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Original Research Paper

The effects of morphology on physicochemical properties, bioactivity and biocompatibility of micro-/nano-bioactive glasses

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ABSTRACT

We prepared micro-/nano-bioactive glasses (MNBG) with controlled morphologies by using a sol-gel method combined with a template (CTAB) centered self-assembly technology, and investigated the effect of the MNBG morphology on physicochemical properties, apatite-forming ability and biocompatibility. Results showed that the specific surface area of rod-like MNBG (RBG) with highly ordered mesoporous structure was higher than that of spherical MNBG (SBG) with irregular worm-like mesoporous structure. Both the MNBG showed the high apatite-forming ability, and the apatite-forming ability of RBG with higher specific surface area was higher than that of SBG. Futhermore, both SBG and RBG had good biocompatibility, could promote proliferation and ALP differentiation of human dental pulp cells (HDPCs), and SBG with a smaller aspect ratio could significantly increase the proliferation and differentiation of the cells, as compared to RBG. This study may motivate the development and applications of MNBG with controllable morphology in dental repair.

1. Introduction

Bioactive glasses (BGs) are promising biomaterials for bone tissue repair and engineering applications, because of their excellent abilities of rapidly bonding to bone and stimulating new bone growth [1,2]. Many studies have demonstrated that the bone-bonding ability of BGs is attributed to the formation of a hydroxycarbonate apatite (HCA) layer on the surface of BGs when contacted with biological fluids [3–5] and osteogenesis is thought to be induced by the ions released from the BGs which can stimulate the production of growth factors and cell proliferation as well as up-regulate the bone regeneration related genes expression in osteoblasts [6–8]. Furthermore, BG can reportedly promote alkaline phosphatase (ALP) activity and collagen type I (Col I) formation which also make functions in dentin formation [9,10]. Dong et al. found that BGs could increase the ALP expression of human dental pulp cells and induce the odontogenic differentiation and dentin formation of dental pulp cells [11,12]. However, the use of BGs in most studies was prepared by melting or sol-gel method, which was inhibited by problems such as severe particle agglomeration, uncontrollable particle size and uncontrollable morphology [13–15].

Particle size can affect the efficiency and pathway of cellular uptake by influencing the adhesion of the particles and their interaction with cell [16–19]. Micro-/nano-BG (MNBG) particles are an attractive alternative to BG micro-particles for dentin tissue regeneration due to their specifical properties such as smaller size and higher specific surface area. The higher specific surface area of the micro-/nano-sized particles should lead to increased interface effects and it should also contribute to improved bioactivity, when compared to mm-sized particles [20–23]. However, most studies about BGs for dentin regeneration were focused on the preparation methods and properties of spherical NBG particles which usually presented as aggregate [24,25]. Furthermore, the morphology of biomaterials also plays an important role in affecting their physicochemical and biological properties. Yang et al. revealed that the shape of hydroxyapatite particles had a strong influence on cellular behavior and the sphere-like particles performed better than the rod-like particles [26]. Huang et al. found that the mesoporous silica nanoparticles with larger aspect ratios had a greater impact on

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cell proliferation, apoptosis, cytoskeleton formation, adhesion and migration [27,28]. Hence, it is of great importance to investigate the morphology effect of BG particles on the physicochemical properties and biocompatibility.

In our previous studies, MNBG with controlled morphologies (from spheres to short or long rods) were successfully synthetized by using a sol-gel method combined with a template (CTAB) centered self-assembly technology [29]. In this work, we further investigated the morphology effect of MNBG particles on physicochemical properties and biocompatibility. Physicochemical properties, apatite-forming bioactivity, cellular uptake, cytoskeleton organization, proliferation and differentiation of spherical MNBG (SBG) and rod-like MNBG (RBG) co-cultured with human dental pulp cells (HDPCs) were evaluated.

2. Materials and methods

2.1. Preparation and characterization of MNBG with controlled morphologies

In our previous studies [29], MNBG with controlled morphologies were successfully prepared by improved sol-gel method using CTAB as the template agent. Spherical and rod-like MNBG could be synthetized by controlling the concentrations of CTAB. The resultant MNBGs with spherical and rod-like morphology were denoted as SBG and RBG, respectively. The morphology and microstructure of samples were determined using transmission electron microscopy (TEM, JEM-2100HR,Japan, 100 kV). Specific surface area was evaluated using multipoint Brumauer-Emmett-Teller (BET) N_2 absorption technique at 77.3 K.

