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Novel bone substitute composed of chitosan and strontium-doped α-calcium sulfate hemihydrate: Fabrication, characterisation and evaluation of biocompatibility

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ABSTRACT

Calcium sulfate is in routine clinical use as a bone substitute, offering the benefits of biodegradability, biocompatibility and a long history of use in bone repair. The osteoconductive properties of calcium sulfate may be further improved by doping with strontium ions. Nevertheless, the high degradation rate of calcium sulfate may impede bone healing as substantial material degradation may occur before the healing process is complete. The purpose of this study is to develop a novel composite bone substitute composed of chitosan and strontium-doped α-calcium sulfate hemihydrate in the form of microcapsules, which can promote osteogenesis while matching the natural rate of bone healing. The developed microcapsules exhibited controlled degradation that facilitated the sustained release of strontium ions. *In vitro* testing showed that the microcapsules had minimal cytotoxicity and ability to inhibit bacterial growth. *In vivo* testing in a mouse model showed the absence of genetic toxicity and low inflammatory potential of the microcapsules. The novel microcapsules developed in this study demonstrated suitable degradation characteristics for bone repair as well as favourable *in vitro* and *in vivo* behaviour, and hold promise for use as an alternative bone substitute in orthopaedic surgery.

1. Introduction

The increasing prevalence of bone defects caused by trauma, osteomyelitis, tumour and other types of bone diseases has created an urgent need for synthetic bone substitutes which are biodegradable and have properties similar to natural bone. Many inorganic materials have been investigated for this purpose due to their chemical similarity to the mineral phase of bone, which promotes the formation of a direct bond with host bone and enhanced osteogenesis [1]. Of these materials, α-calcium sulfate hemihydrate (commonly referred to in the medical literature as calcium sulfate) stands out as a candidate material due to its good biocompatibility, biodegradability, osteoconductive properties and a long history of use in bone repair [2–5]. Upon degradation, α-calcium sulfate hemihydrate forms a slightly acidic environment and results in a high local calcium concentration which promotes osteogenesis [6].

α-Calcium sulfate hemihydrate can be doped with strontium to further improve its ability to promote bone repair. The concentration of strontium ions is maintained at a high level during new bone formation, after which the concentration declines and is held at a constant level in the extracellular fluid of bone cells to facilitate the normal function of bone [7]. The mechanism of action of strontium ions in promoting bone formation is to increase the expression of osteogenic genes and alkaline phosphatase activity in bone marrow-derived mesenchymal stem cells (BMSCs), as well as to inhibit their secretion of receptor activator of nuclear factor-κB ligand (RANKL) which is essential for osteoclast differentiation [8,9]. By releasing strontium ions upon degradation, strontium-doped α-calcium sulfate hemihydrate allows the strontium ion concentration to be maintained at high levels within the defect site, which is advantageous for bone repair as shown in our previous report [10].

The current problem which remains to be solved before strontium-doped α-calcium sulfate hemihydrate can be considered for clinical application is its fast degradation rate. α-Calcium sulfate hemihydrate in current clinical use undergoes complete degradation in 6 to 9 weeks, which is much shorter than the period of time required for satisfactory bone healing to occur (12 weeks) [11]. Following degradation of the implanted material, an empty space remains within the defect site which becomes occupied by fibrous tissue and results in incomplete healing of the defect. The same problem applies to strontium-doped α-

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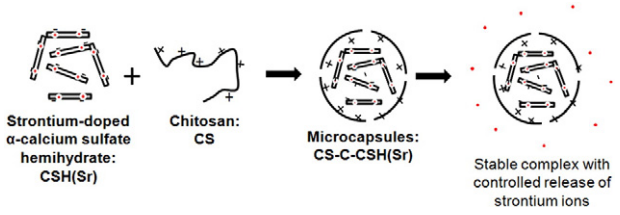

Fig. 1. Schematic illustrating the synthesis of microcapsules (CS-C-CSH(Sr)) composed of chitosan (CS) and strontium-doped α-calcium sulfate hemihydrate (CSH(Sr)).

calcium sulfate hemihydrate, with the additional complication that fast degradation will result in the rapid release of strontium ions at a rate that is mismatched to the rate of new bone formation.

The purpose of this study is to develop a novel composite bone substitute composed of strontium-doped α-calcium sulfate hemihydrate as the base material, with controlled degradation that facilitates the release of strontium ions with a linear release profile to match the rate of new bone formation. To achieve this goal, chitosan was chosen as the material used to encapsulate strontium-doped α-calcium sulfate hemihydrate in order to control its degradation rate. Chitosan is a linear polysaccharide derived from the natural biopolymer chitin, which is found in crustacean exoskeletons [12]. The benefits of using chitosan as a biomaterial, particularly for orthopaedic applications, include biocompatibility and minimal inflammatory potential, intrinsic antibacterial properties, biodegradability, and the ability to support bone formation on its own or when incorporated into various composite systems [13]. Chitosan has also been frequently investigated as a vehicle for drug delivery in the form of micro- and nanoparticles, which can provide controlled and versatile release profiles depending on the method of preparation [14]. A further advantage of using chitosan to form the composite microcapsules in this study is that it has a natural positive charge, which can be anticipated to interact with the negative charges present in strontium-doped α-calcium sulfate hemihydrate to result in a stable complex with controlled release characteristics (Fig. 1).

