha-1 with a RTMS X'Celerator detector. It used Ni-filtered Cu K α radiation over the 2θ range of $20-80^\circ$ at a scan rate of $2.4^\circ/\text{min}$ and with a sampling interval of 0.002° at 40 mA and 45 kV. The surface morphology was analyzed with a Topcon, SM-300 SEM. The copper disc was pasted with carbon tape and the sample was dispersed over the tape. The disc was coated with gold in ionization chamber before microscopic analysis. The implants were made cylindrical in shape with a length to diameter ratio of 2:1 using Instron 4501 model which was designed to minimize the end effect imposed by compressive loading and also with a 0.2 kN load cell or equivalent to check the tensile strength. The compressive strength was calculated from the break load and dimensions of the pellets.

RESULTS AND DISCUSSION

In vitro bioactivity test

The prepared bone graft BCP-AV-CH was kept in SBF solution for 21 days at 37°C . Later the implant was removed from the solution,

washed and dried at room temperature. The SBF supernatant was collected and at regular intervals (3days) and subjected to UV analysis.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of phytonano HAP, β -TCP, CH, BCP-AV-CH implant were shown in fig. 1(A) to fig.1 (D) respectively. The band at 605 cm^{-1} could be assigned to the anti symmetric bending motion of phosphate groups in HA. (14) The ν_1 phosphate bands representing β -TCP was seen at 943 cm^{-1} . The $-\text{OH}$ stretching bands of hydroxyl groups were seen around $3500-3700\text{ cm}^{-1}$. The FTIR spectrum of implant exhibits amide I and II absorption bands at 1649 cm^{-1} and 1682 cm^{-1} , respectively. The hydroxyl groups of CH were merged with those of HA and were seen as broad band at $1030-1095\text{ cm}^{-1}$. FTIR spectra of CH/AV showed the absorption peaks at 3345 cm^{-1} (O-H), 2928 cm^{-1} (C-H), 1722 cm^{-1} (C=O), 1585 cm^{-1} (hydrogen bonded N-H) and 1030 cm^{-1} (C-O).

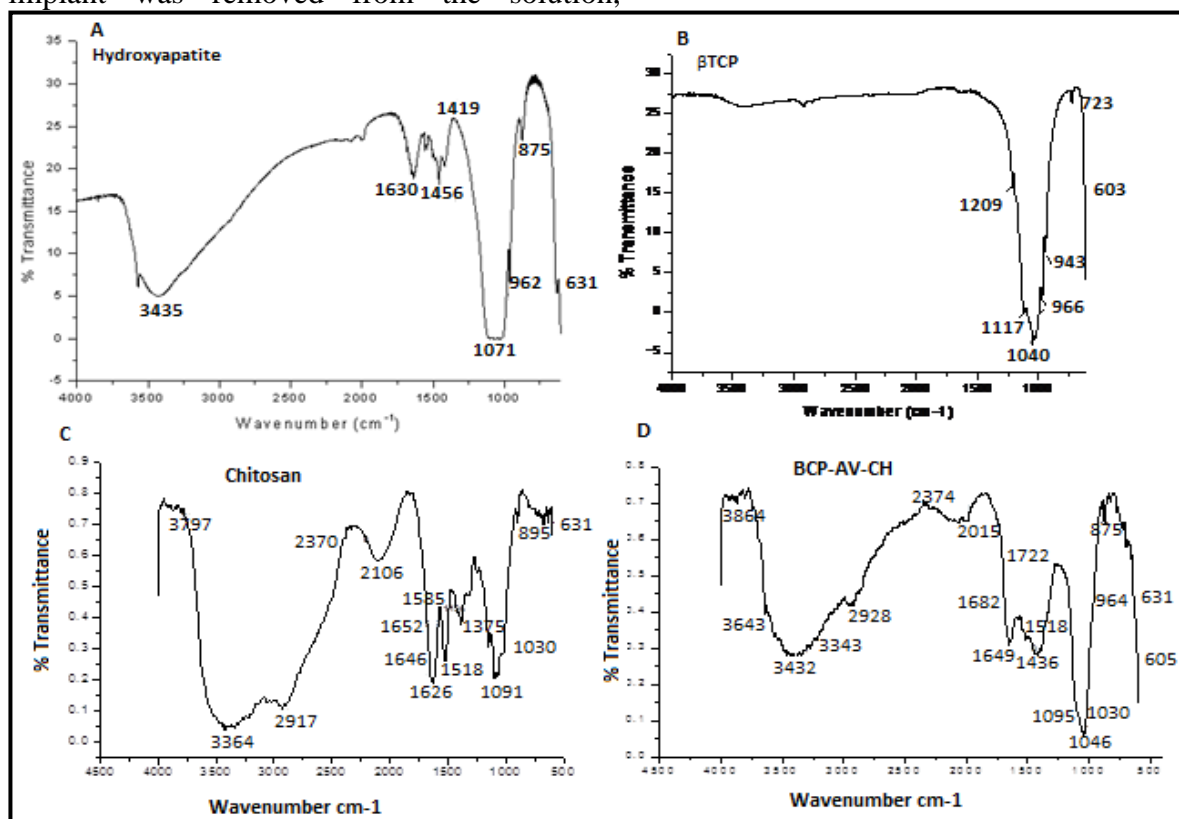


Figure 1: FTIR pattern of (A) phytonano HAP (B) β -TCP (C) CH (D) BCP-AV-CH graft

