Designing biological apatite suitable for neomycin delivery

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Bovine-derived biological apatite has a long history for use in human health care as bone substitute. This article reports an elegant method of designing bovine-derived hydroxyapatite (BHA) blocks suitable for drug delivery. Neomycin sulphate (NS) was selected as a model drug and its loading and release studies were performed under *in-vitro* physiological conditions. The results indicate that phase purity and structural integrity of the BHA are retained even after entrapment of drug molecules. It was noticed that the rate of neomycin delivery was directly proportional to the porosity of the BHA blocks. The *in-vitro* release studies suggest that BHA may be used as a good carrier system for neomycin delivery.

1. Introduction

Bone diseases associated with infection are highly complicated clinical problem, requiring further medical care as they directly affect quality and length of human life. Most of the infectious bone diseases (osteomyelitis, for example) are usually caused by bacteria. Currently, there are a few drug delivery systems (DDS) available for the treatment of infectious bone diseases, based on biodegradable and non-biodegradable polymers. Polymethylmethacrylate (PMMA) has been used as a delivery system [1], but often requires an additional surgery to remove them because of its non-biodegradability. As an alternative, collagen [2] and polylactic acid [3] have been developed as biodegradable DDS. Although these are good delivery systems, they do not have the ability to replace bone grafting. A perfect bone DDS should not only have the ability to deliver the therapeutic molecules at the defective sites but also direct or augment bone grafting upon implantation. Therefore, designing bone DDS using substances that are quite similar in composition to natural bone is a good choice to facilitate efficient delivery of drug and to promote bone growth.

Hydroxyapatite (HA) is a class of bioactive substance rich in calcified tissues. It is widely used as a drug carrier and as a bone substitute owing to its excellent biocompatibility, bioactivity, osteoconductivity and capability of guiding bone regeneration. Accordingly, selection of HA as a candidate DDS for the treatment of infectious bone diseases is highly beneficial rather than polymers. Neomycin sulphate (NS) is one of the most potent broad spectrum antibiotics used extensively in the treatment of bacterial infections associated with the bone diseases. It is an effective therapeutic molecule against gram-positive and gram-negative microorganisms. Accordingly, selection of NS as a model drug is highly beneficial for the treatment of infectious bone diseases. In this study, bovine-derived hydroxyapatite (BHA) was investigated for its suitability in neomycin delivery under *in-vitro* physiological conditions.

2. Experimental procedure

2.1. Sample preparation

BHA was processed from bovine bones by chemical and thermal processes as reported [4, 5]. The porous BHA blocks were fabricated by a die-pressing method using polyvinylbutyral (PVB) as reported [6]. Briefly, the powder mixture containing 500 mg of BHA and 30 to 60 vol.% of PVB was prepared and die-pressed uniaxially at a pressure of 150 MPa for 15 min. The die-pressed blocks were heated to 500°C at a heating rate of 5°C/min to sublime the PVB particles and subsequently sintered at 1000°C at a heating rate of 10°C/min for 6 h. Under ambient sintering conditions, decomposition of HA has been reported

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to begin in the range 1050–1100°C [7]. Further, high temperature sintering may reduce the reactivity of HA [8]; therefore 1000°C was chosen as a sintering temperature. The resulting porous blocks were stored in a vacuum desiccator until assay. The blocks were named as BHA-30 and BHA-60 with respect to their porosity of about 30 and 60 vol.%, respectively.

2.2. Characterization techniques

The phase purity and structural characteristics of the blocks were examined with a powder X-ray diffractometer (Shimadzu XRD-600, Japan). A spectroscopic method was employed to determine the functional groups of the blocks using a Fourier transform infrared (FT-IR) spectrophotometer (ThermoNicolet Avatar 360, USA). The porosity of the blocks was determined by a mercury intrusion porosimeter (Quantachrome Instr., USA) with a minimum filling pressure of 0.5 psi and a maximum of 50 psi. Total porosity was calculated from a total mercury intrusion volume and from a pore size (d) using the Washburn equation $d = -4\nu \text{ Cos } \theta/P$, where ν is mercury surface tension (0.048 N/m), θ is contact angle between mercury and pore wall and P is the pressure above the mercury. The compressive strength was measured by a universal testing machine (Instron, UK) at a cross head speed of 1 mm/min. For this exclusive experiment, four types of the blocks were designed with a volume porosity of 30, 40, 50 and 60% in accordance with ASTM F451-86 testing protocol for the bone materials. The experiments were run in duplicate and the average was taken into account. The loading of NS into the blocks was carried out in phosphate buffered saline (PBS) at pH 7.4 under vacuum condition. The blocks were immersed in 5 ml PBS solution containing 500 mg of NS (100 mg/ml) and decompressed under the vacuum of 250 mm/Hg for 30 min within a high vacuum system. The percentage of loaded NS was calculated as below: % loading $= (X - Y) \times 100/X$. Where, X and Y denotes the initial and final NS concentrations in the loading buffer, respectively. The total amount of NS content was assayed at a wavelength of 257 nm [9] using a UV spectrophotometer (Nicolet UV300, USA). To evaluate its *in-vitro* release characteristics, each NS loaded blocks was placed in a container separately with 5 ml PBS solution of pH 7.4 at 37°C in a thermostatic chamber. The release medium was collected at pre-determined time intervals and the amount of NS released was determined.

