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Biomimetically synthesized polymer-hydroxyapatite sheet like nano-composite

Suprabha Nayar · Ashit Kumar Pramanick · Binay K. Sharma · Gautam Das · B. Ravi Kumar · Arvind Sinha

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Abstract Biomimetic internal architecture is proving valuable for multi-tissue and structural tissue interface engineering. A biomimetic method developed for the in - situ synthesis of hydroxyapatite in poly (vinyl) alcohol, produces nanocomposites in the form of fabric-like sheets, when subjected to lyophilisation. The surface reactive hydroxyl groups of poly (vinyl) alcohol encourage site-specific coupling of hydroxyapatite. This results in improved mechanical properties, which may be better for biomedical applications when compared to a mere blend of the above two.

Introduction

A certain sect of Materials Scientists is always working in collaboration with orthopedic surgeons for the design and development of synthetic counterparts of natural structural components for improving quality of life. Hydroxyapatite [Ca_{10} $(PO_4)_6$ $(OH)_2$; {abbreviated HAp}] is increasingly used for biomedical applications as a bone substitute material in orthopedics and dentistry due to its excellent biocompatibility, bioactivity and osteo-conductive properties. However, the brittleness and low fatigue strength in physiological environment limit its use for load bearing repair or substitute. There are many efforts at developing composites with better mechanical properties, but almost are trying blending HAp with both polar and non-polar organic matrix, which restricts interface binding and mechanical properties of the composites. Here, we have been able to in situ synthesize HAp in Poly (vinyl) alcohol (PVA), a water-soluble synthetic polymer well known for its biocompatibility at ambient conditions. There do even exist reports that PVA hydrogels undergo swelling and can withhold the water volume equivalent to a cartilage [1–5]. In addition, the bioactive apatite content in PVA-HAp composite is nanosized and the in situ synthesis ensures a homogeneous distribution of apatite in PVA, similar to collagen in natural bone. Recent advancements in the field of materials science shows promise to synthesize nanomaterials by mimicking Nature and thus overcome many difficulties with the existing synthesis techniques of nanomaterials. This paper reports such an approach where the in situ biomimetic synthesis of HAp in PVA followed by lyophilisation results in a fabric like sheet. Reports on other biomimetic synthesis of HAp nanoparticles in PVA and cross-linking of PVA during freeze-drying without involving any toxic cross-linker has been the motivating force for the synthesis of this biomimetic-textile that may have applications ranging from wound dressing to tissue engineering [6, 7]. By this biomimetic route, we have an ideal ‘‘organoceramic,’’ not merely a blend. The product of interest is inorganic-organic intercalated structures; the molecular mechanism of which is thought to involve the simultaneous polymerization and ionic gelation of two oppositely charged molecules. A uniform macroporous structure of the fabric with reasonable mechanical integrity suggests that it may find applications as cartilage scaffolds that will guide cells to grow and facilitate the formation of functional tissues and organs.

S. Nayar (✉) · A. K. Pramanick · B. K. Sharma ·
G. Das · B. Ravi Kumar · A. Sinha
Materials Science and Technology Division,
National Metallurgical Laboratory, Burmamines,
Jamshedpur 831007 Jharkhand, India
e-mail: nayar@nmlindia.org

Materials and methods

In situ synthesis of HAp in PVA followed by lyophilisation resulted in nano-composite fabric like sheets. Freshly prepared calcium nitrate tetrahydrate solution of strength 0.4 M was made alkaline using ammonia: water in the ratio 1:2. The pH of the solution was maintained between 9 and 11. About 50 ml of calcium salt solution was added to 200 ml aqueous solution of PVA. The PVA solution was prepared by dissolving 20 gm PVA (obtained from Fluka, India, average molecular weight 1,25000) in 180 ml of doubled distilled water by slowly heating at 80 ± 5 °C. The solution was stirred thoroughly to obtain a homogeneous solution, incubated at a temperature of 30 ± 2 °C for 24 h. 0.156 M-diammonium hydrogen phosphate was made alkaline using ammonia: water in the ratio 1:1, pH between 9 and 11, and the required volume was added gradually to the above-incubated mixture. Milky white coloration was observed almost instantaneously, which was allowed to age for a week at a temperature of 30 ± 2 °C, after which it was decanted and washed thoroughly with de-ionized water. The slurry was then lyophilised at a predetermined subzero temperature. The HAp samples were structurally characterized using the following techniques. Scanning electron microscopy (SEM, JSM-840 A, JEOL) and transmission electron microscopy (TEM, JEOL 200 CX, Philips). The selected area electron diffraction patterns were recorded at 200 kV with a camera length of 86 mm. The camera length was determined by recording the diffraction patterns using a thin gold film. X-ray diffractometry where the angular range of 2θ was from 20 to 80° was done with a scanning rate of 0.5°/min (XRD, PTS 3003, Seifert, targets Co $K\alpha$). Fourier transform infrared spectroscopy (FT-IR-410 (JASCO)) and image analyzer (Metal Power analyser V.3.) were used for the other analysis. Tensile testing was done by using 1 kN load cell in a Universal Table-top Mechanical testing machine.

