Cytotoxicity Evaluation of Three Dimensionally Ordered Macroporous Sol-gel Bioactive Glass (3DOM-BG) Particles*

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Introduction: Sol-gel bioactive glasses (BGs) are a type of third generation biomaterial (i.e., materials that are both bioactive and bioresorbable) [1]. Recently, sol-gel bioactive glasses with three dimensionally ordered macroporous structures (3DOM-BGs) were developed by a sol-gel process combined with a colloidal crystal templating method [2-4]. 3DOM-BGs have been proven to be bioactive (as represented by the bone-like apatite formation) and fully resorbable (as represented by the complete conversion of 3DOM-BG to apatite) in an acellular *in vitro* model (simulated body fluid) [2-4]. This study continues the research of 3DOM-BGs with an emphasis on their *in vitro* cellular responses.

Materials and Methods: 80 % SiO_2 – 20 % CaO (mole fraction) 3DOM-BG particles were prepared as previously described [2-4] and characterized by scanning electron microscopy (SEM) and X-ray micro-diffractometry (XRD). For cell studies, 3DOM-BG particles (size < 1 mm) were sterilized in an autoclave.

Culture of osteoblast-like cells (MC3T3-E1) was performed as previously reported [5]. Three tests were performed: (1) Wst-1assay for cell viability after culturing with leaching products from 3DOM-BG; (2) direct contact cytotoxicity assay; and (3) two-color fluorescence viability assay for imaging cells cultured on 3DOM-BG particles. For Wst-1 tests, 3DOM-BG particles were immersed in culture medium for 24 h [soaking ratio: (0, 0.5, 5 and 25) mg/mL]. The medium was then transferred onto cells in a 24-well plate (10^4 cells/well). Wst-1 tests were performed after 3 d. Triton X-100 (polyoxyethylated octyl phenol, 0.1 % mass fraction) was chosen as a positive/toxic control. Two-way ANOVA with Tukey's multiple comparisons was used to analyze the Wst-1 data. For direct contact cytotoxicity assay, MC3T3-E1 cells were cultured with 0.5 mg/mL of 3DOM-BG particles in a 24-well plate and imaged using phase contrast microscopy after (1, 7 and 14) d. For two-color fluorescence viability assay, cells were cultured (1, 7 and 14) d on and around 3DOM-BG particles, stained with calcein-AM (live cells, green) and ethidium homodimer-2 (dead cells, red) and imaged using fluorescence microscopy.

Results and Discussion: SEM studies (Figure 1A) demonstrated that the CaO-SiO_2 3DOM-BGs have unique 3D ordered macroporous structures [2]. XRD showed that 3DOM-BG is amorphous (data not shown). Wst-1 results showed that there was no statistical difference between negative control and samples, indicating that leaching products of 3DOM-BG are non-toxic (data not shown). Fluorescence images from live/dead studies (data not shown) showed that cells are alive on and around the 3DOM-BG after culturing up to 14 d. Phase contrast microscopy (Fig. 1B) showed that cells proliferated and reached confluence around and adjacent to 3DOM-BG particles after 14 d. This is believed to be an event leading to bone nodule formation [6].

Summary: Wst-1 assay, direct contact cytotoxicity assay and twp-color fluorescence viability assay showed that 3DOM-BG particles are non-cytotoxic and *in vitro* compatible with osteoblasts.

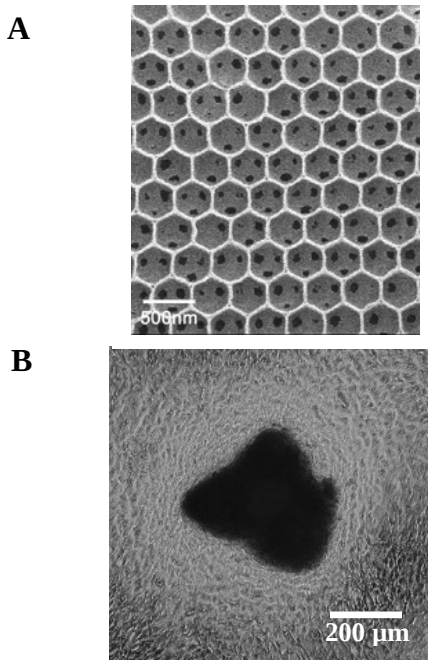

Figure 1. (A) SEM image showing the typical microstructure of the 3DOM-BG particles. (B) Phase contrast image of cells cultured with 3DOM-BG for 14 d.

References

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