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Polymer Ceramic Composite for Bone Regeneration Application

Poulami Basu[1], Bonala Sai Sharan[1], U. Narendra Kumar[2,3], I. Manjubala[1]*

[1]Biomedical Engineering Division, School of Bio Science and Technology, VIT University, Vellore 632 014, India
[2]Manufacturing Division, School of Mechanical and Building Sciences,, VIT University, Vellore 632 014, India
[3]Centre for Excellence in Nano Composites, School of Mechanical and Building Sciences, VIT University, Vellore-632014, India

*Corres.author: i.manjubala@vit.ac.in

Abstract : In this study we report the preparation of a composite scaffolds made from carboxymethyl cellulose (CMC) and hydroxyapatite (HA) via lyophilization technique. The morphology of the CMC/HA scaffold was observed in scanning electron microscope (SEM). The phase composition of hydroxyapatite prepared by sol-gel-microwave technique was characterized by Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). An antibiotic drug, amoxycillin, was incorporated in the composite scaffold and the release test was also performed. These results suggest that the CMC/HA scaffolds can be a promising material for bone regeneration.
Keywords: Carboxymethyl cellulose, hydroxyapatite, lyophilization, scaffold.

1. Introduction

The need to cure bone defects like bone infections and decay, bone tumours, damage and loss of bone due to trauma have influenced the biomedical researchers to open a wide area of research on bone regeneration [1, 2]. Bone is a natural composite-the inorganic constituent of it comprises of hydroxyapatite (HA) nanocrystals whereas the organic components consist of Type I collagen with traces of proteoglycans, glycoproteins and glycosaminoglycans[2]. The emerging field of tissue engineering plays a critical role to repair bone defects and injuries with the biological substitutes that induces the bone cells to regenerate the damaged tissues restoring the bone functions[3, 4, 5, 6]. The osteoconductivity of the HA and the osteoinductive property of the polymer made this composite a suitable platform for bone cells to grow, proliferate and differentiate to form a neotissue [7]. The presence of ceramic in the polymer matrix helps in providing a buffering effect to the polymer to minimize the rate of polymer degradation and helps in preventing unfavorable acidic environment to the surrounding cells and reduces side effects[8]. The chemical and structural properties, highest degree of biocompatibility and bioactivity of HA make it suitable to imitate natural bones[9]. HA has been extensively used in clinical purposes in the form of powder, granules, blocks, coatings and as various hybrid composites due to its properties such as grain size, pore size and wettability[10, 11]. Among natural polymers such as collagen, gelatin, fibrin and chitosan, cellulose based polymers such as carboxymethyl cellulose (CMC) has found application in wound healing as

skin substitute apart from its application in industries of textiles, flocculation, paper, drug and food preservatives[12]. The structure and properties of CMC resembles that of chitosan[13], whereas CMC is water soluble due to the presence of sodium carboxymethyl group. CMC is hydrophilic and highly viscoelastic. The biocompatible, biodegradable behavior of CMC helps to serve as a matrix in repairing severe wounds and injuries. CMC possesses highly organized structure of cellulose which makes it capable of guiding the growth of cells at various stages of development and can stimulate immune response simultaneously[7]. CMC can also influence the nucleation and growth of hydroxyapatite in the polymer matrix[12, 13], similar to influence of chitosan in HA nanocrystals formation[14].

In this study we report a novel composite prepared from CMC and HA via lyophilization technique. The morphology, structure, drug delivery ability and basic physiochemical properties of the resultant composite were investigated.

2. Materials and Methods

2.1 Materials

Carboxymethyl cellulose sodium salt (high viscosity, M.W. 90 kDa) was purchased from HIMEDIA, India. Calcium hydroxide ($Ca(OH)_2$) di-ammonium hydrogen phosphate ($(NH_4)_2HPO_4$) and ammonia were of analytical grade from Sisco Research Laboratories, India.

2.2 Preparation of Hydroxyapatite

Hydroxyapatite nano crystals were prepared using sol-gel technique via microwave irradiation method as reported earlier[15, 16]. Briefly, the calcium hydroxide and ammonium dihydrogen phosphate were mixed at 60 °C to form a sol-gel and then exposed to microwave radiation for 45 min (Microwave Convention system, 2.45 GHz, 800 watts, LG India). The formed hydroxyapatite gel was dried at 40 °C overnight.

2.3 Preparation of CMC-HA Scaffold

A homogenous viscous CMC solution with a concentration of 2 wt% was prepared in double distilled water by stirring for 2h at 50 ℃. The prepared hydroxyapatite powder was added to the CMC solution in ratios of 40 wt% and 60 wt%. The mixture was freezed at -20 ℃ to induce solid liquid phase separation to develop porous material. The samples were then introduced into lyophilizer and freeze dried at -80 ℃. An antibiotic drug, Amoxycillin was incorporated in the scaffold by absorption and the drug release profile was studied in water.

3. Characterizations

The phase composition of HA was studied by X-ray diffraction (XRD) using monochromatic Cu-K_α radiation (λ=1.54Aº) (GE Inspection Technology, Germany) and Fourier Transform Infrared spectroscopy (SHIMADZU Model: IR-Affinity 1, with resolution of 4). The surface morphology and pore structure of the composite was examined with scanning electron microscope in back scattered electron mode (CARL ZEISS EVO MA15, England). Water absorption capacity of the composite was evaluated with de-ionized water, by soaking for 4 hrs to determine its swelling capacity. The drug release profile was carried out in water and the stability tests were done in serum.

