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Fabrication of 3D porous silk scaffolds by particulate (salt/sucrose) leaching for bone tissue reconstruction

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ABSTRACT

Silk fibroin is a biomaterial being actively studied in the field of bone tissue engineering. In this study, we aimed to select the best strategy for bone reconstruction on scaffolds by changing various conditions. We compared the characteristics of each scaffold via structural analysis using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), the swelling ratio, water uptake, porosity, compressive strength, cell infiltration and cell viability (CCK-8). The scaffolds had high porosity with good inter pore connectivity and showed high compressive strength and modulus. In addition, to confirm bone reconstruction, animal studies were conducted in which samples were implanted in rat calvaria and investigated by micro-CT scans. In conclusion, the presented study indicates that using sucrose produces scaffolds showing better pore interconnectivity and cell infiltration than scaffolds made by using a salt process. In addition, *in vivo* experiments showed that hydroxyapatite accelerates bone reconstruction on implanted scaffolds. Accordingly, our scaffold will be expected to have a useful application in bone reconstruction.

1. Introduction

Q2 Bone reconstruction is clinically important due to the large number of patients who have bone defects of critical size. Various materials have been clinically used for bone reconstruction such as allograft bone, ceramic powder and demineralized bone. However, lack of bone replacement supplements has posed a problem. The emergence of tissue engineering has provided a promising alternative for bone reconstruction [1,2].

Typically, a graft of artificial tissue is intended to replace the tissue's function while the cells reconstitute a natural replacement. One of the most important goals in tissue engineering research is to design scaffolds with the capability of biomimicking the natural extracellular matrix (ECM), which guides cellular migration, provides mechanical support and regulates cellular activities [3]. Cells must be able to attach and proliferate on the new ECM throughout the entire scaffold to successfully graft [4].

There are a variety of scaffold fabrication methods in tissue engineering, including gas foaming [5], freeze-drying [6], particulate-leaching [7], and electrospinning [8]. Particulate-leaching is a simple technique for the fabrication of a 3D porous scaffold that uses a polymer solution uniformly mixed with salt particles or sucrose particles of a specific diameter. The solvent then evaporates, leaving behind a polymer matrix with salt particles or sucrose particles embedded throughout. When the composite is immersed in water, the particles leach out to produce a porous structure [9]. These methods are also called salt leaching and sucrose leaching. By changing the particle size, this technique can easily control pore size and thickness of the septum of a 3D porous scaffold.

Adult stem cells have emerged as a cell source for tissue engineering [10,11]. MSC can be isolated from a wide variety of tissues including bone marrow and adipose. Bone marrow is the major source of MSCs, and bone marrow-derived MSCs have been used to treat a variety of defects and diseases, including critical size segmental bone defects, and are also the MSCs most used for bone tissue engineering research [12,13].

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Hydroxyapatite (HAP) is the major mineral constituent of the bone matrix [14]. It is very useful for bone defects because it has good osteoconductivity. However, there are limits and restrictions on fabricated porous materials composed of hydroxyapatite materials because of their poor mechanical properties. Silk fibroin (SF), a natural polymer, has many advantages such as good biocompatibility, low immunogenicity, and a controllable degradation rate [15–17]. It therefore has been used in tissue engineering research for artificial bone a variety of organs. It is possible to improve the biocompatibility and osteointegrative capacity by mixing the bone conductive ceramic and the biodegradable polymer to overcome the defects of each material.

In this study, we fabricated an artificial bone scaffold for various tissue engineering methods. Finally, we developed a hybrid artificial bone scaffold by mixed hydroxyapatite as a bone-conductive ceramic and silk fibroin as biodegradable polymer. We evaluated the mechanical and physical properties and cell adhesion, proliferation and differentiation of osteogenic-induced BMSC on each scaffold *in vitro* and *in vivo*.

2. Materials and methods

2.1. Preparation of SF solution and HAP nanoparticles

Silk fibroin (SF) was derived from the cocoons of *Bombyx mori*. The procedure for the preparation of a silk solution is normally composed of three steps. The first process is a degumming step. Cocoons were finely chopped and boiled in 0.02 M sodium carbonate at 95 °C for an hour to be degummed. The silk fibers were then washed with distilled water and dried. Second, degummed silk fibers were dissolved with $CaCl_2$, Et-OH and H_2O ($CaCl_2$:Ethanol:H_2O = 1:2:8) at 98 °C for 40 min. In the final step, this solution was dialyzed against distilled water by using a dialysis membrane for two days. Its molecular weight was MWCO 12–14 kDa. The concentration of the final silk fibroin solution was calculated to 6 wt%. This solution was then concentrated by using PEO [18]. Rod-shaped hydroxyapatite nanoparticles were kindly gifted by Cell & Gene Biotechnology with a particle size of 30–60 nm.