3. Results and discussion

As physicochemical properties of the delivery system play a vital role in determining stability and release rate of the drug, it is noteworthy to evaluate these properties. The powder XRD technique was engaged to appraise phase purity and structural integrity of the porous BHA blocks. Fig. 1 represents a series of XRD patterns of BHA before and after NS loading. The XRD pattern of BHA shows a few crystalline diffracted peaks approximately at 26, 28, 29, 30–35, 39, 46, 49 and 50° (2θ) corresponding to the characteristic peaks of apatite (Fig. 1a), which is in accordance with the standard data of International centre

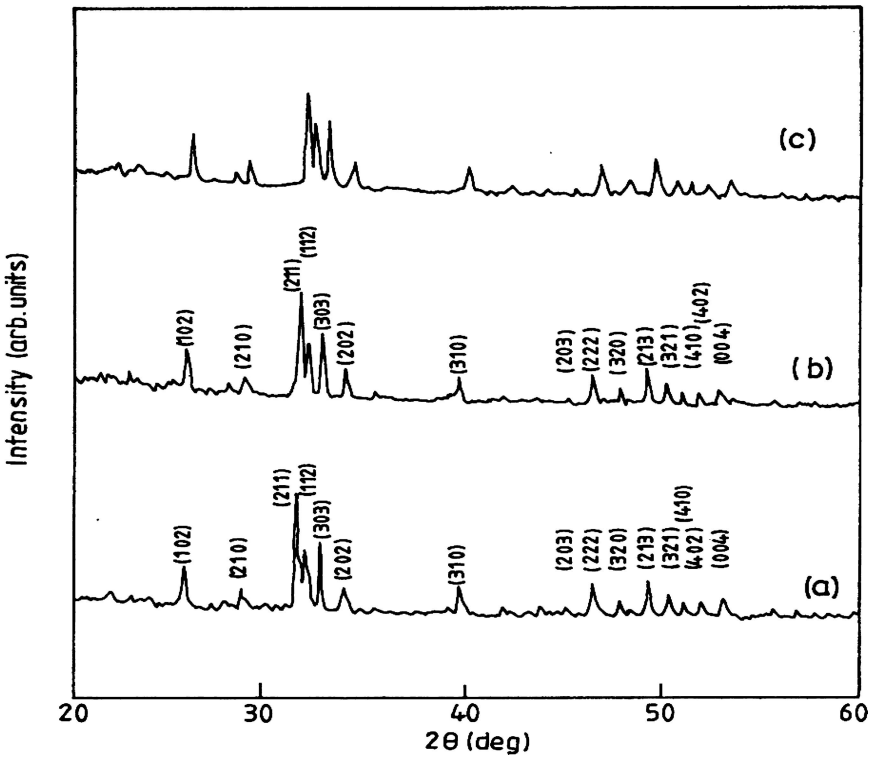

Figure 1 XRD patterns of (a) BHA, (b) porous BHA before drug loading, (c) after drug loading.

