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Effect of addition of hyaluronic acids on the osteoconductivity and biodegradability of synthetic octacalcium phosphate

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ABSTRACT

The present study was designed to investigate whether three sodium hyaluronic acid (HyA) medical products, Artz®, Suvenyl® and a chemically modified derivative of sodium HyA Synvisc®, can be used as suitable vehicles for an osteoconductive octacalcium phosphate (OCP). OCP granules (300–500 μm diameter) were mixed with these sodium HyAs with molecular weights of 90×10^4 (Artz®), 190×10^4 (Suvenyl®) and 600×10^4 (Synvisc®) (referred to as HyA90, HyA190 and HyA600, respectively). OCP–HyA composites were injected using a syringe into a polytetrafluoroethylene ring, placed on the subperiosteal region of mouse calvaria for 3 and 6 weeks, and then bone formation was assessed by histomorphometry. The capacity of the HyAs for osteoclast formation from RAW264 cells with RANKL was examined by TRAP staining in vitro. Bone formation was enhanced by the OCP composites with HyA90 and HyA600, compared to OCP alone, through enhanced osteoclastic resorption of OCP. HyA90 and HyA600 facilitated in vitro osteoclast formation. The results suggest that the osteoconductive property of OCP was accelerated by the HyAs-associated osteoclastic resorption of OCP, and therefore that HyA/OCP composites are attractive bone substitutes which are injectable and bioactive materials.

1. Introduction

Octacalcium phosphate (OCP; $Ca_8H_2(PO_4)_6{\cdot}5H_2O$) is an osteocondutive ceramic used as a bone substitute that has demonstrated quantitative bioactivity in several in vitro studies [1–3]. Osteoblastic differentiation of mouse bone marrow stromal cells is enhanced when they are in contact with OCP crystals [1,2]. Differentiation is augmented by OCP in a dose-dependent manner based on the quantification of mRNA expression of osteoblast differentiation markers, such as alkaline phosphatase (ALP) and osterix [1]. Another particular feature of OCP is the capability to induce osteoclast formation from a co-culture of bone marrow cells (osteoclast precursor cells) with osteoblasts on OCP crystals even in the absence of $1,25(OH)_2D_3$, which is an agent that increases the osteoclast differentiation factor receptor activator of NF-κB ligand (RANKL) in osteoblasts [3]. Hydroxyapatite (HA) does not possess this bioactive property [1,2]. OCP tends to progressively convert to the most thermodynamically stable HA phase when implanted in bone defects [2,4] or subcutaneous tissue [4–6]. This transition process is thought to induce the bioactive property of OCP that is associated with physicochemical changes [2], such as the controlled diffusion of calcium and inorganic phosphate ions around the crystals [7] as well as the progressive increase in affinity of serum protein adsorption onto the crystals [8,9]. OCP appears to have a greater ability for enhancing bone regeneration in vivo compared to HA materials [2,4,10–12]. In addition, OCP is biodegraded by osteoclastic cells when implanted in various bone defects [10,12–15] through various forms, such as granules [2,4,10,12,13,16], even in critical-sized defects [2,11,16], which are defined as defects not spontaneously repaired during the lifetime of the animal [17].

We have found that the capability of OCP in a granule form to regenerate bone in vivo can be markedly controlled not only by the conversion process of OCP to HA [2] but also by physical and chemical factors, including the dose [18], the microstructure of the individual crystals assembling the each granule [19], the granule size [16] and the stoichiometry of OCP (Ca/P molar ratio of OCP) [14], which may be important factors for designing OCP-based materials for bone regeneration. One disadvantage of using OCP as a filling material for bone defects, however, is the difficulty in forming the correct shape [20–22], not only for filling large defects,

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but also for injecting into randomly shaped defects. This is due to the presence of large water molecules in the OCP structure [23,24], which means that OCP cannot be molded through a general sintering process, unlike HA or β-tricalcium phosphate (β-TCP) ceramics [25,26]. Sintering of OCP leads to the decomposition of the OCP structure as the temperature increases [23,24]. In order to overcome the poor moldability of OCP, the granules or crystals of OCP need to be combined with natural polymers, such as collagen [21], gelatin [20] and alginate [27]. Using this approach, the composites are able to form a three-dimensional scaffold and can be used in large bone defects with relatively better handling performances and excellent bone regenerative properties compared to OCP granules without a polymer [20,21,27]. However, the composites still cannot be used to fill randomly shaped defects by injection, which may result in less bone formation reaction compared to defects filled with a sufficient amount of material [28–32]. Therefore, improvements in the injection characteristics of OCP without loss of bioactivity or osteoconductivity characteristics could lead to the widescale use of these materials in bone tissue engineering.

