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Degradability of injectable calcium sulfate/mineralized collagen-based bone repair material and its effect on bone tissue regeneration

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ABSTRACT

The nHAC/CSH composite is an injectable bone repair material with controllable injectability and self-setting properties prepared by introducing calcium sulfate hemihydrate (CSH) into mineralized collagen (nHAC). When mixed with water, the nHAC/CSH composites can be transformed into mineralized collagen/calcium sulfate dihydrate (nHAC/CSD) composites. The nHAC/CSD composites have good biocompatibility and osteogenic capability. Considering that the degradation behavior of bone repair material is another important factor for its clinical applications, the degradability of nHAC/CSD composites was studied. The results showed that the degradation ratio of the nHAC/CSD composites with lower nHAC content increased with the L/S ratio increase of injectable materials, but the variety of L/S ratio had no significant effect on the degradation ratio of the nHAC/CSD composites with higher nHAC content. Increasing nHAC content in the composites could slow down the degradation of nHAC/CSD composite. Setting accelerator had no significant effect on the degradability of nHAC/CSD composites. *In vivo* histological analysis suggests that the degradation rate of materials can match the growth rate of new mandibular bone tissues in the implanted site of rabbit. The regulable degradability of materials resulting from the special prescriptions of injectable nHAC/CSH composites will further improve the workability of nHAC/CSD composites.

1. Introduction

Bone often undergoes damage or suffers defects resulting from trauma or diseases. Traditional therapies employed in bone defect repair consist in the use of autografts, allografts or xenografts. Despite all the satisfactory results they have had, these therapies present limitations such as donor site morbidity, material availability, anatomic problems and the risk of inducing transmissible diseases [1]. These complications promote to develop alternatives to conventional bone grafting.

At present, autogenous bones as the best bone repair materials have widely been accepted as the gold standard for the treatment of bone defects though they have the drawbacks of donor shortage and secondary damage. Therefore, as a surrogate of autogenous bone, an ideal bone substitute should simulate both the components and the microstructure of normal bone. Bone matrix is a 3-dimension composite with an intricate hierarchical structure of collagen fiber mineralized by nano-sized hydroxyapatite (nHA) crystals [2]. By biomimetic strategy, a biomimetic bone repair materials (nHA/collagen, nHAC for short) with a hierarchical structure was prepared by the self-assembly or biomineralization of collagen and nHA in our previous work [3]. Briefly, collagen fibrils were formed by self-assembly of collagen triple helices. The nHA crystals were aligned with their respective crystallographic *c*-axes generally parallel to one another and to the long axis of the molecules and fibrils in which they were located. The mineralized collagen fibrils aligned parallel to each other to assemble into mineralized collagen fibers, which is similar to the microstructure of autogenous bone. Cellular functions of these materials have demonstrated that the composites support well cellular growth and related functions, and lead to new bone formation [4,5]. More importantly, the mineralized collagen composites have now been successfully used for tens of thousands of cases in clinic, including various types of hard tissue repair [6]. They can thus be used as a surrogate or supplement of autogenous bones. However, the materials lack some handling characteristics because of its solid-preformed block shape, which can result in increased bone loss, trauma to the surrounding tissue, and a longer surgery time when the material is implanted into the damaged tissues by surgery [7]. One of the major

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Table 1
The prescription of injectable nHAC/CSH composite paste.

Content of nHAC (%)	0	5	10	20
L/S ratio (ml/g)	0.5–0.7	0.5–0.7	0.7–0.9	0.8–1.0

improvements is to develop an injectable material by a minimally invasive technique. Not only this material can mold to the shape of the bone cavity and set *in situ* when injected, but also it can decrease the surgical operation time, reduce the damaging effects of large trauma and the size of the scars, and relieve post-operative pain. The patient can achieve rapid recovery in a cost-effective manner [8].

Because the properties of good biocompatibility and promoting bone healing, calcium sulfate hemihydrate (CSH, $CaSO_4 \cdot 0.5H_2O$) has been used in clinic for a long time [9–13]. More importantly, CSH mixed with water has the ability to undergo *in situ* setting after filling the defects by injection [14]. Herein, CSH was introduced into nHAC to form nHAC/CSH composites and to develop an injectable bone repair material. In our previous studies, we have reported that the injectable bone repair material has controllable injectability, regulable self-setting properties, good biocompatibility, suitable mechanical properties [15] and strong ability to promote bone formation [16,17]. However, for an ideal bone implant or repair material, the degradability is also an important factor for its usage in clinic [18]. The nHAC/CSH composites mixed with water will transform into mineralized collagen/calcium sulfate dihydrate (nHAC/CSD) composites after they set [14,16]. In the present work, we have investigated *in vitro* degradability of nHAC/CSD composites in simulated body fluid (SBF) at 37 °C in detail. Moreover, the effect of material degradability on bone tissue repair and regeneration has been discussed. These properties are essential to evaluate the availability of biomaterials in guiding tissues regeneration and repair.