Statistical analysis

The results reported are an average of three different experimental set-ups.

Results and discussion

Lyophilization or freeze-drying, is a method of drying biological materials to stabilize them for storage. However, here we have used lyophilization to achieve a porous, friable structure. The results obtained in this study revealed that in situ synthesis of nanosized HAp in PVA followed by lyophilization resulted in a significant retention of porosity. The desirable physical characteristics of biomaterials scaffolds for tissue engineering applications include high porosity, large surface area, large pore size, and uniformly distributed interconnected porous structures throughout the matrix. In addition, the scaffold has to provide spatial signals to modulate the organization of the cells as well as that of the extra-cellular matrix. Our studies revealed that in situ synthesis of HAp in PVA followed by lyophilisation produced a hybrid fabric sheet (Fig. 1). Microstructural studies using SEM confirmed the formation of a macroporous structure with pore size in the range of 1–3 μm (Fig. 2). The microstructure confirms a cross-linked structure of PVA tubules of 20–40 nm in diameter and few microns in length. The formation of PVA tubules can be attributed to the partial solubility of PVA in water that results in the formation of micelle like structure that under goes self-assembly to minimize their surface energy [8]. Volume fraction of the pores in Fig. 2 was determined using quantitative metalography. Using Image Analysis software on the SEM micrograph of Fig. 2, the volume% of the pores was determined as 62.5, calculated on the basis of four different frames of reference. The emergence of the porosity in the polymeric matrix is attributed to the gelation assisted phase separation at low temperature. The PVA tubules get cross-linked as a result of phase separation stabilized by hydrogen bonds. Phase separation of polymer solution is a type of self -assembly, where instead of small molecules, big molecules arrange themselves to produce a porous microstructure. One can easily modulate the pore size by changing the temperature of freezing; generally lower the temperature the finer is the size of the pores. The PVA tubules containing hydroxyl functional groups attached to carbon backbone acts as specific sites for the adsorption of calcium ions in the solution. This attraction is similar to the interfacial molecular recognition in the process of biomineralization. The subsequent treatment of