Hyaluronic acid (HyA) is an extracellular matrix that is involved in most connective tissues of the body [33,34]. HyA is a linear natural polymer with repeating unsulfated glycosaminoglycans consisting of D-glucuronic acid and *N*-acetyl-D-glucosamine [34]. Due to the involvement of HyA in several biological processes, such as osteoconduction and wound healing [35–39], several studies have assessed the utility of injectable HyA gels for the treatment of bone-related diseases, such as osteoarthritis [40,41], as these gels have a relatively high molecular weight that determines their viscoelastic properties [33]. Other studies have explored the possibility of developing scaffold materials with or without cross-linking of HyA [42–45]. Special attention has focused on maintaining the osteoconductivity of HyA by adding calcium phosphate materials through the deposition of HA crystals biomimetically onto the HyA matrix [44,45] or simply by mixing β-TCP granules with HyA gels [42,46,47]. These studies suggest that HyA-containing scaffold materials are efficacious in bone-repair processes. Other studies have attempted to promote the binding between abundant carboxyl groups present in HyA and the calcium sites in calcium phosphate crystal surfaces [45], resulting in the formation of relatively firm composites. The structure of OCP stacks apatite layers alternately with hydrated layers [48,49], which is why OCP has been proposed as a precursor of HA formation in experimental calcium and phosphate solutions in vitro [48,50] and hypothesized to be a precursor of bone apatite crystals in vivo [51]. Some properties of OCP, such as the serum protein adsorption affinity [9], resemble those of HA. Although the chemical compatibility of HyA with OCP crystals has never been assessed, OCP may work as a better adsorbent for HyA molecules in a similar manner to HA.

The aim of the present study was to examine whether the viscoelastic properties of HyA gels with relatively high molecular weights (ranging from 90×10^4 to 600×10^4), using commercially available HyA medical products, can be used as suitable vehicles for OCP granules from the point of view of osteoconductivity as well as the handling properties during surgical procedures. Unexpectedly, the HyA gels not only enhanced the handling properties of OCP but also increased the osteoconductivity of OCP granules, accompanied by an increase in osteoclastic activity to resorb OCP, when the gels were combined with OCP and implanted onto mouse calvaria as composite materials consisting of OCP and HyA. It is likely that the OCP–HyA combination may have a synergistic effect with regard to the osteoconductivity of OCP.

2. Materials and methods

2.1. Preparation of OCP and OCP–HyA composites

OCP was prepared by mixing calcium and phosphate solutions as previously described [4]. The granules, consisting of an OCP crystal aggregate, were prepared from OCP precipitates by passing them through a standard testing sieve. Granules with diameters ranging from 300 to 500 μm were used (Fig. 1A). The sieved OCP

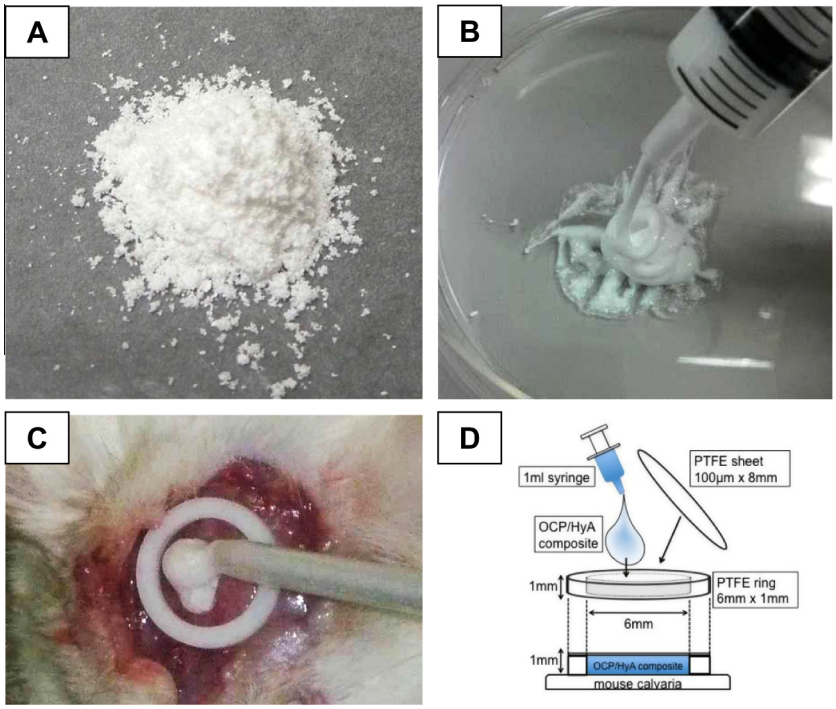

Fig. 1. A photograph of the sieved OCP granules (300–500 μm) (A) and the injectable OCP–HyA90 composite (B). A photograph (C) and schematic illustration (D) of the experimental animal model.