2.2. Assessment of in vitro apatite-forming ability

The assessment of the *in vitro* apatite-forming ability of the obtained MNBG was tested by immersing samples in simulated body fluid (SBF) described by Kokubo et al. (Na^+ 142.0, K^+ 5.0, Mg^{2+} 1.5, Ca^{2+} 2.5, Cl^- 147.8, HCO^{3-} 4.2, HPO_4^{2-} 1.0, and SO_4^{2-} 0.5 mmol L^{-1}) at a concentration of 1 mg/mL at 37 °C to monitor the formation of HCA on the surface of the samples with time [30]. The samples were soaked in SBF for 5 d without refreshing solution. Once removed from the incubation, the solids were separated by centrifugation, washed three times with acetone and deionized water, then dried at ambient temperature and characterized using FE-SEM (Nova NanoSEM430, FEI, USA, and 15 kV), Fourier transform infrared spectroscopy (FT-IR, Nexus, Nicolet Co., USA) and powder X-ray diffraction (XRD, X'pert PRO, Panalytical, the Netherlands) with Cu K_a (1.548 Å).

2.3. Culture of human dental pulp cells

The use of primary human dental pulp cells (HDPCs) in this study was approved by the Oral Surgery Department of Peking University School and Hospital of Stomatology, Beijing, People's Republic of China, and all patients provided written informed consent and ethics permission. HDPCs were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, Invitrogen, Catalog No. 12430) supplemented with 10% fetal bovine serum (FBS, Gibco, Invitrogen, Catalog No. 12657029), 100 U/mL penicillin, and 100 mg/mL streptomycin. The cells were cultured at 37 °C in a 5% CO_2 atmosphere and 95% of relative humidity to allow the cells to adhere. The culture media was changed every 2–3 d and the non-adherent cells were washed away by phosphate buffer solution (PBS). When the HDPCs were cultured until 90% confluence, the confluent cells were detached by trypsin and used for the seeding, attachment, proliferation and differentiation assays. Cultured HDPCs in Passages 4–6 were used in subsequent experiments.

2.4. Cellular uptake

The particles internalized by the cells were characterized by using TEM. For TEM analysis, the cells were treated with 50 μg/mL of SBG and RBG for 24 h. The cell monolayers were then rinsed with PBS three times and digested with 0.25% trypsin-EDTA solution. After digestion, the cells were centrifuged into pellets and washed with PBS, then fixed in 0.1 M phosphate buffer containing 2.5% glutaraldehyde and 2% paraformaldehyde for 24 h. The samples were then fixed again with 1% osmium tetroxide for 2 h, routinely dehydrated through gradient ethanol (30%, 50%, 60%, 70%, 80%, 90%, 95%, 100%, 5 min each), and embedded in epoxy resin. Resin-embedded blocks were cut into 60–80 nm ultrathin sections with an ultra-microtome. The ultrathin sections were placed on 200-mesh copper and examined with TEM.

2.5. Cell proliferation assay

MNBG solutions were prepared by dispersing SBG and RBG in culture medium at a concentration of 100 μg/mL, respectively. Immediately prior to their use, the samples were thoroughly homogenized by ultrasonic dispersion. The cells cultured without the particles were used as controls. The cells were seeded into a 96 well plate with 8000 cells per well and incubated at 37 °C in 5% CO_2 for different times. The cells were exposed to SBG and RBG solutions by adding 200 μL of SBG and RBG solutions to the monolayer of cells in each well, respectively. The cells proliferation was quantitatively determined by using a cell counting kit (CCK-8) in accordance with the manufacturer's instructions. At the time point, samples were washed with PBS and 100 μL CCK-8 mixture solutions (0.5 mg/mL) were added to each well, followed by incubation for 2 h. The absorbance was measured at a wavelength of 450 nm using a micro-plate reader (Thermo 3001, Thermo Co., USA). The study was repeated three times and six replicates were used within each study.

2.6. Cell cytoskeleton formation

F-actin was stained with FITC-phalloidin (AAT Bioquest, Inc.). Briefly, the cells were plated on the confocal dish and incubated with SBG and RBG at concentrations of 100 μg/mL for 24 h. After incubation, the cells were fixed with 4% formaldehyde and permeabilized with 0.1% Triton X-100. The dishes were then incubated at room temperature with FITC-phalloidin for 1 h. The cells were washed three times with PBS, stained for 5 min in DAPI (Beyotime) at room temperature, and washed five times with PBS again. Cells were directly visualized by confocal microscopy (LSM710, Zeiss, Germany) and representative photographs were taken.

2.7. ALP activity assay

The differentiation of HDPCs cultured on SBG, RBG particles and without particles was assessed by measuring the ALP activity using p-nitrophenyl phosphate substrate (pN) (ALP kit, Thermochem). At 7 and 10 d, the culture medium was removed and the cell layers were rinsed gently 3 times using cold PBS. The cells were lysed and lysate was assayed with the hydrolysis of pN in the presence of ALP enzyme. This evaluation was performed in accordance to the manufacturer's instructions (Sigma, USA) and the absorbance was measured at a wavelength of 405 nm using a microplate reader. A sample cultured under the same conditions without cells was used as a blank to correct the absorbance values. After normalizing to the total protein content, the ALP activity was calculated

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