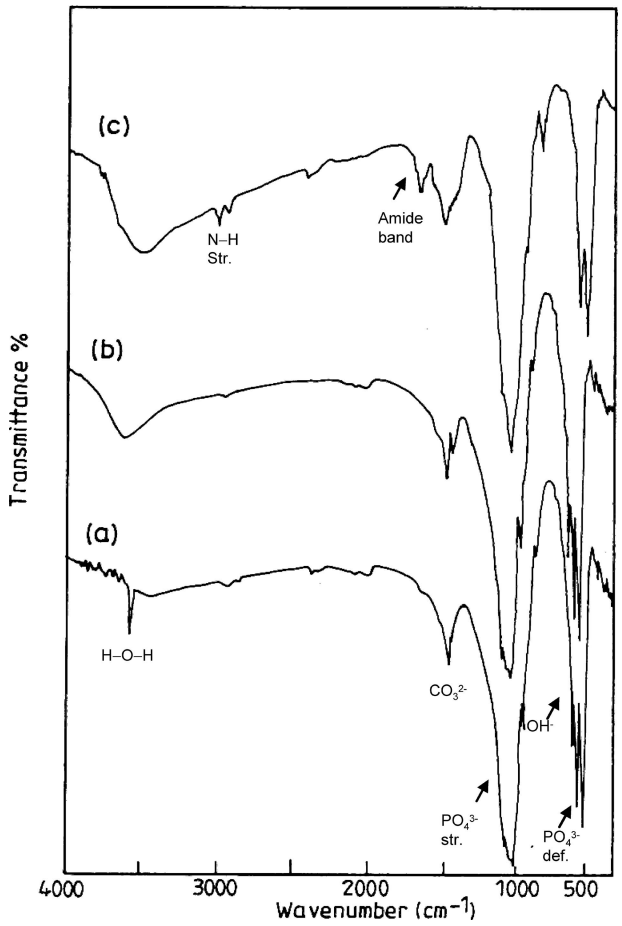

Figure 2 FT-IR spectra of (a) BHA, (b) porous BHA before drug loading, (c) after drug loading.

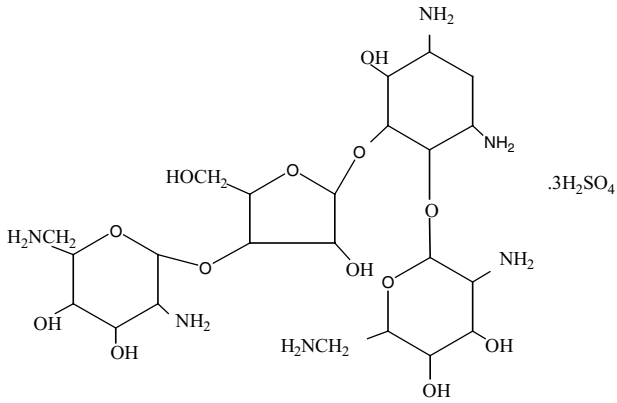

Figure 3 Chemical structure of neomycin sulphate.

for diffraction data (ICDD) file 9-432 [10]. There were no significant changes observed between the porous BHA blocks before and after drug loading (Fig. 1b and c, respectively), which suggests that the entrapment of drug molecules did not alter the structure of apatite phase. Further, it indicated that the influence of drug molecules into the pores of BHA has not persuaded any phase transformation, which means the drug has not chemically reacted with carrier system. The resolved XRD peaks were indexed on the basis of hexagonal crystal system of space group $P6_3/m$. These results clearly indicated the retention of phase purity and structural integrity of the BHA system.

Fig. 2 illustrates FT-IR spectra of BHA and porous BHA before and after drug loading. The results of FT-IR of BHA (Fig. 2a) and porous BHA (Fig. 2b) displayed all the characteristic absorption peaks corresponding to the apatite phase. The peaks at 1043 and 970 cm^{-1} can be assigned to the stretching vibrations of PO_4^{3-} ions and peaks at 605 and 569 cm^{-1} can be assigned to the deformation vibrations of PO_4^{3-} ions. The peaks due to the variations of OH^- ions appeared at 3570 and 640 cm^{-1}. The spectrum of drug loaded porous BHA (Fig. 2c) shows a specific peak at 1632 cm^{-1} pertaining to N-H bending band and a peak 2920 cm^{-1} corresponding to N-H stretching band in addition to the apatite absorption peaks. These two peaks originated from the amine group of NS, which provides a qualitative proof of drug loading into the BHA blocks. The chemical structure of NS is given in Fig. 3 to identify its reactive functional groups. On the other hand, a peak detected at 1450 cm^{-1} indicate the presence of carbonate ions in all the BHA samples, suggesting that the BHA processed under the above experimental condition is of carbonate-substituted HA similar to a natural bone apatite. As bone minerals of human body contain a substantial amount of carbonate ions (3–8 wt%), substitution of carbonate in the apatite phase is an added advantage, which may enhance bioactivity and mechanical strength of the delivery system [11, 12].

As porosity of the delivery system is a critical factor, which not only determines the drug loading but also essential for the satisfactory bone tissue in-growth and for other histological responses associated with a bone metabolism, the present study carefully measured the porosity of the BHA blocks. The percentage porosity of the blocks was found to be 30% for BHA-30 and 60% for BHA-60. The volume porosity of 40% (named as BHA-40) and 50% (named as BHA-50) were also fabricated for the exclusive measurement of this compressive strength in order to correlate their relationship with the porosity. The increase in porosity may frequently be accompanied by a significant reduction in the mechanical strength of biomaterials. Further, the relationship between compressive strength and porosity can be unified in terms of a polynomial equation, which describes the role or influence of macro/micro porosity on the compressive strength of porous HA [13]. Keeping the above factors in view, the compressive strength of porous BHA blocks (30 to 60 vol.%) was measured in this study using a universal testing machine and the data obtained are depicted in Fig. 4. The compressive strength was found to be 60.6 ± 2.6 MPa for BHA-30, 53.4 ± 1.8 MPa for BHA-40, 32.6 ± 2.0 MPa for BHA-50, and 17.1 ± 2.2 MPa for BHA-60. These values are quite comparable to the compressive strength of cancellous or trabecular bone [14], which is in the range of 10–50 MPa. The overall results suggest that increase

