Uniform Beads with Controllable Pore Sizes for Biomedical Applications

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Statement of Purpose: Porous beads are attractive for applications in tissue engineering and cell delivery. The advantages of porous beads include large surface area, good mechanical strength, and high interconnectivity; all of them could facilitate cell seeding and migration, as well as transportation of nutrient/oxygen and metabolic wastes, in addition to the protection of cells in porous beads from physical damages. The cell/beads constructs are also injectable into the body using a needle without surgical operation. A number of techniques have been demonstrated for producing porous beads.[1] However, these methods were mainly based on the use of a gas forming agent and the resultant beads were often limited in terms of structure openness and interconnectivity, porosity, and size uniformity for the beads. In the present study, we seek to demonstrate a simple method for fabricating uniform porous beads with different pore sizes based on an unstable emulsion using a fluidic device, and evaluate the effect of pore size on cell growth.

Methods: The W-O emulsion was prepared by emulsifying an aqueous solution (2 g) of gelatin (7.5 wt%) and PVA (1 wt%) in 6 g of a PLGA solution (2 wt%) with a homogenizer at 20,000 rpm for 3 min. In 5 min after homogenization, the W-O emulsion was introduced as a discontinuous phase into the fluidic device,[2] where an aqueous PVA solution served as the continuous phase. A syringe with a luer tip offset from the center of the barrel was employed to selectively introduce the top or bottom layer of the phase-separated emulsion. Water-in-oil-in-water (W-O-W) droplets were collected in a 1-L tall beaker containing 900 mL of ice-cold water and gently stirred overnight to allow the organic solvent to evaporate. Afterwards, the beads with gelatin were gently stirred in 900 mL of warm water held at 45 °C for 3 h to completely remove residual gelatin, and then washed with water. NIH/3T3 fibroblasts were seeded into the beads using a spinner flask for 24 h. The beads with cells were then washed with PBS, and transferred into a 96-well plate (one bead per well). Cells were characterized with fluorescence microscope by 4,6-diamino-2-phenylindole (DAPI, for nuclei) and LIVE/DEAD staining. Cell proliferation was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.

Results: We could selectively introduce the bottom (Fig. 1A) or top (Fig. 1B) layer of the phase-separated emulsion into the fluidic device using a syringe with a luer tip offset from the center of the barrel. The insets in Fig. 1A and 1B, show optical micrographs taken from the bottom and top layers of the emulsion, where the gelatin/water droplets were dispersed in an oil phase. Small gelatin/water droplets were mainly found in the bottom layer of the emulsion at a low volume ratio relative to the oil phase, whereas the large, coalesced gelatin/water droplets dominated the top layer at a high volume ratio. This observation could be ascribed to the high density of DCM (1.33 g/cm^3) and the absence of an oil-soluble surfactant. The selective introduction of each layer resulted in porous beads with different pore sizes (Fig. 1, C and D, 13.1 ± 7.3 and 35.8 ± 12.5 μm, respectively). The average overall diameters of the beads with small and large pores were 321.5 ± 12.6 and 368.5 ± 9.6 μm, showing good uniformity. Figure 1, E and F, show fluorescence micrographs of microtomed sections of the cell/bead constructs stained with DAPI for nuclei after 1 day of culture. It appeared that more cells could penetrate and reach the center for the beads with large pores than those with small pores. MTT assay indicated that, the proliferation rate of cells in the beads with small pores was slightly faster than in the beads with large pores up to 5 days of culture, which was probably due to the large outer surface area of the beads with small pores; however, they dramatically lost their viability after 7 days, whereas the cells in the beads with large pores kept growing with high viability (data not shown). LIVE/DEAD staining of cell/bead constructs (Fig. 1, G and H) also confirmed the high cell viability in the beads with large pores.

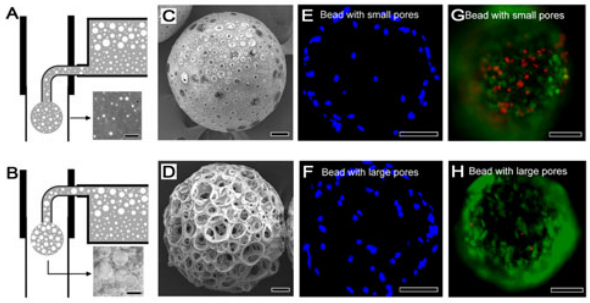

Figure 1. A, B) Schematic diagrams of the fluidic devices. Insets show optical micrographs of the emulsion in each phase. C, D) Scanning electron microscopy images of porous beads made from the phases shown in (A) and (B). Fluorescence micrographs of nuclei staining (E, F) and LIVE/DEAD staining (G, H) of cells in porous beads with (E, G) small pores and (F, H) large pores. Scale bars: 10 μm in A, B, and 50 μm in C-H.

Conclusions: By employing an unstable emulsion and selectively introducing the top or bottom layer of the emulsion into a fluidic device, we successfully obtained uniform beads with controlled pore sizes. High viability and loading of cells in the beads with large pores were confirmed by culturing fibroblasts, compared to the beads with small pores. We believe that the uniform, porous beads have great potential for applications in tissue engineering and cell delivery.

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