UV Spectrophotometric analysis

Fig. 2(A) shows the UV Spectrophotometric analysis of the implant in which a gradual deposition of calcium and phosphorous attains a stationary phase from 12-21 days. This indicates that the implant once placed *in vivo* exhibits the diffusion of calcium and phosphorous along with the phytochemicals and causes osteogenesis. (13)

X-ray diffraction (XRD) Analysis

Fig.2(B) shows the X-ray diffraction (XRD) pattern of BCP-AV-CH and suggest the presence of phytonano HAP and its

crystallinity decreases with increasing CH content. The peaks at 19.54° , 21.26° , 26.12° , and 28.77° indicates the reflection from 111, 202, 002, and 210 crystal planes, respectively, thereby indicating the presence of phytonano HAP. The peaks at 31.17° , 33.119° , 48.66° and 52.7° indicates the reflection from 222, 112, 130 and 315 crystal planes, respectively thereby indicating the presence of β TCP and CH. (15) All the samples show only characteristic peaks of β TCP suggesting that its phase did not change into other phases during preparation. This is important for achieving good mechanical and biological properties of produced scaffolds.

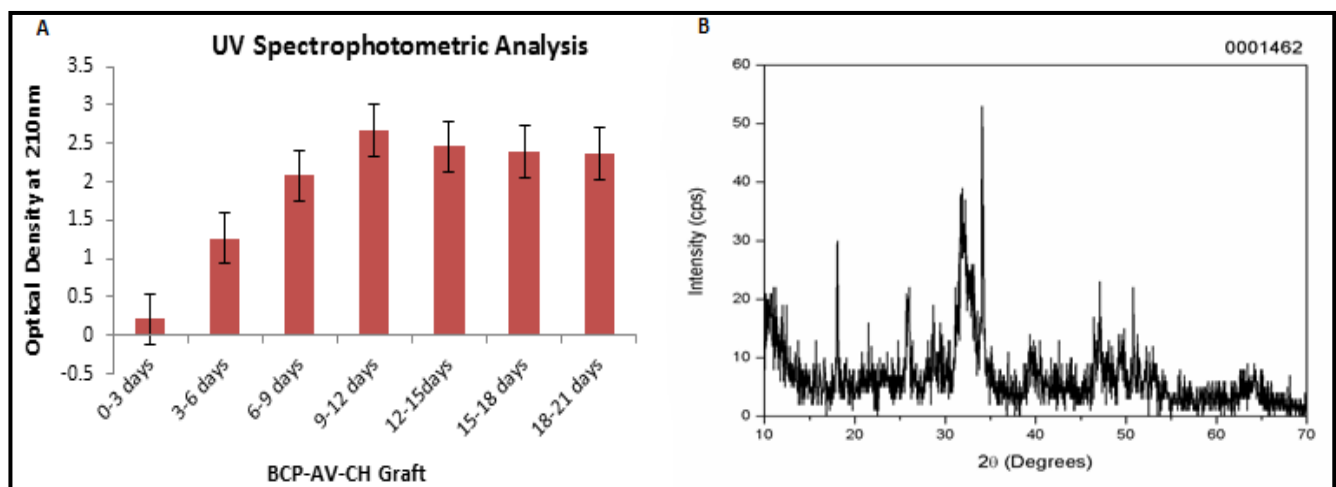


Figure 2: (A) UV spectrophotometric analysis of the graft (B) XRD pattern of the implant

Mechanical strength

Compression tests were performed to characterize the mechanical properties of the prepared scaffolds. The strength of the composite depends upon various factors like composition of the graft, particle size, nature of the additives and percentage moisture present in sample. (13) Fig. 3(A) showed the compression strength of BCP-AV-CH implant before immersing in SBF. The implant depicts the maximum compressive load of 193.40N which proves that the material can be used for non-load bearing bone graft material which can be attributed to the presence of BCP and CH. Tensile properties indicates how the material will react to forces being applied in tension. (16) Fig. 3(B) shows the tensile strength of the implant having 0.49 mPA and has a maximum load been applied 13.82 N which is suitable to

be a non-load bearing bone graft. Here the strength of the material is increased by the presence of CH which crosslinks with BCP.

