Directing Cell Migration with Engineered Two-Dimensional Concentration Gradients of Fibroblast Growth Factor-2

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Statement of Purpose: Spatial patterns of growth factors, such as concentration gradients, play critical roles directing cell migration, proliferation, and differentiation during embryonic development and wound healing. Engineering arbitrary and persistent spatial growth factor patterns to study and control biological patterning relevant to tissue morphogenesis, homeostasis, and repair is vital to understanding tissue development. We have developed an inkjet printing method capable of creating biologically-inspired concentration modulated patterns of growth factors. We demonstrate this methodology using a model hormone, fibroblast growth factor-2 (FGF-2), naturally immobilized onto a fibrin substrate. We have already shown that uniform printed patterns of FGF-2 direct cell proliferation in a dose-dependent manner. In this study, we focused on creating concentration gradients of FGF-2 to determine the effects of gradients on directing cell migration and proliferation.

Methods: FGF-2 patterns were printed onto fibrin substrates using a custom inkjet printer. The inkjet is a MicroJet (MicroFab Technologies Inc., Plano, TX) piezoelectric drop-on-demand device with a 30 µm diameter nozzle that delivers approximately 14 pl drops of a dilute FGF-2 bioink. To verify pattern accuracy and quantify the retention of the printed patterns, FGF-2 labeled with Cy3 dye was printed and desorption experiments were performed using iodinated FGF-2. To assess the bioactivity of the printed FGF-2, uniform square patterns of FGF-2 with varying surface concentrations modulated by overprinting were printed first. The patterns were rinsed to remove unbound FGF-2 and a uniform distribution of MG-63 human osteosarcoma cells were seeded. Images of the patterns were acquired every thirty minutes and cell counts were performed on the patterns. To specifically address cell migration, experiments were also performed with concentration gradient patterns as well as uniform patterns and reverse gradients to serve as controls. After printing, unbound FGF-2 was removed and Swiss3T3 mouse fibroblast cells were seeded to form a starting line at one end of each of the patterns. The patterns were imaged every five minutes for up to five days. With both the uniform pattern and concentration gradient experiments, individual cell movement needed to be tracked to properly quantify the cellular response to the patterns. With the image stack of each pattern consisting of more than 1000 images, manual cell tracking was impractical. Computer vision was used to automate tracking of individual cell movement and cell proliferation to characterize the cell response to each of the patterns.

Results / Discussion: Imaging of the patterns printed using Cy3 labeled FGF-2 demonstrated that inkjet printing can be used to accurately deposit gradient patterns. Desorption experiments performed with iodinated FGF-2 showed an initial 90% loss of the applied FGF-2 during the post-printing rinse steps. However, the remaining 10% was bound to the fibrin substrate for up to 9 days with negligible subsequent desorption. Since the cells are seeded on the substrates after the initial rinse period, the cells are interacting with immobilized growth factor patterns and not soluble FGF-2. The MG-63 cells proliferated in register with the uniform printed square patterns demonstrating that the immobilized FGF-2 was biologically active. Cell counts on the patterns indicated that the cells proliferated in a dose-dependent manner up to a level of saturation where increased FGF-2 surface concentrations did not result in increased cell density. Computer vision analysis of the images demonstrated that the primary response of the cells to the uniform patterns of FGF-2 was proliferation of cells that were on the patterns at the beginning of the experiment. In addition, cells that encountered the pattern during the experiment did not move off but remained on the pattern. For the migration studies, computer vision analysis demonstrated that the gradient of increasing concentration from the cell source directed the cell migration front, defined arbitrarily as the line that 90% of the cells are behind, further than the control patterns. Individual cell trajectories on the gradients were able to be tracked demonstrating migration persistence using computer vision analysis. The slope of the gradient as well as gradient design was found to influence the level of cellular response.

Conclusions: We have demonstrated an inkjet printing methodology that can be used to create immobilized patterns of native growth factors on physiologically-relevant substrates. The surface concentration of FGF-2 can be controlled by overprinting and remains bound to the substrate for up to 9 days *in vitro* while retaining its biological activity. The technique was used to create both uniform and concentration gradient patterns of FGF-2. Analysis with computer vision demonstrated that uniform concentration patterns direct proliferation while concentration gradients direct movement of the cell population front consisting of a combination of cell migration and proliferation. These studies verify the relevance of immobilized growth factor patterns in directing cell organization. Future studies will focus on expanding this methodology. This patterning technique is not limited to FGF-2 and fibrin but is readily extensible to other extracellular matrix substrates and hormones.

Reference: (Campbell PG. Biomaterials. 2005;26:6762-6770.)