3D Rapid Assays to Detect Differences in Morphology and Migration for Combinatorial Screening of Biomaterials: Proof of Concept

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Statement of Purpose: In tissue engineering, biomaterials are designed to modulate desirable cell responses [1]. Following injury, many tissues possess an inherent regenerative capacity, inducing changes in the local microenvironment. These key changes offer scientists a biomimetic strategy to alter cell behavior using engineered constructs. The in vivo complexity is a challenge due to the prohibitively large experimental space. Current methods are limited by throughput and cost, and examine cell response to a very narrow experimental space. Biomaterials are typically tested for a single cell type, rather than evaluating the cellular response of all resident cells. Understanding the synergistic effects of extracellular matrix (ECM) and growth factors (GFs) in modulating cell responses in 3D cultures is critical for the development of advanced composite biomaterials [3]. We present a rapid assay to detect differences in cell response within model 3D biomaterials by first analyzing cell morphology, followed by migration on a narrowed group of composite materials, narrowing the experimental space to identify optimal combinations that elicit desired response for tissue regeneration. To illustrate, we present a proof of concept, wherein combinations of 2 materials are generated to support cell spreading and cell migration in 3D.

Methods: 1.5mm thick polypropylene sheets with circular holes were used to house the arrays. Collagen type I (COL) was chosen as a base material because it is the structural basis for many tissues *in vivo* but is not found in Schwann cell (SC) basal lamina. Matrigel™ (BD Biosciences, San Jose, California) (MAT) was chosen as an additive because it is reconstituted basement membrane, primarily comprised of laminin and collagen type IV, known components of the natural SC basal lamina. For morphology screens, primary SC were seeded $0.1x10^6$ cells/mL into a library of scaffolds. Briefly, SC were suspended in 15µL of 2 mg/mL COL, mixed with a varying volume of MAT, and deposited into arrays. The number of samples is limited only by array size, however previous power analysis indicates that 4 samples are enough to determine significance. For migration experiments, 10µL of either COL only or COL-MAT (2 mg/mL and 0 or 0.35 vol. fraction, respectively) were deposited into arrays, partially polymerized (<10 min.), a second layer added, partially polymerized (<5 min.), and $2.5x10^3$ SCs injected into the center of the construct. 35% MAT was identified in the screen as an optimal material for SC spreading. In both assays arrays were fixed, stained for actin and nuclei, and imaged using a Typhoon Trio+ fluorescent scanner and a Zeiss LSM 510Meta confocal microscope. The scanner provides a platform for rapidly assessing cell cytoskeletal response to composite materials and confocal microscopy is used to verify that scanned results accurately represent the cell response. Image analysis was performed using ImageJ.

Results: SCs were seeded in COL-MAT gels, fixed at day 3, scanned and analyzed as described. MAT volumetric percentage varied from 0% to 100%. Scanned images were converted to binary and total area of fluorescence measured. In COL-only gels, SCs did not spread and remained rounded (1A-B). Inclusion of MAT (10, 20, 35, 50, 75%) induces statistically higher levels ($p<0.05$) of SC spreading (1A-B), while Matrigel-only constructs supported little cell spreading (not shown). It is interesting to note a non-linear response is observed, further supporting this screening to evaluate changes in cell spreading. Scaffolds are imaged using a more accurate but more costly manner and similar trends are observed with the greatest spreading at 20-35% MAT. Using this concentration, migration was evaluated relative to COL only constructs. Increases in migration were measured relative to changes in the COL-only biomaterials. We observe similar changes in macroscale migration assays (not shown).

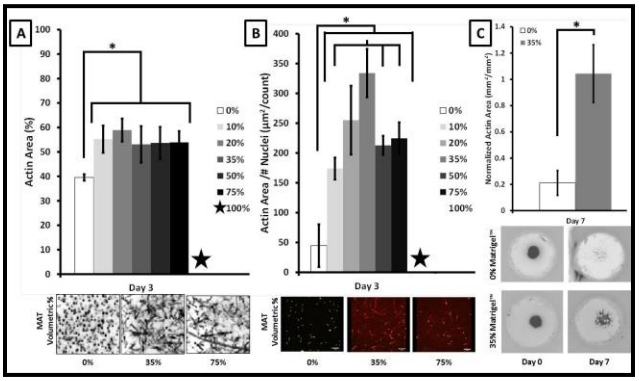

Figure 1. Scanned images and morphological analysis (A). Confocal images and morphological analysis (B). Scanned images and analysis of migration (C). (n=4; $p<0.5$)

Conclusions: To assess the synergistic impacts on cell migration and morphology of large combinations of soluble and insoluble factors, a HTP strategy for identifying positive "hits" is necessary for success. Fluorescent scanning is a tool capable of rapidly measuring rough changes in cell cytoskeletal response to a large number of composite materials, giving comparative data of the quality of multiple independent composite materials in a resource and time efficient manner. Both growth permissive and inhibitory hits are identified for further examination in the migration assay. These two assays together provide a robust, rapid, HTP analysis of multiple cell metrics to large combinations of composite materials are underway. Hits from individual cell screens can be re-combined to develop materials that are supportive of multiple cells types (e.g. neural and non-neural cells). The inherent flexibility in this setup enables smooth transition into other areas of tissue engineering and provides a powerful tool for investigating the synergistic effects between biological materials.

References: (Hulkower KI. Pharmaceutics. 2011 3: 107-124) (C.E. Schmidt. Annu. Rev. Biomed. Eng .2003 5:293-347)