SEM analysis

Fig. 3(C & D) depicts the SEM images of BCP-AV-CH composites before and after SBF respectively. The SEM images showed that spherical shaped surface indicating the presence of CH and the BCP imparts porosity to the material which is due to the presence of β -TCP in the graft. CH as a whole creates a porous matrix with BCP through which phytochemicals along with HA were embedded. The SEM picture of sample after SBF clearly shows the deposition of mineral phase (calcium phosphate) onto the crystals. (17) The aggregation was seen more due to the interconnection of the crystals via calcium

phosphate deposition. In addition, the polymer network seemed to be porous and ceramic

polymer interaction was not disturbed.

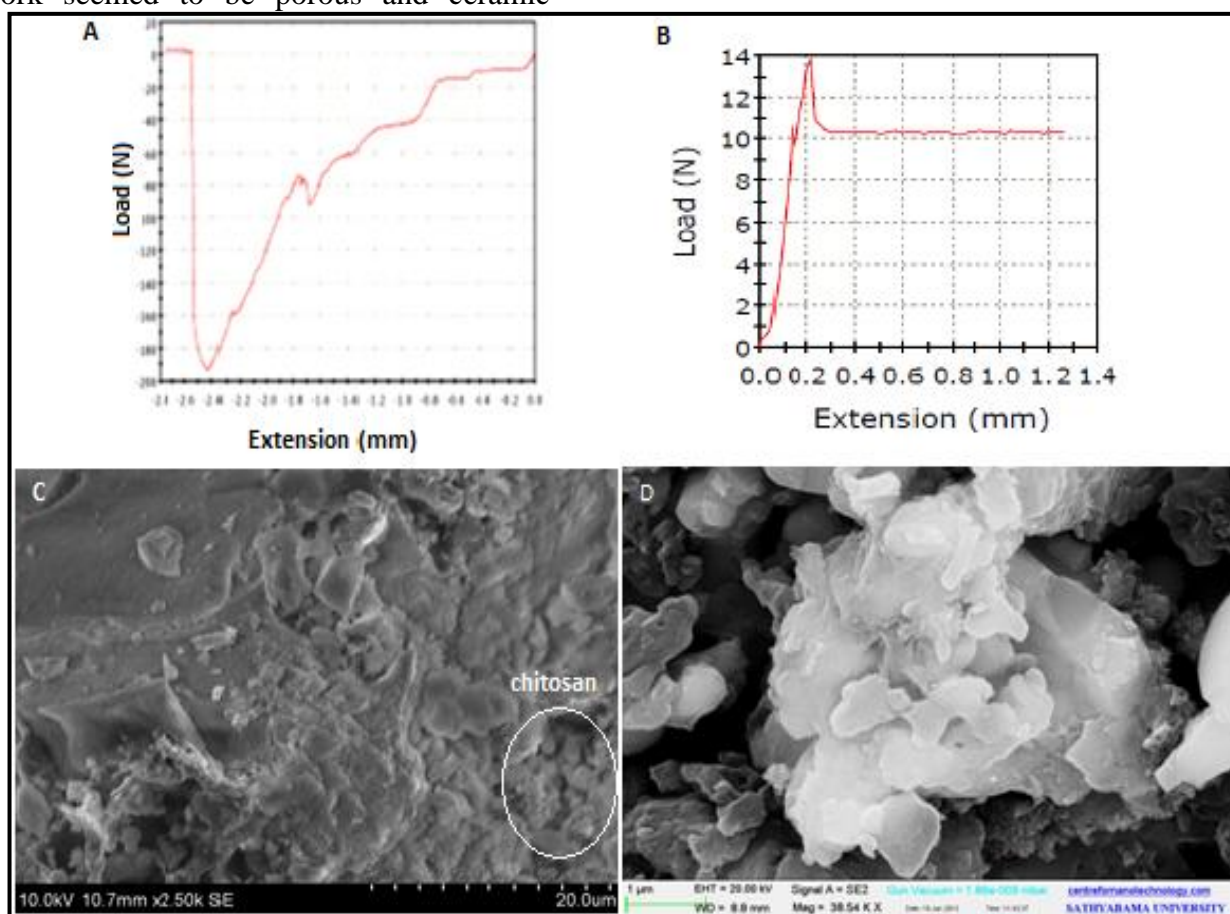


Figure 3: (A) Compression strength of the graft (B) Tensile strength of the graft (C) SEM image of BCP-AV-CH graft before SBF (D) SEM image of BCP-AV-CH graft after immersion in SBF

CONCLUSION

In this study, a novel bone graft containing Biphasic calcium phosphate was prepared with the phytochemicals of *Aloe vera* and it was conjugated with chitosan. The bone graft possessed better osteoconductivity and mechanical properties. Hence it can be used for fracture healing and other biomedical applications.

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CONFLICT OF INTEREST

Author declares that there are no conflict of interest.

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