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Preparation and characterization of silk fibroin/strontium carbonate film through rapid formation

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ABSTRACT

Silk fibroin/strontium carbonate (SF/$SrCO_3$) composite films fabricated from a simple biomineralization process is reported. These composite films with different $SrCO_3$ concentrations are achieved and can change from transparent to opaque as the $SrCO_3$ content increased. The resulting $SrCO_3$ demonstrates different morphologies, chrysanthemum pattern grown on film surface and micro-globule composed nano-particle grown in film. Moreover, SF/$SrCO_3$ composites show excellent biocompatibility with rat marrow stem cells (MSCs), making it a valuable biofunctional silk material for bone engineering applications.

1. Introduction

Silk fibroin (SF), extracted from *Bombyx mori* cocoons, is a widely used protein polymer for biomedical applications. Silk has many excellent biological characteristics used for osteogenesis, bone induction and conductibility application. Therefore, complex inorganic/organic composite materials have been proposed for bone tissue engineering, and become a rapidly growing research area [1].

Recently, the positive effect of strontium (Sr) is recognized in the treatment of osteoporotic bone and bone defects [2–4]. Sr can play an important role in bone remodeling, such as osteoblast promotion and inhibition [5]. Despite the known beneficial effect of Sr on bone remodeling, the feasibility of Sr-incorporated biomaterials in clinical applications relies largely on Sr content and local release kinetics [3]. For this reason, Sr has been tested in different forms, such as carbonate, chloride, phosphate, to realize local release while diminishing the administered Sr dose [6,7]. Moreover, it has been widely reported that calcium was mineralized in SF materials [8–10] to form hydroxyapatite/SF scaffold for bone tissue engineering. Due to the similar characteristics between calcium and Sr (size and charge), it is possible to incorporate Sr into SF material using biomineralization process. The study presents a simple methods based on the above idea of biomineralization to incorporate Sr into SF films. The morphology, secondary structure and biological characteristics of the Sr-containing SF film were characterized, aiming to develop a biofunctional silk material for bone tissue engineering.

2. Experimental

The preparation process of silk fibroin/strontium carbonate (SF/$SrCO_3$) composites is illustrated in Fig. 1. *Bombyx mori* silk solution (~8 wt%) was prepared as our earlier procedure [11]. $SrCl_2$ was added into SF solution with the concentration of 0.1 wt%, 1 wt% and 5 wt%. The mixed solution was cast on 6-well cell culture plate and left for 24 h at room temperature. The dried films were kept in a wet vacuum environment containing water and $(NH_4)_2CO_3$ for 3 days to induce $SrCO_3$ biomineralization. Finally, the resulting composite films were immersed in 75% ethanol solution to induce structural transition for 30 min, and then rinsed with deionized water and dried at room temperature.

The surface and cross-section of SF/$SrCO_3$ composite films were observed with S-4800 SEM (Hitachi, Tokyo, Japan) at 3 kV. For cross-section imaging, the composite films were fractured in liquid nitrogen to avoid deformation. The specimens were sputter coated with gold prior to imaging. XRD (X'PERT PRO MPD, PANalytical Company, Netherlands) was performed on SF/$SrCO_3$ composite films with CuKα radiation at 40 kV and 30 mA.

Cell culture was performed to characterize the biocompatibility of materials. Pure SF and SF/$SrCO_3$ films were formed in 24-well cell culture plate as above procedures. These materials were irradiated with γ–ray before use. Animals were used and approved by the Animal Care and Use Committee of Soochow University, China. Rat marrow stromal cells (MSCs) were obtained as described in our previous report [12]. MSCs were seeded at a density of 5.0×10^3 cells on films prewetted with

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Dulbecco's eagle medium (DMEM). The cell culture medium was composed of L-DMEM with 10% Fetal Bovine Serum (FBS, Gibco, Carlsbad, CA, USA) and 1% penicillin–streptomycin, and was replaced first at 24 h, and then every 72 h. MSCs were observed using light microscope and SEM. MSCs proliferation was determined using MTT assay conducted as described in our previous report [13].

All experiments were performed with a minimum of N=3 for each data point. Statistical analysis was performed by one-way analysis of variance (ANOVA). Differences were considered significant when $p \leq 0.05$.