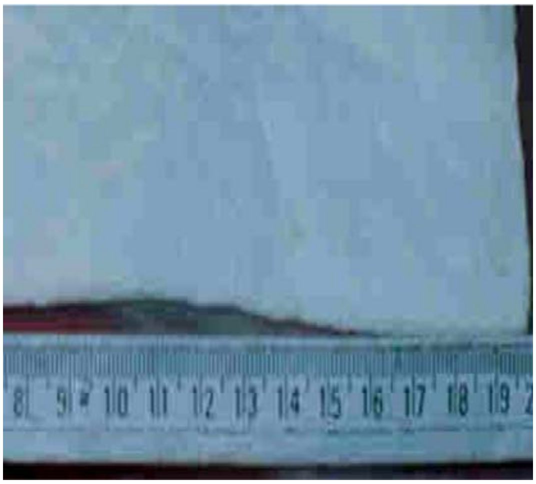

Fig. 1 PVA-HAp nanocomposite sheet as produced by freeze-drying

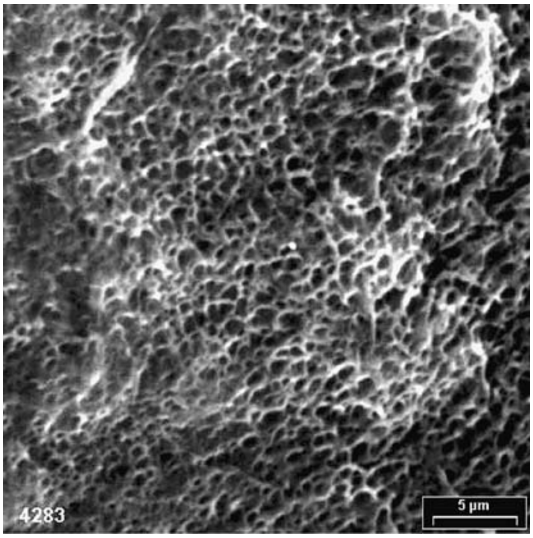

Fig. 2 Microstructure of the PVA-HAp sheet exhibiting macroporous structure

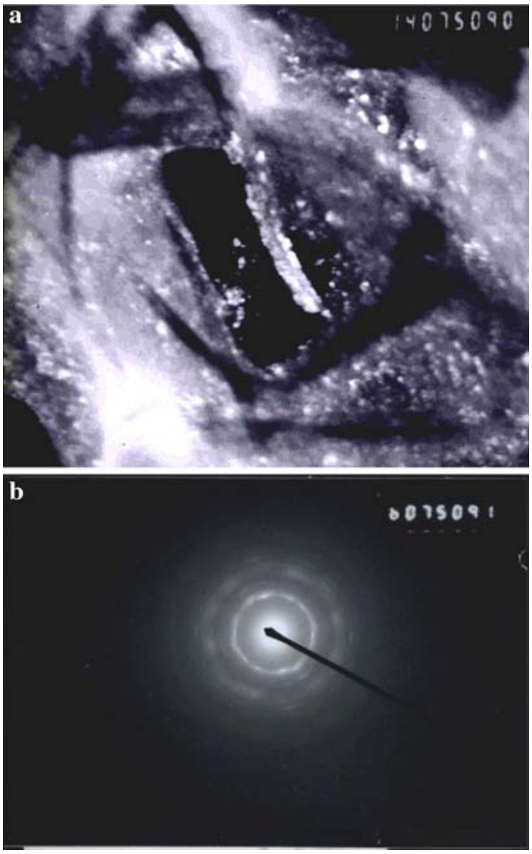

Fig. 3 (**a**) Dark field image of PVA-HAp composite revealing the precipitation of nanosized HAp particles in the polymeric matrix. (**b**) Selected area diffraction pattern of the precipitated nano particles corresponding to (112) and (222) reflections of HAp

calcium ions with phosphate ions in presence of ammonium hydroxide led to the precipitation of HAp nano-particles in PVA tubules. The presence of HAp particles was finally confirmed by TEM (operated at 160 kV) analysis that ensured the in situ precipitation of HAp nanoparticles in the size range 5–10 nm inside the PVA matrix (Fig. 3a). PVA mediated synthesis of HAp nanoparticles not only yielded the particles of uniform size but also kept them in monodispersed form. Crystalline nature of the precipitated particles could be confirmed by the selected area diffraction pattern revealing a polycrystalline structure with the diffraction rings corresponding to (112) and (222) reflection of HAp (Fig. 3b). XRD analysis also confirmed the formation of the hydroxyapatite phase in the synthesized nanocomposite with (002), (102), (210), (211), (300), (202) and (301) ,(222), (213) and (004) reflections of the HAp (Fig. 4). The peak widths are indicative of the nanosize of the HAp crystallites. It is interesting to note that in place of (211) as the peak with maximum intensity, our result shows (300) as the peak with maximum intensity. It indicates a preferential growth or rod like morphology of HAp as also confirmed by the superimposition of standard stick diffraction pattern of HAp. FTIR analysis of biomaterials show typical absorption bands at 3572 and 3433 cm^{-1} that correspond to the stretching mode of the OH group, this is also shown in the fabric (as indicated by the arrow in the figure) The hydroxyl vibration mode is found to be present near 632 cm^{-1} Other absorption bands at 3133.76 and 1638.23 cm^{-1} also correspond to the presence of hydroxyl groups in HAp. A band at 2397.08 cm^{-1} may be attributed to the trace amount of ambient water and carbon dioxide. Band at 1387.53 cm^{-1} reveals the presence of $–CH_2$ asymmetric bending and bands at 1033.66 and 565.04 cm^{-1} reveal the presence of phosphate group in the composite system. Bands at 828.28 and 602.65 cm^{-1} indicate the out of plane vibration of O–H and C–H of PVA. The band at 1764.85 cm^{-1} is for the C=O group, a shift from the 1700–1740 cm^{-1} wave number range may be attributed to a chemical bond formation across the inorganic-organic interface (Fig. 5). The mechanical property measurements revealed a typical ductile failure of the fabric at a stress of 0.30 MPa. The slope of initial linear portion of stress-strain curve exhibits a Young modulus of 7.5 MPa (Fig. 6). The obtained values of modulus and strength do not speak highly about the structural integrity of the composite under loading conditions. Optimizing the relative percentage of HAp and PVA in the system and also modifying the gelation kinetics to increase the degree of cross-linking can improve modulus.

