Combinatorial Screening of Osteoblast Response to 3D Nano-Composite Tissue Scaffolds Using Gradients and Arrays

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Statement of Purpose: Current methods for combinatorial and high-throughput (CHT) screening of cell-material interactions utilize a two-dimensional (2D) format where cells are presented on flat materials surfaces [1]. However, cell response in 3D scaffolds is more representative of their behavior in vivo [2]. Therefore, biomaterials must be fabricated into a 3D scaffold to generate a 3D tissue since cells are sensitive to topographical differences between 2D surfaces and 3D scaffolds. Thus, the objective in this work was to develop CHT methods to screen cell-material interactions in 3D. We used a bone tissue model to study osteoblast attachment and proliferation on porous poly(ε-caprolactone) (PCL) scaffolds containing amorphous calcium phosphate nanoparticles (nACP). nACP is highly soluble in water and releases calcium and phosphate ions that are known to enhance osteoblast response.

Methods: nACP particles (Ca:P ratio 1.5, 100 nm diameter) were prepared by spray drying [3]. A two-syringe pump CHT platform was adapted to prepare scaffold libraries [4]. Using a pure PCL (relative molecular mass 65000 g/mol, Sigma) solution (10 % mass/volume in dioxane) and a PCL solution supplemented with 30 mass % nACP, libraries were deposited in gradient and array formats on NaCl (225 μm-425 μm diameter). Polymer solution was deposited along a trough measuring 7 cm x 8 mm x 6 mm for gradients and in 36 wells of a 96-well plate for arrays (Fig. 1). Scaffolds were lyophilized and soaked in water to leach salt. For characterization, gradients were cut into seven segments along their length and array scaffolds were removed from the 96-well. nACP content was determined by thermogravimetric analysis (TGA). Amount of soluble calcium and phosphate ions released from scaffolds was measured by a spectrophotometric method. MC3T3-E1 cells (Riken) were cultured in Eagle's α-minimum essential medium supplemented with 10 volume % of fetal bovine serum and 0.6 % by volume kanamycin sulfate. Osteoblasts were cultured on gradients and arrays and DNA content was measured by Picogreen at 1 d (cell attachment) and 14 d (cell proliferation). For imaging, cell nuclei were stained with Sytox Green.

Results: TGA indicated that nACP in gradients spanned a range of 1 mass % to 14 % and in the arrays from 3 % to 23 % (not shown). Significant release of calcium and phosphate ions was measured from PCL scaffolds containing nACP (not shown). Cell numbers at 1 d increased monotonically with increasing nACP for gradients but were nearly the same for all compositions with arrays (Fig. 2). For arrays, scaffolds occupied the entire well bottom and cells were forced to adhere to the scaffolds. For gradients, cells could adhere to the scaffold or settle on the bottom of the dish. The availability of two outcomes (adherence to scaffold or to dish) made it possible for nACP to have an effect on adhesion (1 d) to scaffolds with the gradients. At 14 d proliferation, osteoblast numbers were enhanced in PCL scaffolds containing nACP for both gradients and arrays, but the magnitude of enhancement was higher for arrays (Fig. 2). For gradients, all scaffold compositions were in a single dish where released calcium and phosphate could diffuse to all gradient positions dampening differences in osteoblast proliferation between nACP-rich and nACP-poor segments. For arrays, discrete scaffold compositions were in individual wells where only the scaffolds within each well could release calcium and phosphate to activate osteoblasts. Calcium and phosphate ions released from array scaffolds were concentrated in the 0.2 mL of medium in each well leading to stronger enhancement of proliferation for array scaffolds with > 12 mass % nACP.

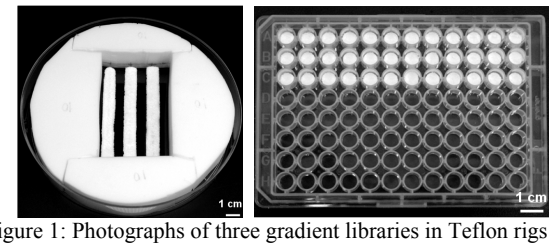

Figure 1: Photographs of three gradient libraries in Teflon rigs (left) and one array library in 96 well plate (right).

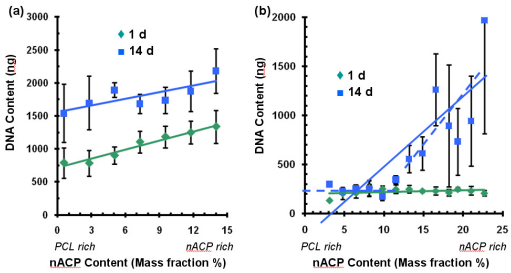

Figure 2: DNA content in (a) gradients and (b) arrays measured at 1 d (green) and 14 d (blue). Lines are linear fit to the data.

Conclusions: A new gradient approach for screening cell response to 3D scaffolds was used to demonstrate that nACP enhanced osteoblast adhesion and proliferation on composite scaffolds. Results for gradients and arrays were similar, but differences were observed that can be explained by effects of the cell culture designs on calcium and phosphate ion diffusion and concentration. Gradients were better suited for measuring cell adhesion (1 d) and arrays were better for proliferation (14 d).

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