A Systematic Approach to Bioprinting Growth Factor Gradients and Interpreting Cell Responses

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Statement of Purpose: Immobilized patterns of growth factors bound to the cell surface or to the extracellular matrix (ECM) have been shown to occur *in vivo.* These patterns provide cues for directing cell fate including migration, proliferation, and differentiation during development and wound healing. Therefore, engineering arbitrary and persistent growth factor patterns on a biologically-relevant substrate is important for studying the role of biological patterning during tissue repair. One approach that can be used to create such patterns is our inkjet bioprinting technology. Our technique consists of depositing native growth factors on fibrin coated substrates whereby retention of the printed patterns is dependent on native binding affinities. For example, we have previously demonstrated the utility of this method by patterning the proliferation of MG-63 preosteoblast cells in response to uniform and concentration gradients of fibroblast growth factor-2 (FGF-2). Although the bioprinting concept is straightforward, the practical utilization of this technique requires attention to many details and the integration of complimentary technologies to realize an effective printing system with interpretable results. Issues include: characterizing the printing substrate; quantifying the retention of the growth factor patterns; determining the appropriate printing process parameters such as drop spacing and surface concentration; and, categorizing and quantifying cell behaviors. In this study, we provide an example of our systematic approach to bioprinting to determine how human mesenchymal stem cells (hMSCs) respond to immobilized concentration gradients of FGF-2 and heparin-binding epidermal growth factor (HB-EGF).

Methods: The fibrin printing substrates were first investigated with immunostaining, SEM, and AFM to determine uniformity of coverage as well as surface roughness. Substrate preparation was optimized using these results. Since the affinity of each growth factor for fibrin is different, it is important to determine pattern persistence for each new growth factor used. To quantify the delivered surface concentrations of growth factor and determine retention of the patterns over time, the growth factors were iodinated and desorption experiments were performed. Growth factor patterns were printed using a MicroJet (MicroFab Technologies Inc., Plano, TX) piezoelectric drop-on-demand device with a 20 μm diameter nozzle that creates a printed spot size of approximately 50 μm. Computer vision-based calibration was used to print onto specific locations on the substrates to facilitate subsequent image analysis. To determine the range of surface concentrations that the cells respond to, including the minimum and saturation level, 3 by 3 arrays of uniform square patterns of varying surface concentration were printed. The patterned substrates were rinsed to remove unbound growth factor and a uniform distribution of hMSCs was seeded on the substrates. Images of the patterns were acquired every ten minutes using time-lapse video microscopy and average speed and cell counts were determined on and off the pattern for each square in the printed array. The dosage information obtained from the uniform pattern experiments was then used to design linear growth factor concentration gradients. The gradient patterns as well as uniform patterns were printed at a 90° angle relative to a starting line of seeded hMSCs. The patterns were imaged every ten minutes for up to seven days. Individual cell movements and proliferation of the entire cell population was tracked using an automated computer vision system to quantify the cellular response to the patterns.

Results / Discussion: The smoothness and uniformity of the substrate surface was found to be dependent on the preparation of fibrinogen. Aggregated fibrinogen resulted in a surface with large clusters of fibrinogen which attenuated the cell response to the printed patterns. Desorption experiments performed with the iodinated FGF-2 and HB-EGF showed an initial loss of the applied growth factor during the post-printing rinse steps. However, the retained growth factor was bound to the fibrin substrate for up to 10 days with negligible subsequent desorption. The hMSCs proliferated in register with the uniform printed square patterns of FGF-2 and HB-EGF in a dose-dependent manner up to a level of saturation. The uniform pattern arrays were used as a high throughput screening method to determine the relevant surface concentration range for creation of the concentration gradients. For the migration studies, computer vision analysis demonstrated that the concentration gradients directed cell alignment when compared with the control patterns of uniform concentration and no growth factor. Individual cell trajectories on the gradients were tracked demonstrating persistence of alignment and migration using computer vision analysis. The slope of the gradient and gradient design was found to influence the cellular response.

Conclusions: We have demonstrated a systematic approach for determining how cells respond to immobilized growth factor concentration gradients. We have carefully examined each aspect of the process from creation of the printing substrates to the cellular responses to the printed patterns. This study verifies the relevance of our inkjet bioprinting technology in directing stem cell fate. Future studies will focus on using this approach to include other ECM molecules as the printing substrate as well as patterning additional growth factors.

References:

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