Conclusions

A biomaterial scaffold produced under ambient conditions creates a milieu within which cells may be instructed to form a tissue or organ in a highly controlled way. The

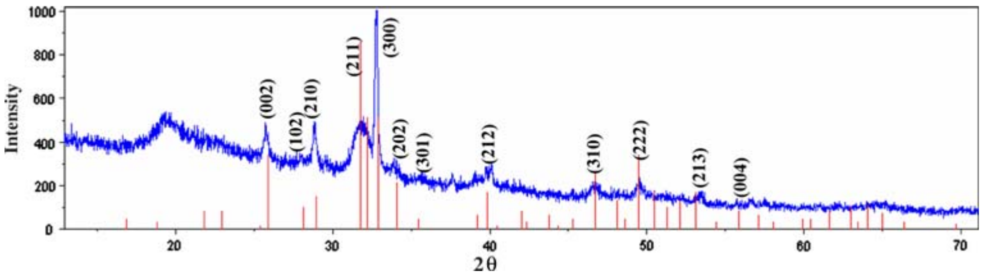

Fig. 4 X-ray diffraction pattern of the composite confirming the formation of HAp phase

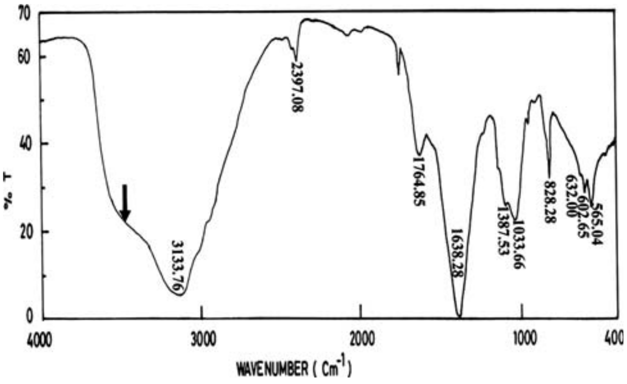

Fig. 5 FTIR spectrum of the PVA-HAp nanocomposite sheet

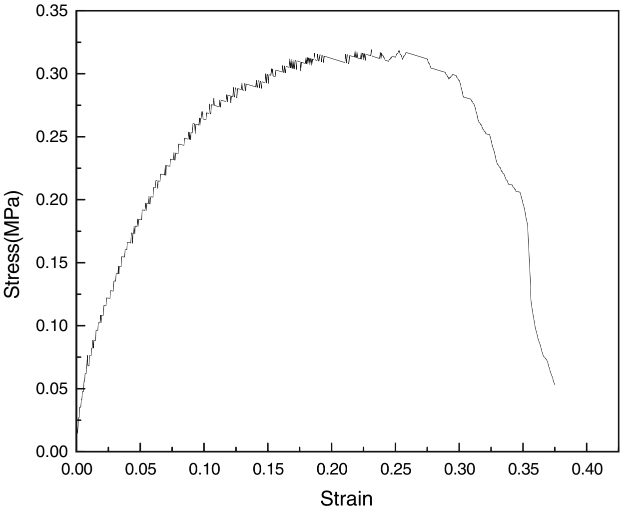

Fig. 6 Stress-strain curve of the PVA-HAp nanocomposite sheet

possibility of realizing a chemical bonding between HAp and PVA provides a wide scope for integrating HAp in varied composites. Advantages of in situ synthesis of nanosized HAp in poly (vinyl alcohol) are many; nanoparticles in scaffolds enhance biocompatibility, improve contact guidance and even reduce friction. Better is the interface binding; better is the mechanical behavior of the composites. AFM and FTIR studies indicate an intimate relationship between the organic and inorganic phase. Incubation of the fabric sheet in simulated body fluid (SBF) at 30 ± 2 °C for a month followed by atomic absorption spectroscopy (AAS) of SBF revealed no leakage of calcium and phosphate ions showing stability of the fabric. Altering physical or chemical characteristics of the scaffold may even promote tissue growth around the implant. Attempts are on to collaborate for such kind of studies.

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