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Synthesis of strontium chondroitin sulfate and the evaluation of its capability to attenuate osteoarthritis

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ABSTRACT

Osteoarthritis (OA) is the most prevalent musculoskeletal disorder and the leading cause of joint disability in elderly patients. In this study, we fabricated strontium chondroitin sulfate (SrCS), a new polysaccharide-metal ion complex that is the combination of chondroitin sulfate and strontium, which are two widely adopted chemicals in OA clinical management. The structural, chemical compositions and morphology of as-fabricated SrCS were systematically investigated. Cell proliferation test, RT-PCR and preliminary animal studies were conducted to evaluate the clinical potential of SrCS on OA treatment. The materials characterization results verified that the Sr was successfully integrated into CS by replacing sodium in the original structure and formed a new polysaccharide-metal ion complex. The cell proliferation results indicated that the SrCS has excellent biocompatibility for both chondrocyte and osteoblast. The RT-PCR results showed that the SrCS can significantly increase the expression of COLII and ACAN, decrease MMP1 and MMP13 in chondrocyte and decrease the IL-6 and IL-1β in both chondrocyte and osteoblast. Preliminary animal studies demonstrated that SrCS can effectively simulate the articular cartilage formation in SD-rats after modified Hulth's OA modeling surgery. We therefore believed that the SrCS should be a rather effective chemical for OA clinical management as well as a beneficial component for various biomaterials in cartilage tissue engineering.

1. Introduction

Osteoarthritis (OA) is the joint disorders that generally characterized by articular cartilage (AC) degradation, subchondral bone sclerosis and osteophyte formation. It is prevalent worldwide and now is the leading cause of physical disability among aging adults (Clegg et al., 2006; Gutierrez et al., 2015; Wandel et al., 2010). There is no satisfactory clinical treatment for OA so far: pharmacological treatment of OA is limited to only relieve the symptom but fail to slow down the OA's progression, and total joint arthroplasty that might yield various complications is the final solution (Henrotin et al., 2001; Sharma, 2016). Although the etiology of OA is still unknown, it is generally accepted that the entire joint, including AC, subchondral plate and trabecular bone, are altered by OA and the cross-talk between these joint tissues should be rather important in OA's pathology (Grover & Samson, 2016; Provenza et al., 2015; Xiao et al., 2016). Recent studies reported that the disequilibrium of bone metabolism will result in abnormal osteoid islets on subchondral bone, which is one of the critical steps in OA progression (Xiao et al., 2016; Zhen et al., 2013). Hence, both the AC and subchondral bone should be well considered for effective clinical treatment of OA.

Chondroitin sulfate (CS) is a complex, heterogeneous polysaccharide. It is the basic component of all connective tissues (Barnhill et al., 2006; du Souich, 2014; Ronca et al., 1998). Commercially available chondroitin sulfate extracted from cartilage generally exists in the form of sodium salt. The degree of sulfation and the chain length of extracted CS vary from different species and tissues, with the molecular weight range from 10 to 100 kDa (Tat, Pelletier, Mineau, Duval, & Martel-Pelletier, 2010). Chondroitin sulfate is anti-inflammatory, beneficial in collagen production and the anabolic/catabolic balance of chondrocytes (Ronca et al., 1998). Therefore, chondroitin sulfate currently is widely adopted on pharmacological treatment for OA, which was reported to be able to

