Inverse Opal Scaffolds for Regenerative Engineering: Precision Control and On-Demand Fabrication

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Statement of Purpose: Three-dimensional (3D) scaffolds are finding widespread use in applications related to tissue engineering and regenerative medicine due to their pivotal role in supporting the attachment, migration, proliferation, and differentiation of the cells. Among various types of scaffolds, those with interconnected pores are usually advantageous in facilitating the transport of oxygen, nutrients, and wastes, which is highly important for maintaining high viability for the cells residing in the bulk of the scaffold. Despite many years of research, the use of 3D porous scaffolds has been largely limited to those fabricated using stochastic porogen methods, which often result in foams with random pore sizes, disordered structures, and non-uniformity in interconnectivity. Here we present a novel type of 3D porous scaffolds, the inverse opal scaffolds possessing well-controlled pore sizes, a long-range ordered structure, uniform interconnections, and great reproducibility among different batches. More importantly, we demonstrate that their pore sizes and related physical properties can be precisely controlled, which, can then affect the behaviors of cells and tissues both *in vitro* and *in vivo*.

Methods: Poly(D, L-lactide-*co*-glycolide) (PLGA) inverse opal scaffolds were fabricated according to our previously published protocol [1]. Briefly, uniform gelatin microspheres was packed into a cubic-close packed (ccp) lattice in a 50-mL centrifuge tube filled with methanol. After heating in an oven at a pre-set temperature (65, 80 or 100 ^{o}C) for certain period of time (0.5, 3, or 5 h), the lattice was harvested, placed on a filter paper to let methanol evaporate completely, and infiltrated with a PLGA solution in 1,4-dioxane (10%, 18%, 25% or 30%, in weight percentage). After removing the excess solution with a filter paper, the lattice pellet was frozen at -20 ^{o}C for 5 h, and lyophilized in a freeze-dryer overnight. The sample was then placed in a beaker containing 900 mL of water heated at 43 ^{o}C for 3 h under gentle stirring to dissolve the lattice of gelatin microspheres.

Results: In this work, we focused on inverse opal scaffolds made of PLGA because of the widespread use of PLGA as a scaffolding material owning to its biodegradability and biocompatibility. Fig. 1A shows a 3D schematic of an inverse opal scaffold. Three types of pores in different sizes are present. The first type (*i*) is the spherical pores comprising the majority of the scaffold, whose dimension is essentially the same as the diameter of the templating microspheres. We refer to this parameter as the "pore size", which is consistent with the definition used by other researchers working on porous scaffolds. The pore size is easily adjusted by using gelatin microspheres with different, uniform sizes. For example, Fig. 1, B and C, shows PLGA inverse opal scaffolds with pore sizes of 224 μm and 312 μm, respectively. The second type (*ii*) of pores is the circular pores interconnecting adjacent pores in the bulk, whose dimension is defined as the "window size". The window size of a scaffold can be controlled by changing the annealing condition of the gelatin lattice. As shown in Fig. 1, D-H, at a constant annealing time of 3 h, the window size increased from *ca.* 25 μm at 65 ^{o}C to *ca.* 43 μm at 80 ^{o}C and *ca.* 66 μm at 100 ^{o}C, corresponding to 13%, 23% and 35%, respectively, of the pore size (190 μm). The third type (*iii*) of pores is the circular openings on the surface of a scaffold, whose dimension we define as the "surface pore size". The surface pore size is dependent on the viscosity of the infiltrating PLGA solution in 1,4-dioxane. At a concentration of 10 wt.%, the surface pores had the same size as the pore size of 190 μm (Fig. 1G)); at 18 wt.% and 25 wt.%, the surface pore sizes were *ca.* 162 μm and 73 μm, respectively, corresponding to 85% and 38% of the pore size (Fig. 1, D and H); at 30 wt.% or higher, however, the surface of most pores became completely sealed by the polymer. We further found in an *in vitro* cell invasion experiment that, the migration of the cells into the inverse opal scaffolds was mainly determined by the window size, rather than the pore size of a scaffold. The different pore size could also affect the degree and pattern of neovascularization in PLGA inverse opal scaffolds *in vivo* [2].

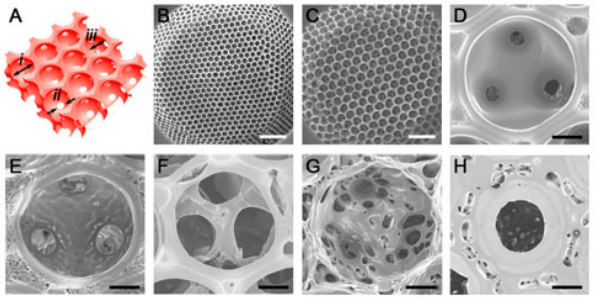

Figure 1. (A) A 3D schematic showing an inverse opal scaffold and three different sizes: *i*) pore size, *ii*) window size, and *iii*) surface pore size. (B, C) SEM images of PLGA inverse opal scaffolds with pore sizes of 224 μm and 312 μm, respectively. Scale bars: 1 mm. (D-H) PLGA inverse opal scaffolds with a pore size of 190 μm but window sizes of (D-F) 25 μm, 43 μm, and 66 μm, respectively, and surface pore sizes of (G, D, H) 190 μm, 162 μm, and 73 μm, respectively. Scale bars: 50 μm.

Conclusions: We have fabricated PLGA inverse opal scaffolds with precisely controlled pore sizes, window sizes, and surface pore sizes by adjusting the fabrication conditions. The behaviors of cells/tissues in the scaffolds are dependent on these physical properties of a scaffold. Therefore, it is highly significant that we can achieve precision control over the sizes of an inverse opal scaffold and on-demand fabrication for specific applications.

References: [1] Zhang Y. Biomaterials. 2010;31:8651; [2] Choi S-W. Adv Healthcare Mater. 2012; in press.