The present study explores, for the first time, the synthesis of novel microcapsules composed of chitosan and strontium-doped α-calcium sulfate hemihydrate, and reports their composition, morphology and degradation characteristics, as well as the evaluation of their *in vitro* and *in vivo* biocompatibility. The main novelties of the microcapsules are: 1) preservation of the good bioactivity and biodegradation properties of strontium-doped α-calcium sulfate hemihydrate, which are advantageous in promoting bone healing, 2) simultaneous introduction of the properties of chitosan as a sustained release vehicle with antibacterial effects, and 3) ability to treat osteomyelitis as well as the bone defect when applied, as these two conditions frequently occur together in the clinical situation, due to the combined properties of the two materials.

2. Materials and methods

All reagents were obtained from Sigma-Aldrich, USA unless otherwise specified.

2.1. Preparation of strontium-doped α-calcium sulfate hemihydrate (CSH (Sr)) powder

Strontium-doped α-calcium sulfate hemihydrate (Sr-α-$CaSO_4 \cdot 0 \cdot 5H_2O$) was synthesised from Sr-$CaSO_4 \cdot 2H_2O$ powder. Sr-$CaSO_4 \cdot 2H_2O$ and Sr-α-$CaSO_4 \cdot 0 \cdot 5H_2O$ powders were prepared according to our previously published procedures [10]. Briefly, to prepare Sr-$CaSO_4 \cdot 2H_2O$ powder, $Ca(OH)_2$ and $Sr(OH)_2$ (molar ratio: $Ca(OH)_2$/$Sr(OH)_2$ = 9:1) were dispersed in a mixture of ethanol and water (volume ratio: ethanol/water = 4:3). At room temperature and under rapid mechanical stirring at 800–1000 rpm, 2 mol H_2SO_4 solution was added dropwise into the mixture and the mixture was stirred for another 8 h for the reaction to reach completion. The reaction product was filtered, washed with deionised water and ethanol, and dried at 65 °C. The resulting Sr-$CaSO_4 \cdot 2H_2O$ powder was ground and sieved through 200 meshes.

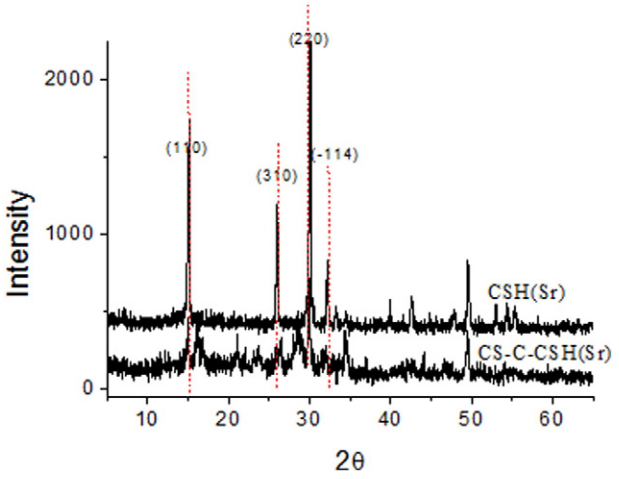

Fig. 2. XRD patterns of CS-C-CSH(Sr) and CSH(Sr).

To prepare Sr-α-$CaSO_4 \cdot 0 \cdot 5H_2O$ powder, 300 mL of 15% NaCl solution was heated to 104 °C while stirring, and the solution was adjusted to pH = 5 using diluted HCl. 45 g of Sr-$CaSO_4 \cdot 2H_2O$ powder was added into the solution while stirring at room temperature, and the mixture was stirred for another 4 h for the reaction to reach completion. The reaction product was filtered while hot, washed several times with boiling deionised water and then ethanol, and dried at 100 °C. The resulting Sr-α-$CaSO_4 \cdot 0 \cdot 5H_2O$ powder, hereon referred to as CSH(Sr), was used for subsequent experiments.

2.2. Preparation of microcapsules composed of chitosan and strontium-doped α-calcium sulfate hemihydrate (CS-C-CSH(Sr))

To prepare microcapsules composed of chitosan and strontium-doped α-calcium sulfate hemihydrate, chitosan solution adjusted to pH = 5 was heated to 100–102 °C while stirring, and strontium-doped α-calcium sulfate hemihydrate powder was slowly added into the solution (molar ratio: CSH(Sr)/H_2O = 0.15:1). The mixture was stirred for 1 h for the reaction to reach completion. The reaction product was filtered while hot, washed several times with boiling deionised water and then ethanol, and dried at 80 °C for 8 h. The resulting microcapsules, hereon referred to as CS-C-CSH(Sr), was used for subsequent experiments.

2.3. Physical properties of the samples

Samples of CS-C-CSH(Sr) and CSH(Sr) were examined by X-ray diffraction (XRD; MaxRC, Rigaku, Japan) with a voltage of 36 kV and current of 20 mA, at a scanning rate of 4° min^{-1}, step width of 0.02° and 2θ range from 7° to 65°. Morphology and microstructure of the samples were examined by scanning electron microscopy (SEM; Ultra 55, Carl Zeiss, Germany) after sputter coating with gold.

2.4. Degradation and ion release test

Samples of CS-C-CSH(Sr), CSH(Sr) and α-calcium sulfate hemihydrate (CSH) were soaked in deionised water for 12 weeks. At the end of each week, the concentration of strontium ions in solution was determined using inductive coupled plasma atomic emission spectroscopy (ICP-AES; Optima 2000DV, PerkinElmer, USA) to obtain the release profile of strontium ions for each of the materials over 12 weeks. The release rate of strontium ions is the most important outcome resulting