2. Materials and methods

2.1. Materials

Calcium sulfate dihydrate (CSD), medicine grade, was obtained from Merck & Co., Inc. (Germany). nHAC was prepared by self-assembly of nHA and collagen as previously reported [3], and calcium sulfate hemihydrate (CSH) was prepared by the hydrothermal synthesis of CSD as reported in our previous work [19].

2.2. Materials preparation and characterization

By mixing CSH with nHAC, the nHAC/CSH composites with various nHAC contents (0%, 5%, 10% and 20%, weight ratio) were prepared. Various contents of CSD (0%, 5%, 10%, 15% and 20%, weight ratio) as setting accelerator was also added into the composites. These composites were mixed with deionized water at various ratios of liquid to solid (L/S, ml/g) according to the prescription as shown in Table 1 in order

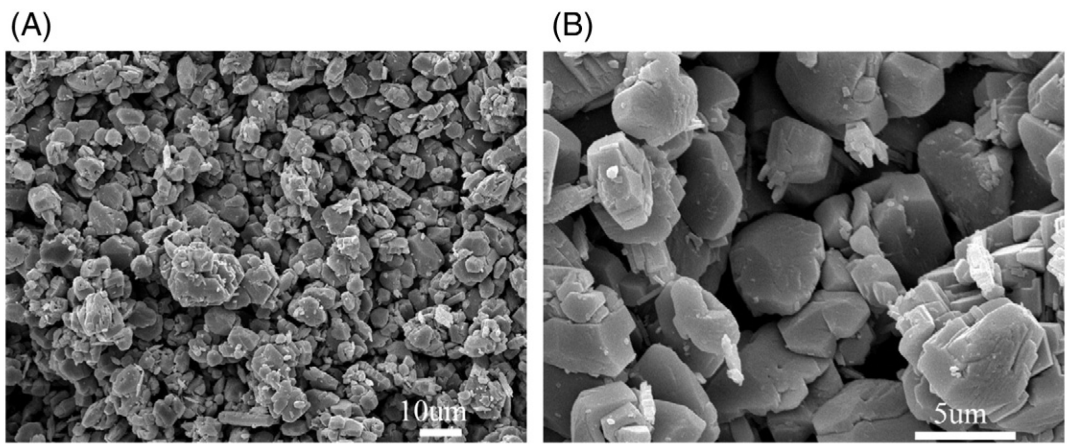

Fig. 1. SEM micrographs of ground CSH crystals (A) ×1000, and (B) ×5000.

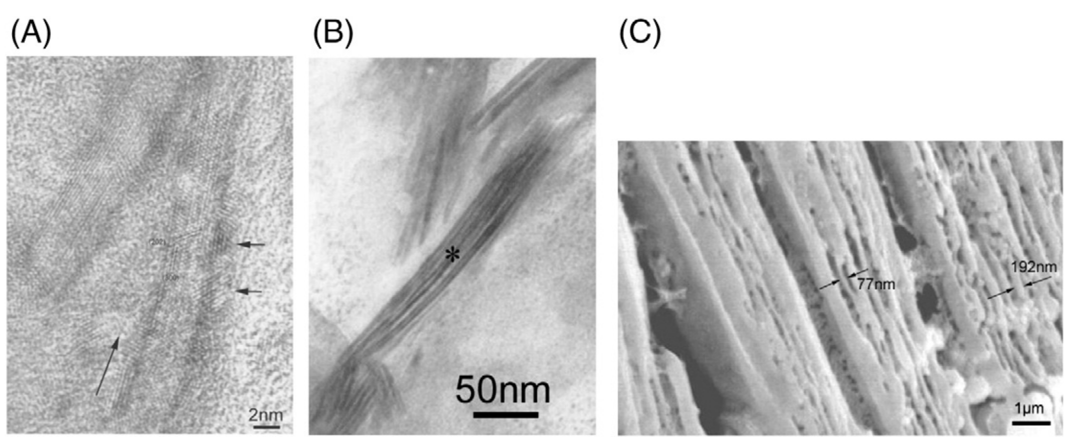

Fig. 2. Hierarchical structure of biomimetic mineralized collagen [3]. (A) Long arrow indicates the longitude direction of collagen fibril. Two short arrows indicate HA nanocrystals; (B) Mineralized collagen fibrils. (C) Mineralized collagen fibers.