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Interaction of bone progenitor cells with silica-based calcium phosphate glass powders prepared by sol–gel template-free and template-structured processes

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Abstract

The main objective of this study was to compare the response of bone progenitor cells (D1) to silica-based calcium phosphate glass (80:15:5) powders prepared by sol–gel template-free (SCP) and template-structured (SCP-T) processes. The SCP-T powders exhibited an approximately three times larger specific surface area compared to that of SCP powders. The formation of a hysteresis loop also signified the presence of mesoporous structures in the SCP-T samples; however, a hysteresis loop was not observed for the SCP samples, resulting in 1/5 of the pore volume of the SCP-T samples. The viability of the cells cultured in media containing the SCP-T powders for 24, 48, and 72 h was greater than 90%. D1 cells cultured on SCP and SCP-T substrates showed the highest cell proliferation after 1 h of culture. The number of cells decreased from 24 h to 168 h for all experimental groups ($p > 0.05$). Importantly, both the SCP and SCP-T substrates had positive effects on the osteogenic differentiation and mineralization of the progenitor cells. However, cells cultured on SCP-T had approximately 3.4 times higher ALP activity.

Keywords: Bone progenitor cells; Osteogenic; Silica-based calcium phosphate glass

1. Introduction

Silica-based calcium phosphate glasses (SiO_2–CaO–P_2O_5) were first prepared using the sol–gel process in the early 1990s [1]. Using this method, porous silica-based calcium phosphate glasses were prepared from the hydrolysis and polymerization of metal hydroxides, alkoxides or inorganic salts. This synthesis method and application indicated that sol–gel chemistry could be a potential processing method for molecular and textural tailoring [2–6]. In contrast to melt-derived bioglasses, sol–gel glasses are prepared at low processing temperatures. Due to the high surface area and porosity derived from the sol–gel process, a wide range of bioactive compositions can be prepared to achieve higher bone bonding rates, as well as excellent degradation/resorption properties [7,8].

Recently, mesoporous structures containing silica-based calcium phosphate glass (SiO_2–CaO–P_2O_5) have been proposed as potential bone implant materials. These materials have a high specific surface area, as mesopores ranging from 2 to 50 nm are distributed throughout the structural matrix. As a result, these materials are capable of fast reactions, specifically

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dissolution and re-precipitation of bone-like apatite mineral [9–11].

Nanostructured mesoporous silica matrices can be produced using an evaporation-induced self-assembly method (EISA) [12]. In this method, the evaporation of an organic solvent triggers silicon and a high molecular weight tri-block polymer surfactant, which is used as a templating agent, to self-assemble into micelles. Assembly into a liquid-crystalline mesoporous phase structure is the next step. Finally, high temperature heat processing is performed to remove the surfactants, leaving behind a mesoporous structure consisting of silica-based calcium phosphate glass material [10,13–15,]. In this process, the parameters associated with the templating agents are critical for the preparation of large surface areas and pore structure features [16].

To assess the biocompatibility of mesoporous structures of bioactive glasses, *in vitro* testing has been conducted using human Saos-2 osteoblasts and murine L929 fibroblasts. High viability was observed for both cell types in the presence of these materials, and signs of cell damage were not observed. However, a reduction in the proliferation of both osteoblasts and fibroblasts was detected [17]. Interestingly, it has previously been reported that silica-based calcium phosphate glass materials with a mesoporous structure could improve the *in vivo* bioactivity of polymer-based scaffolds[18]. Nevertheless, prior studies on silica-based calcium phosphate glasses have not examined the relationship between the biological response and the effects of the template agents.

In this study, we hypothesized that powders created with templating agents to have high specific areas and mesoporous structures would have a more positive impact on biological response. To test this hypothesis, $80SiO_2$–$15CaO$–$5P_2O_5$ powders prepared using a template free process (SCP) or with templating agents (SCP-T) were used to evaluate the response of progenitor bone cells.