4. Results and Discussions

The scaffold for bone regeneration should be porous to serve as a platform for cells to proliferate, differentiate, form new tissue, act as channel for vascularity and supply of nutrients for healthy in-growth of bone cells[5, 6, 16]. In this study, lyophilization technique was used to prepare scaffold of CMC/HA composite in a suitable mould.

The X-ray diffraction pattern of hydroxyapatite nanocrystals is shown in Figure 1. The peaks related to the HA were observed at (211), (112), (300) and a distinctive peak at (002). The peaks indicate the crystalline nature of hydroxyapatite. The crystal size as determined using Scherrer formula is about 7.38 nm.

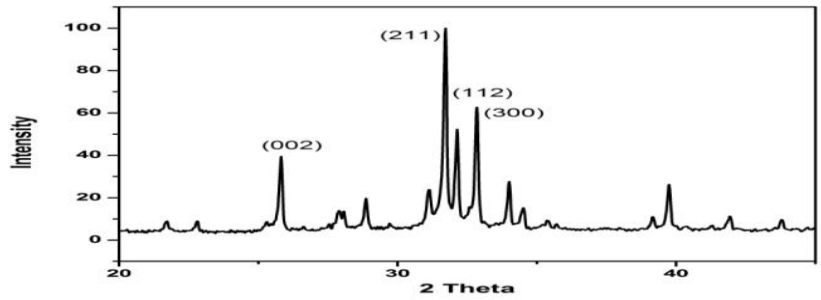

Figure.1. shows the X-ray diffraction pattern of the HA nanocrystals; the peaks are indexed according to the standard JCPDS 9-432.

The FTIR spectrum of nano-hydroxyapatite is shown in figure 2. The characteristic peak at 3571.8 cm^{-1} denotes OH^- stretching vibration and the band at 1048.2 cm^{-1} indicates the presence of phosphate ions. The other bands between 500 cm^{-1} to 850 cm^{-1} attribute to unique characteristic vibration of PO_4. These results suggest that the sol-gel-microwave method yield a pure form of HA nanocrystals in a semi-crystalline state. This HA powder was further utilized for composite formation with CMC.

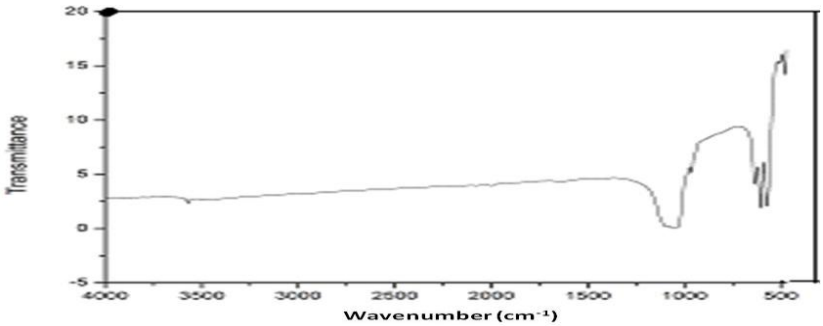

Figure.2. FTIR spectrum of the HA along with the prominent peaks of hydroxyl and phosphate groups.

The morphology of CMC/HA composite produced by the lyophilization technique is shown in Figure 3. As observed from Figure 3a, the surface of the scaffold is not smooth. It is a network of cellulose fibres formed from CMC which are coated or entangled with hydroxyapatite ceramic. The individual fibres of CMC are covered with hydroxyapatite cluster or agglomeration as shown in Figure 3b. The lyophilisation of the composite has induced the segregation of HA coated CMC fibres, compared to a matrix wall formation as in chitosan/HA scaffold[5].

The swelling behavior of CMC/HA scaffold in water was found to be 100 % whereas the release of the amoxicillin was constant for a period of 72 hrs in water.

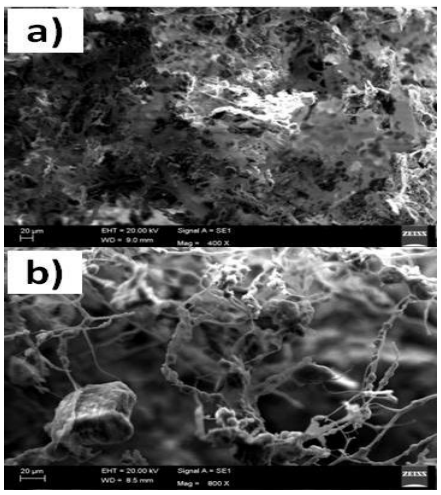

Figure.3. shows the SEM microphotographs of CMC/HA composite at a) 400X and b) 800X magnification level.

Conclusion

CMC/HA scaffold fabricated via solid liquid phase separation technique of lyophilisation yields porous network of CMC fibres coated with hydroxyapatite clusters. These fibrillar scaffolds seems to deliver the drugs for short duration and probably can be a delivery vehicle for antibiotics in inflammatory bone defects. The swelling behavior in water proves it to be a better wound healing material. Further in vivo studies are needed to evaluate its efficacy in bone regeneration.

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