Biomaterials 144 (2017) 176–187

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Europium-doped mesoporous silica nanosphere as an immune-modulating osteogenesis/angiogenesis agent

Mengchao Shi [a, b, 1], Lunguo Xia [c, 1], Zetao Chen [d], Fang Lv [e], Huiying Zhu [a], Fei Wei [d], Shengwei Han [d], Jiang Chang [a, b], Yin Xiao [b, d, **], Chengtie Wu [a, b, *]

[a] State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai, 200050, China
[b] Australia – China Centre for Tissue Engineering and Regenerative Medicine, Queensland University of Technology, Brisbane, Queensland, 4059, Australia
[c] Center of Craniofacial Orthodontics, Department of Oral and Cranio-maxillofacial Science, Ninth People's Hospital Affiliated to Shanghai Jiao Tong University, School of Medicine, Shanghai, 200011, China
[d] Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, 4059, Australia
[e] Shanghai Key Laboratory of Regulatory Biology, Institute of Biomedical Sciences and School of Life Sciences, East China Normal University, Shanghai, 200241, China

ARTICLE INFO

Article history:
Received 26 April 2017
Received in revised form
4 August 2017
Accepted 14 August 2017
Available online 16 August 2017

Keywords:
Mesoporous silica nanospheres
Osteogenesis
Angiogenesis
Bone tissue engineering

ABSTRACT

Although much research has gone into the design of nanomaterials, inflammatory response still impedes the capacity of nanomaterial-induced tissue regeneration. *In-situ* incorporation of nutrient elements in silica-based biomaterials has emerged as a new option to endow the nanomaterials modulating biological reactions. In this work, europium-doped mesoporous silica nanospheres (Eu-MSNs) were successfully synthesized via a one-pot method. The nanospheres (size of 280–300 nm) possess uniformly spherical morphology and mesoporous structure, and well distributed Eu elements. The nanospheres show distinct fluorescent property at 615 nm for potential bio-labeling. Noticeably, the Eu-MSNs stimulate pro-inflammatory response of macrophages and induce a modulated immune microenvironment, which further activates the osteogenic differentiation of bone marrow stromal cells (BMSCs) as well as angiogenic activity of human umbilical vein endothelial cells (HUVECs). During the process, osteogenesis-related genes (*e.g.* ALP, OCN, OPN and COL-I) of BMSCs, and angiogenesis-related genes (*e.g.* CD31, MMP9, VEGFR1/2, and PDGFRα/β) of HUVECs were significantly upregulated by Eu-MSNs modulating immune environment of macrophages. The *in vivo* study further demonstrated that the Eu-MSNs could not only stimulate osteogenesis by accelerating the new bone formation at critical-sized cranial defect site, but also support the blood vessel formation as well as collagen deposition and re-epithelialization at chronic skin wound sites, showing an improved angiogenesis activity when comparing with MSNs alone. Given the easy handling characteristics and extensive application potential, the results suggest that Eu-MSNs could be used as immunity-modulated osteogenesis/angiogenesis agent for skin and bone regeneration.

1. Introduction

Mesoporous silica nanospheres (MSNs), as one of the most popular nano-scale biomaterials, have been widely applied in drug delivery owning to the unique physicochemical properties including tunable particle size and pore volume, high specific surface area and favorable biocompatibility [1–3]. Particularly, osteogenesis/angiogenesis-stimulated drugs, cytokines and various nanoparticles such as contrast agents and luminescent materials have been loaded into MSNs to achieve multifunction in tissue repair and bio-labeling [4–8]. However, the relatively low loading

* Corresponding author. State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai, 200050, China.
** Corresponding author. Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, 4059, Australia.
E-mail addresses: yin.xiao@qut.edu.au (Y. Xiao), chengtiewu@mail.sic.ac.cn (C. Wu).
[1] Mengchao Shi and Lunguo Xia are co-first authors.

http://dx.doi.org/10.1016/j.biomaterials.2017.08.027
0142-9612/

efficiency, poor targeting ability, complex synthesis process and side effect caused by remaining additives limit the further application of MSNs as drug carrier for tissue engineering [9]. Previous studies demonstrated that Si ions can be effectively released with a sustained profile from MSNs [10]. The specific surface area of MSNs is the main factor for controlling the dissolution rate. With the higher surface area exposed to the dissolution media, the diffusion rate in MSN matrix could be distinctly enhanced [11]. Thus, MSNs, the silica-based nanomaterial with tunable high specific surface area, can be applied as a degradable resource for Si ions release. It is known that the effect of Si itself, as an important factor in skeletal development and repair, could interfere with the mineralization process of bone growth [12,13]. At cellular level, it is indicated that Si-containing ionic products can improve the proliferation and osteogenic differentiation of bone marrow stromal cells (BMSCs) and periodontal ligament cells by stimulating the osteogenesis-related gene expression and bone matrix protein of BMSCs [14]. Si ions also present a positive effect on angiogenesis by enhancing cell migration, chemotactic homing, tubular networking, *in vivo* neo-blood vessel sprouting through the activation and stabilization of HIF-1α, which is a master event in a down-stream signaling of angiogenesis [15,16]. To construct multifunctional biomaterials for tissue regeneration, more and more attention has been paid on taking advantage of these inorganic ions with favorable biological activities instead of the high-cost drugs and cytokines [17].

