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Enhancing osteoconductivity and biocompatibility of silver-substituted apatite *in vivo* through silicon co-substitution

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ABSTRACT

The success of bone substitute can be undermined by poor osseointegration and infection. To counter both issues, many researches attempted to incorporate antibacterial agents such as silver into biocompatible materials like hydroxyapatite. However, the balance between both properties is often fraught with compromise. Silver, silicon co-substituted hydroxyapatite (Ag,Si-HA) was developed to create bioactivity in tandem with antibacterial properties. The favourable *in vitro* results prompted us to study further Ag,Si-HA *in vivo*. Haematoxylin and eosin, Masson's trichrome and immunohistochemistry staining of type I collagen results demonstrated that the co-substitution of Si in Ag-HA to form Ag,Si-HA, could enhance its osteoconductivity and biocompatibility in the rabbit femoral condyle model.

1. Introduction

Bone substitutes are widely used and highly successful treatments for bone degeneration issues have been reported, and this translate potentially to a projected market value of USD 3.2 billion by 2022 [1]. However, their success can be undermined by poor osseointegration and infection, which are the two leading causes of implant failure and revision surgeries. As such, the ability to osseointegrate, and deter bacteria adhesion for bone substitute are beneficial, and many researches looked into incorporating antibacterial agents such as silver into biocompatible materials like hydroxyapatite [2–4]. However, the balance between both properties is often fraught with compromise [2–4]. In our previous study, silver and silicon were co-substituted into hydroxyapatite (Ag,Si-HA), to create antibacterial properties in tandem with biocompatibility so as to promote bone regeneration [5,6]. The Si component in Ag,Si-HA has been demonstrated to mitigate the effect of Ag component towards mesenchymal stem cells without compromising its antibacterial properties against *Staphylococcus aureus* (*S. aureus*) in the *in vitro* studies [5]. Ag,Si-HA was also shown to have enhancing bone differentiation properties *in vitro* than HA [5].

Nevertheless, *in vitro* study is only a fundamental step in evaluating the biocompatibility of a biomaterial. Animal models play an indispensable role in testing bone substitute biomaterials for understanding their osteoconductivity, biocompatibility, and interaction with host tissues. To demonstrate the relevance of Ag,Si-HA for clinical application, an *in vivo* study of implanting Ag,Si-HA, fabricated in the form of microbeads in a rabbit femoral condyle model is performed. The present study will build upon earlier *in vitro* work to demonstrate the osteoconductivity and biocompatibility of Ag,Si-HA, bringing one step closer in developing advanced biomaterial for tissue repair and regeneration. Implanting Ag,Si-HA in the form of microbeads exhibits a number of advantages over powders and porous blocks. Powders are difficult to handle, keep in place after implantation, and will get disintegrate/dissolve, while macroporous blocks are brittle, difficult to shape and cannot fit tightly to the surface of the defects preventing osteoconductive process [7]. In addition to ease of handing, microbeads confer greater surface areas that allow the cells to grow on the particles as well as at the interparticles.

2. Materials and methods

2.1. Preparation of apatite microbeads

The synthesis of apatite, fabrication and characterization of the apatite microbeads were reported in detail in [8]. Briefly, apatite

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powders were synthesized via a wet precipitation reaction, and added to 0.03 g/ml alginate (Sigma) solution at a ratio of 1:1 w/w to form a suspension. Apatite microbeads were then extruded at a rate of 200 drops/min into a 0.5 M calcium chloride (Sigma) cross-linking solution, using an in-house drop-on-demand microvalve extrusion device. Lastly, apatite microbeads were washed, dried, and heated to 1150 °C for 2 h in air.

2.2. Animals and ethics

All experiments were performed in accordance with Chinese Animal Protection Network (CAPN) guidelines regarding the protection of animals. The principles of laboratory animal care were strictly adhered to follow ethical guidelines set by IACUC in the assured institution.

2.3. In-vivo implantation

A 5 mm (diameter) × 8 mm (depth) defect was created at the femoral condyle of each female New Zealand white rabbit (six-months old, weighing 1.8–2.2 kg), and 150 µl of the microbeads were implanted into the defect site. Three groups of implants: HA (group 1), Ag-HA (group 2) and Ag,Si-HA microbeads (group 3) were prepared, with HA microbeads as the control.

Rabbits were euthanized using carbon dioxide, and samples were retrieved for examinations post 1 and 3 months of implantation, with a sample size of n = 4 at each time point, resulting in a total of 24 rabbits.

2.4. Histology and immunohistochemistry

For haematoxylin and eosin, Masson's trichrome analyses and immunohistochemical staining of type I collagen (COL-I), tests were performed according to standard procedures [9,10].

