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Original Article

Anti-inflammation performance of curcumin-loaded mesoporous calcium silicate cement

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Background/Purpose: Calcium silicate (CS) cements have excellent bioactivity and can induce the bone-like apatite formation. They are good biomaterials for bone tissue engineering and bone regenerative medicine. However, they have degradability and the dissolved CS can cause the inflammatory response at the early post-implantation stage. The purpose of this study was to design and prepare the curcumin-loaded mesoporous CS (MesoCS/curcumin) cements as a strategy to reduce the inflammatory reaction after implantation.
Methods: The MesoCS/curcumin cements were designed and prepared. The characteristics of MesoCS/curcumin specimens were examined by transmission electron microscopy (TEM), X-ray diffraction (XRD) and scanning electron microscopy (SEM). Their physical properties, biocompatibility, and anti-inflammatory ability were also evaluated.
Results: The MesoCS/curcumin cements displayed excellent biocompatibility and physical properties. Their crystalline characterizations were very similar with MesoCS cements. After soaking in simulated body fluid, the bone-like apatite layer of the MesoCS/curcumin cements could be formed. In addition, it could inhibit the expression of tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) after inflammation reaction induced by lipopolysaccharides and had good anti-inflammatory ability.

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Conclusion: Adding curcumin in MesoCS cements can reduce the inflammatory reaction, but does not affect the original biological activity and properties of MesoCS cements. It can provide a good strategy to inhibit the inflammatory reaction after implantation for bone tissue engineering and bone regenerative medicine.

Introduction

With an increasing aging population, bone defects and disorders have become a global health care problem and thus leading to a tremendous clinical need for bone repairs.[1,2] Using of autografts, allografts or artificial materials are some of the current approaches for the replacement of damaged bones. However, there is a limitation to the volume of autologous implants that can be obtained from a patient. Furthermore, allografts can sometimes induce innate immune responses in a patient.[3] Artificial materials (e.g. stainless steel and titanium alloys) have low bioactivity and thus would not be able to form a good association with the living bones.[4] Therefore, current research has basically focused on the development of new optimal bone substitutes.

Engineered bone tissues have been regarded as an alternative potential strategy for bone regeneration.[5,6] Cells, scaffolds and growth regulatory signals represent the major elements of bone tissue engineering. Scaffolds are the main platform for cell proliferation and their physical properties are expected to closely mimic the mechanical properties and functions of the extracellular matrix (ECM) in bones. Ceramics have been widely used in bone tissue engineering. Studies have shown that ceramics are highly bioactive and regarded as one of the most promising and interesting candidate to be used as a biomaterial for bone tissue scaffolds.[7,8] The calcium silicate (CS) material displays a high level of bioactivity and it has the ability to bind to not only soft tissues but also living bones.[9,10] Furthermore, the capability of Si ion in forming bone-like apatite layer is also observed *in vitro* using simulated body fluid (SBF).[11,12] Even though there are numerous undoubted advantages in using CS cement, they still have fast degradation rates. Degraded CS have a strong tendency to increase the alkalinity of its surroundings, thus inducing immune and inflammatory response during the early stages of post-implantation.[13–15] The mesoporous CS (MesoCS) nanoparticles have been shown to have a high specific surface area, pore volume, and internal mesopores that can induce the apatite to precipitate and exhibit excellent mineralization behavior.[16,17]

Curcumin is a hydrophobic polyphenol and is a main component of tumeric. Cyclocurcumin, demethoxycurcumin and bisdemethoxycurcumin are some of the other curcuminoids that can be extracted from turmeric.[18,19] However, there are previous reports showing that curcumin has a higher level of activity than demethoxycurcumin or bisdemethoxycurcumin.[20] It has been further revealed in other studies that curcumin has other pleiotropic effects (e.g. anti-inflammatory, anti-oxidation, and anti-tumor effects).[21,22] This is due to that curcumin can target, interact, and regulate multiple molecular targets. These targets include inflammatory cytokines, kinases, growth factors, transcription factors, adhesion molecules, apoptosis-related proteins, etc.[23,24] Curcumin has been found to have an astonishing influence on the anti-inflammatory response, because of its inhibitory effect on the production of cytokines and induction of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and lipooxygenase (LOX) formation.[25,26] In this study, curcumin-loaded mesoporous CS (MesoCS/curcumin) cements demonstrated not only anti-inflammatory responses but also excellent characteristic bone-like apatite formation and good biocompatibility. We hope that this study can be used as an alternative strategy to solve the problem of inflammatory responses in future applications of MesoCS cements as implants. This study can also facilitate the development and understanding of bone tissue engineering and bone regenerative medicine.

For this purpose, we specially designed and prepared MesoCS/curcumin cements to increase the physical properties of MesoCS cements and to reduce the post-implantation inflammatory responses. In addition, we also investigated the inflammatory marker interleukin-1 beta (IL-1β) and biologic changes of lipopolysaccharide (LPS)-treated human mesenchymal stem cells (hMSCs) directly cultured on MesoCS cements.

Materials and methods

Preparation of MesoCS/curcumin specimens

The method of the preparation of MesoCS particles has been described previously.[16] MesoCS nanoparticles were prepared using a template method. Briefly, 3.3 g cetyltrimethylammonium bromide (CTAB, Sigma–Aldrich, St. Louis, MO, USA) and 6 mL $NH_3 \cdot H_2O$ were mixed in double-distilled water (ddH_2O, 300 mL) and then stirred for 15 min at 60 °C. Next, 15 mL tetraethyl orthosilicate (TEOS, Sigma–Aldrich) and 15.6 g calcium nitrate were added with vigorous stirring for 3 h. The precipitate products were then collected by filtration and washed three times each with 1 N hydrochloric acid and ethanol. After this, the collected powders were dried at 60 °C overnight and sintered at 800 °C for 2 h to remove remaining traces of CTAB. Curcumin (Sigma–Aldrich) was dissolved in 0.5 M NaOH as a stock (20 mg/mL), to achieve a concentration of 2–10 mg/mL in ddH_2O. The curcumin concentration of liquids in this

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