Inspired by the *in-situ* incorporation of therapeutic ions in silicate-based bioceramics and bioactive glasses, which showed improved stimulatory effect on tissue repair [18,19], it is of great interest to introduce such functional ions into MSNs. We speculate that the combination of these foreign ions with the intrinsic Si ions can award the materials with improved biological properties for tissue regeneration. It has been reported in previous studies that europium (Eu), one of the lanthanides, can functionally mimic calcium (Ca), influence the bone remodeling cycle and treat bone density disorders such as osteoporosis [20,21]. Previous study showed that the Eu hydroxide nanorods (EHNs) could enhance the proliferation of HUVECs *in vitro* and stimulate vascular sprouting *in vivo* [22]. Additionally, Eu ions possess satisfactory luminescent property with increased brightness and prolonged signal intensity that has potential for bio-labeling [23]. Till now, as for the research of Eu ions for the biomedical application, much attention has mainly been paid on the fluorescence property and the application as imaging agent. Most of the Eu-containing biomaterials have been used for luminescent cellular/tissue imaging [24], as well as to track and monitor the drug release behavior [25]. Although the cytotoxicity of some Eu-containing biomaterials has been studied recently [26,27], the exact biological role of Eu ions, especially the effect on tissue regeneration process, has not been investigated yet. On the other hand, most of the present Eu-containing nanomaterials are either regular-shaped crystalloid such as Eu hydroxide nanorods and NaF_4:Eu^{3+} that have poor degradability [22,26], or the irregular-shaped amorphous glasses used for luminescent devices [28,29]. The preparation of Eu-containing nanoparticles for application of tissue engineering with uniform morphology and satisfactory degradation ability still remains a great challenge. Considering its attractive performance and potential in tissue engineering, Eu is a promising candidate to be incorporated into MSNs to achieve degradable and homogeneous nanospheres and to further explore the biological properties.

A great amount of previous studies on nanomaterials used for tissue regeneration have focused only on either the osteogenesis or angiogenesis-stimulatory properties on BMSCs or HUVECs [30,31], very few attention has been paid on the interaction between nanoparticles and immune cells as well as the following tissue regeneration [32]. It is widely recognized that the bone regeneration process is a result of multiple tissue responses involving early inflammation, angiogenesis and osteogenesis. Neglecting the inter-connection of these factors could lead to the discrepant results between *in vivo* and *in vitro* studies and thus the failure of clinical trials [33]. Particularly, as the first line of defense against foreign bodies, the innate immune response by macrophages is critical to new bone and neo-vessel formation by sharing a number of cytokines, receptors, signaling molecules and transcription factors [34]. Inflammatory cytokines interleukin-4/10/13 (IL-4, IL-10, IL-13) can inhibit osteoclast differentiation and bone resorbing activity, and enhance the osteoblast activity and bone formation while IL-1, IL-6 and tumor necrosis factor-α (TNF-α) possess the opposite effects [35]. Similarly, it has been recognized that during inflammatory reactions, immune cells could enhance neovascularization via secreting pro-angiogenic factors including VEGF, TNF-α and IL-8 and angiogenesis-modulating enzymes including matrix metalloproteinases (MMPs) and cycloxygenase-2 (COX-2) [36]. However, there are very few studies related to how the interactions of nanobiomaterials and immune cells further affect the subsequent osteogenesis and angiogenesis of stem cells. Thus, it is of great importance to investigate how Eu-MSNs modulate the immune response of macrophages, and its following influence on skin and bone regeneration.

In this study, we applied a facile and efficient approach to incorporate therapeutic ion Eu into MSNs to obtain uniform Eu-MSN nanospheres and investigated the influence of the nanomaterial on the response of macrophages, osteogenic differentiation of BMSCs, angiogenic differentiation of HUVECs, as well as the *in vivo* bone regeneration and skin would healing.

2. Materials and methods

2.1. Synthesis and characterization of mesoporous silica nanospheres (MSNs) and europium containing mesoporous silica nanospheres (Eu-MSNs)

In typical synthesis of MSNs, 2 mL of ammonium hydroxide (NH_3H_2O) and 0.728 g of cetyltrimethylammonium bromide (CTAB) were added to 200 mL of distilled water and stirred for 1 h at 40 °C. 4.167 g of tetraethoxysilane (TEOS) was dissolved in 100 mL of ethanol and added to the above water solution, stirring for 5 h at 40 °C. Eu-MSNs with different concentrations of Eu doping (1 mol%, 2 mol% and 3 mol%) were acquired by adding 0.09 g, 0.182 g and 0.276 g of europium nitrate hexahydrate ($Eu(NO_3)_3 \cdot 6H_2O$) with 4.167 g of TEOS in 100 mL of ethanol, respectively. The solution was then cooled at room temperature overnight. The precipitate was collected by centrifuge at 7000 rpm for 15 min and washed 3 times with distilled water and ethanol. After drying the as-synthesized materials under vacuum for 24 h, it was then calcined at 600 °C in air for 6 h at a heating rate of 1 °C/min to remove the structure directing template CTAB. The morphology and structure of MSNs/Eu-MSNs was characterized by scanning electron microscopy (SEM, Magellan 400, FEI, USA), transmission electron microscopy (TEM, JEM-2100F, JEOL, Japan) and small-angel X-ray diffraction using CuKα radiation at a scanning rate of 0.4°/min with a step width of 0.002° from 1.0° to 5.0° (SAXRD, Rigaku D/Max-2550V, Geigerflex, Japan). The element distribution was analyzed by the SEM accessary energy dispersive spectrometer (EDS) system while the quantitative analysis was conducted by inductively coupled plasma-atomic emission spectrometry technique (ICP-AES, Thermo Fisher X series 2, USA). The isotherm pattern, specific surface area and pore volume were tested by N_2 adsorption–desorption (Micromeritics ASAP 2010 analyzer, Micromeritics, USA) using BET and BJH model calculation. The surface charges of MSNs and Eu-MSNs in distilled water were tested by Zeta-potential (Zeta sizer