2. Material and methods

2.1. Material preparation

The testing group, silica-based calcium phosphate glass only (SCP), was fabricated without the F127 agent. The control group, mesoporous structured silica-based calcium phosphate glass (SCP-T), was prepared using a typical synthesis method with F127 as a templating agent. Briefly, F127 (7.0 g), tetraethyl orthosilicate (TEOS, 6.7 g), $Ca(NO_3)_2 \cdot 4H_2O$ (1.4 g), triethyl phosphate (TEP, 0.73 g; 80:15:5 Si:Ca:P molar ratio), and 0.5 M HCl (1.0 g) were dissolved in ethanol (60 g) and stirred at room temperature for 1 day [11]. Then, a polyurethane foam was immersed in the sol, compressed, and then released. This process forced the sol into the pores of the foam and allowed for uniform coating with the appropriate sol. The raw porous scaffold bodies were fully dried and then thermally treated at a constant heating rate (10 °C/min) to calcination temperatures in the range of 600 °C for 2 h. After cooling, the powders were ground, sieved through #325 meshes and then subjected to characterization.

2.2. Textural characterization

Nitrogen adsorption and desorption isotherms were measured at 77 K on a Quantachrome Autosorb 1 sorption analyzer. All samples were purged for 12 h at 150 °C under high vacuum in the degas port of the adsorption analyzer. The specific surface areas of the samples were measured using the BET method (ASAP 2010, Micromeritics, USA), with nitrogen as an absorbent.

2.3. Cytotoxicity of SCP-T

The sample powders were sterilized under high pressure at 121 °C for 20 min in an autoclave. Then, 1 g of SCP-T powder was incubated in 10 mL of culture medium at 37 °C and 5% CO_2. After 4 days of incubation, the conditioned media was centrifuged at 2000 rpm for 5 min. The supernatants were subsequently filtered and used for cytotoxicity testing with fibroblast cells (NIH 3T3, abbreviated 3T3). The cells were provided by the National Institute of Health (NIH) in Taiwan and were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Invitrogen Taiwan Ltd., MD) containing 10% bovine serum (BS) (Biological Industries, Haemek, Israel). An XTT Cell Viability Assay Kit was used to quantify the number of live cells using an absorbance reader. Briefly, 3T3 cells were plated at a seeding density of 1×10^4 cells/well in a 96-well microplate and cultured for 24 h. The media was then replaced with SCP-T conditioned media (100 μL/well) for another 24 h. For analysis, the cells on the sample surface were washed with phosphate-buffered saline (PBS) and then treated with 100 μL of culture medium containing 50 μL of XTT reagent for 4 h. The reaction medium was then measured spectrophotometrically at 490 nm using a microplate reader UVM-340 (ASYS Hitech GmbH, Eugendorf, Austria). Finally, the cell numbers were determined from a plot of absorbance (OD values) *versus* cell number. Each experiment was performed five times ($n=5$).

2.4. Cell seeding and SEM specimen preparation

Bone marrow mesenchymal stem cells (MSCs) cloned from Balb/C mice (D1 cells) were purchased from the American Type Culture Collection (ATCC). The cells were cultured under standard conditions (*i.e.*, 37 °C, humidified atmosphere with 5% CO_2) in DMEM media supplemented with 10% FBS, 100 IU/mL penicillin, and 100 mg/mL streptomycin. The cells were not used beyond passage 8.

D1 cells were seeded onto SCP and SCP-T disks at an initial density of 1×10^5 cells/cm^2 and placed in a 48-well culture plate. The cells were incubated for 1, 24, 72, or 168 h. Afterwards, the SCP AND SCP-T disks were fixed in a solution of 25% glutaraldehyde with 4% paraformaldehyde for 1 h at 4 °C. The cells were then washed in wash buffer containing 4% sucrose in PBS and post-fixed in 1% osmium tetroxide in PBS for 1 h at 4 °C. Samples were then dehydrated sequentially in graded ethanol (30%, 50%, 70%, 95%, and 100% ethanol), dried in hexamethyldisilizane (HMDS) for