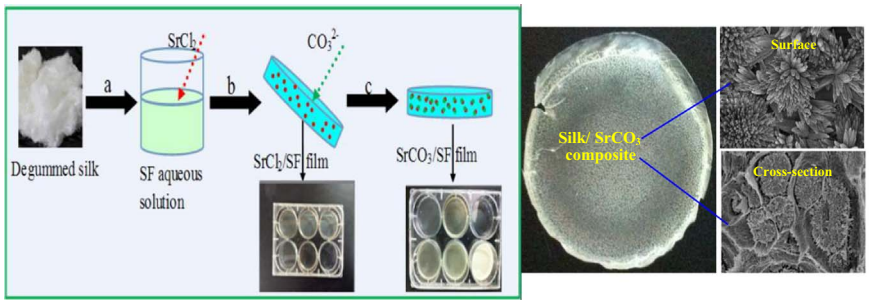
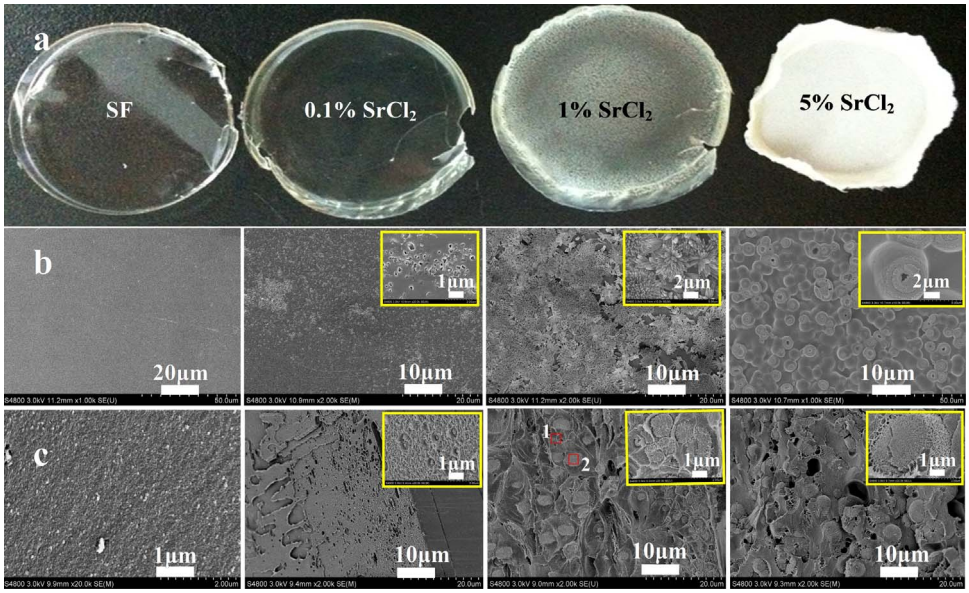


Fig. 1. Schematic diagram of biomineralization process of $SrCO_3$ in SF film.



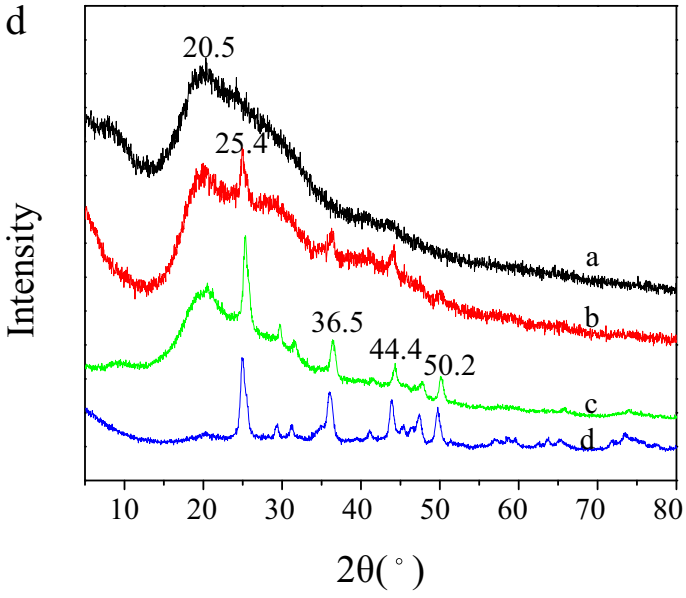


Fig. 2. Optical photos (a) and SEM images of surface (b) and cross-section (c) of $SF/SrCO_3$ composite film derived from SF solution with 0%, 0.1%, 1% and 5% $SrCl_2$ content. (d) XRD patterns of $SF/SrCO_3$ composite films derived from SF solution contained (a) 0% $SrCl_2$, (b) 0.1% $SrCl_2$, (c) 1% $SrCl_2$ and (d) 5% $SrCl_2$.