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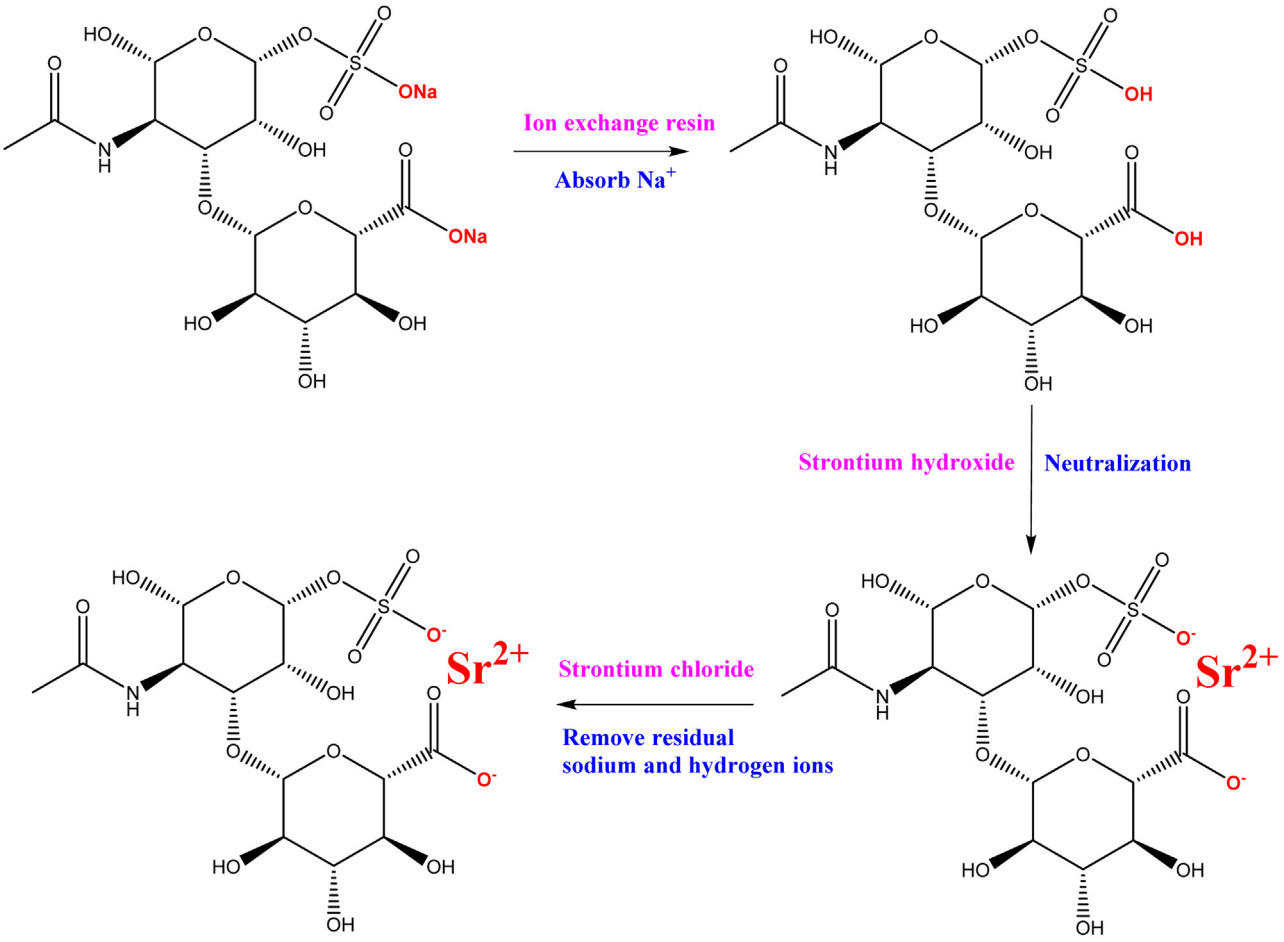

Fig. 1. Synthesis pathway of SrCS (95% ethanol was applied to precipitate SrCS powder from SrCS liquid). The Sr^{2+} ions will substitute Na^{+} ions and bond either $–SO_4–$ or –COO– site (Cael, Winter, & Arnott, 1978).

effectively relieve the pain, improve the AC function and slow down the radiological progression of OA (Grover & Samson, 2016; Provenza et al., 2015; Roubille et al., 2015; Wandel et al., 2010; Zhang et al., 2008).

Strontium (Sr) is a trace element of human body that mainly exists in skeleton (Li et al., 2007). Sr is known as anti-inflammatory and antioxidant, and has been employed as antirheumatic drug for decades (Morandi, 1956). Strontium has beneficial effects on both bone and cartilage metabolism (Henrotin et al., 2001; Marie, 2006; Reinholt et al., 1985). Recent clinical studies suggested that Sr can reduce bone resorption, increase bone formation and simulate the formation of collagen matrix. Therefore, Sr has been gradually adopted in the OA treatment (Sharma, 2016; Wang et al., 2015; Wyland, 2015; Yu et al., 2013).

In this study, strontium chondroitin sulfate (SrCS), a new chemical combining chondroitin sulfate and strontium was first fabricated through ion exchange method. We systematically investigated the chemical composition, structure and morphology of as-prepared SrCS and evaluated its potential capability on OA treatment. The results suggested that the proposed fabrication protocol can successfully integrated Sr into CS to form SrCS with high purity. Moreover, the SrCS is anti-inflammatory, rather effective in simulation collagen formation and helping the growth of cartilage. We therefore believed that SrCS fabricated in this work should be a promising chemical for various biomedical applications, especially those related to OA.

2. Materials and methods

2.1. Materials

The chondroitin sulfate used in this study was supplied by Henan Xingyuan Chemical products Co. Ltd., which is chondroitin sulfate A extracted from bovine trachea, with molecular weight 18 kDa (Supplementary Fig. 1). The cation exchange resin and strontium chloride used were purchased from Sinopharm Chemical Reagent Co., Ltd. Strontium hydroxide used in this study was purchased from Shanghai Chemical Reagent Co., China.

2.2. Synthesis of SrCS

Fig. 1 illustrates the synthesis pathway of SrCS. The fabrication process includes ion exchange, pH adjustment, CS-strontium chloride reaction and the SrCS precipitation. In the ion exchange procedure, a chromatographic column filled with pre-processed ion exchange resin (732 sodium cation resin) was employed to absorb sodium ions in CS. The CS (10 wt.%, 10 mL) solution was gradually added into the column with carefully controlled flow rate (1 mL/min) to ensure the maximum absorption. The output liquid that mainly contains chondroitin sulfate acid was collected using a clean beaker. With the purpose of improving the reaction conversion rate, pH adjustment was performed by adding suitable amount of strontium hydroxide to ensure the pH value of the collected liquid to be around 6.5. After that, strontium chloride solution was added into the pH-adjusted liquid to exchange residual sodium and