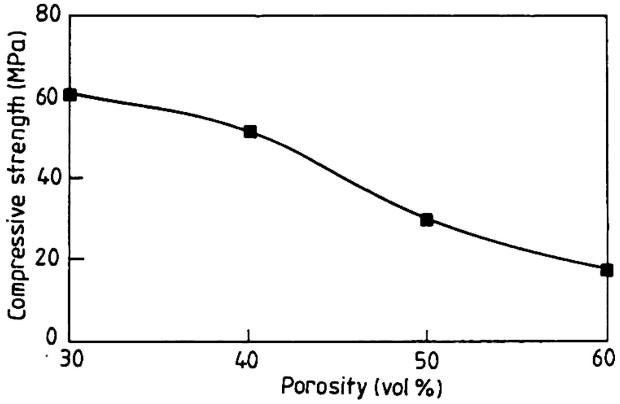

Figure 4 Porosity-compressive strength relationship of BHA blocks.

in volume porosity decreases the compressive strength of the BHA blocks.

To obtain the absorption rate of NS, changes observed in the weight of BHA block from before soaking to after soaking was divided by its weight before soaking. It was determined that the BHA-30 and BHA-60 blocks were loaded with 160 and 325 mg of NS, respectively when immersed in 5 ml PBS solution having a NS concentration of 100 mg/ml. The percentage loading of NS was found to be $32 \pm 2.14\%$ for BHA-30 and $65 \pm 1.64\%$ for BHA-60. These results clearly indicated that the BHA-60 has taken-up a higher amount of NS as compared to BHA-30. It is believed that the higher amount of drug loading could be attributed to the nature of their higher porosity. Hence, it can be concluded that the drug loading efficiency is directly proportional to porosity of the blocks.

The *in-vitro* release profiles of NS are plotted in Fig. 5. The graph shows that the amount of NS released from the BHA-60 was quite higher than BHA-30 due to its higher porosity. The initial burst release (42%) was observed in the case of BHA-60 for the first three days. As NS loading content increased, the fraction of exposed drug molecules in the BHA blocks were also increased, which might have induced the initial rapid drug release. It was found that 48% of NS was released in 30 days from the BHA-30, whereas 94% was released from the BHA-60 for

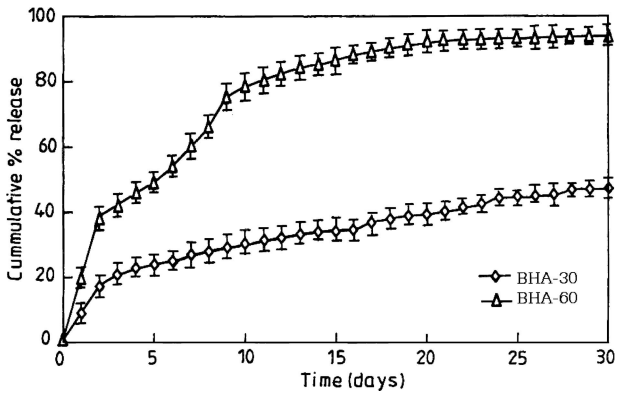

Figure 5 The *in-vitro* release profiles of neomycin sulfate from the BHA blocks.

the same period. Here, the initial burst release followed by a sustained release observed for 30 days may be attributed to the entrapment of NS into blocks. It may even help to avoid toxicity due to the over dosage of drug. The drug release profiles obtained from the two blocks show a relatively similar trend, but the characteristic parameters such as 50% drug release time and total drug release time are significantly different from each other. The total amount of drug released from the BHA-60 was quite higher than BHA-30 owing to the higher porosity. It is also appreciated to have 60 to 80% volume porosity for the occurrence of extensive bone in-growth and to transpire a strong interfacial fixation [15]. It is also recognized that high concentration of antibiotic at the bone-implant interface is essential in order to prevent diseases associated with bacterial infections [16]. Further, it is anticipated that the remaining BHA, after completion of the NS release, would function as a temporary replacement until a new bone forms. Therefore, the BHA could play a dual role as a drug carrier and as a bone graft substitute.

4. Conclusions

This study demonstrated the feasibility of using porous BHA as a carrier system for the delivery of neomycin under *in-vitro* physiological conditions. The results indicated that strength of the BHA blocks decreased with increasing porosity. It was also noticed that porous BHA-60 allows higher loading and release of neomycin than porous BHA-30, indicating that rate of drug delivery is directly proportional to the porosity of the blocks. Based on the experimental results, BHA may be used as a good carrier system for neomycin delivery.

Acknowledgments

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