2.2. Fabrication of SF and HAP hybrid scaffold

We fabricated four types of hybrid scaffolds by particulate leaching using salt and sucrose (Fig. 1). According to the solution and particle type, they were simply called SS (Silk scaffold by Sucrose leaching), HSS (Hap mixed Silk scaffold by Sucrose leaching), SN (Silk scaffold by Salt leaching) and HSN (Hap mixed Silk scaffold by Salt leaching). "SS" was scaffold of the fabricated by using silk fibroin solution and sucrose particles. "HSS" was scaffold of the fabricated by sucrose leaching and used 10% of HAP particles in 16% silk fibroin solution. "SN" was scaffold of the fabricated by salt leaching and used 16% silk fibroin solution. "HSN" was scaffold of the fabricated by salt leaching and used 10% of HAP particles in 16% silk fibroin solution. A three layer particulate leaching process was set up with the lower and upper layers composed of particles (salt or sucrose) and the middle layer was a silk fibroin solution or a mixed silk fibroin and hydroxyapatite solution. The three-layered

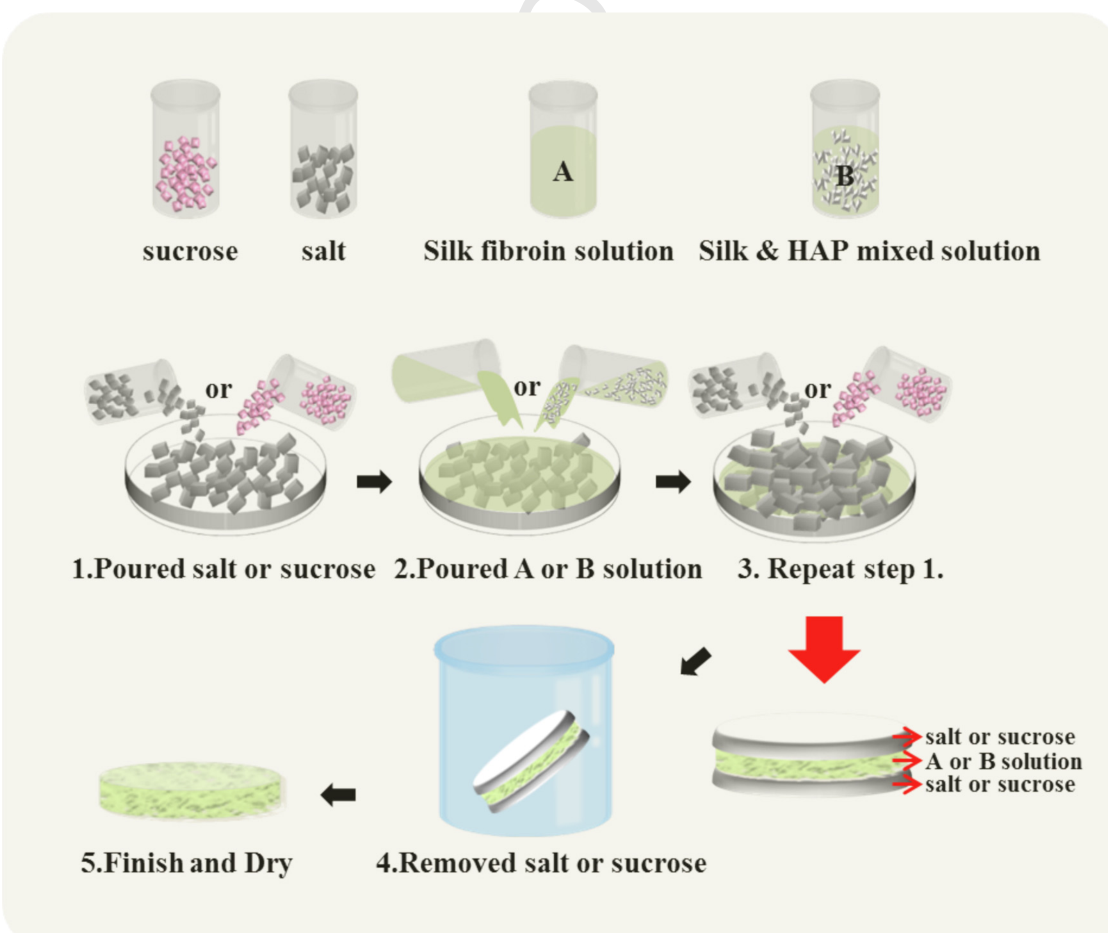

Fig. 1. The fabrication process for four types of scaffold.

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