3. Results

The contact of surrounding tissue to the microbeads was intimate and direct without fibrous tissue encapsulation (Fig. 1). With increasing implantation period from month 1 to 3, tissue formation in groups 1 and 3 increased, and lesser nucleus was also observed surrounding the microbeads. Furthermore, tissue formation resembling mineralizing bone (indicates as M) was also formed around the microbeads for groups 1 and 3 (Fig. 1g and i). On the other hand, there were still a lot of nucleus surrounding the microbeads in group 2, and newly formed bone was little seen (Fig. 1h).

Comparing tissue formation in Masson's trichrome histology staining (Fig. 2), it was evident that the tissue type formed by group 2 was different from groups 1 and 3. Groups 1 and 3 exhibited greater production of tissue formation resembling mineralizing bone (indicates as M) and connective tissue (blue coloration suggested to be stained by collagen I fibers, indicated by red triangle) than group 2. This was further confirmed with immunohistochemistry staining of COL-I.

The microbeads were stained with COL-I, and staining became more intense with increasing implantation period as they were made up of apatite, which were biocompatible to provide a good surface for protein absorption (Fig. 3). Comparing among the

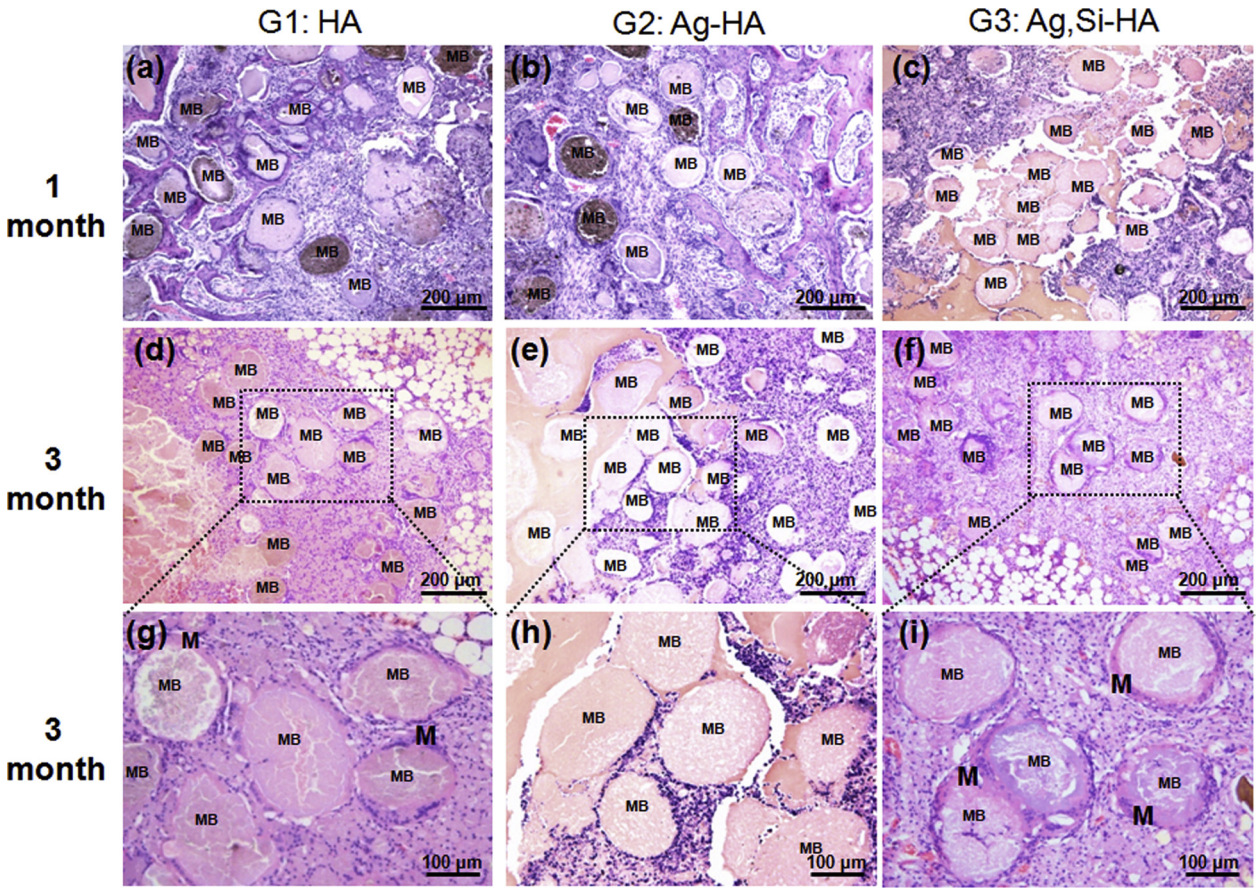

Fig. 1. Haematoxylin and eosin staining of implanted (a) HA, (b) Ag-HA, (c) Ag,Si-HA microbeads at month 1; (d) and (g) HA, (e) and (h) Ag-HA, and (f) and (i) Ag,Si-HA microbeads at month 3. M indicates mineralizing bone, and MB indicates microbeads.