Identifying chemical moieties to control hMSC differentiation using a high-throughput methodology

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Statement of Purpose: Cell-matrix interactions play a critical role in development, regeneration, and disease[1,2], orchestrating cellular functions from migration to guided differentiation of progenitor cells[3,4]. The need for culture environments that direct and control differentiation and subsequent function of stem cells has become increasingly important due to the potential of these cells to be used in regenerative medicine. In particular, understanding the role of the ECM environment (e.g., interaction with cell surface receptors, binding of growth factors) is critical for a materials approach to successfully re-create the stem cell niche. To this end, chemical functional groups that mimic different extracellular environments were incorporated into PEG-based arrays to test their effect on human mesenchymal stem cell (hMSC) proliferation and differentiation.

Methods: Using an automated system (Bio-Rad VersArray ChipWriter Pro System) copolymers hydrogel arrays (spot diameter = 600 μm) were created on standard glass microscope slides by photopolymerization of the following (macromolecular) monomers: poly(ethylene glycol) (PEG) dimethacrylate (MW~550) and 50 mM of the monomethacrylated monomers 2-aminoethyl methacrylate (amino), tert-butyl methacrylate (t-butyl), ethylene glycol methacrylate phosphate (EGMP), 2,2,3,3 tetrafluoropropyl methacrylate (fluoro), and methacrylic acid. Since PEG is known to limit non-specific interactions with biological molecules, this technique allows us to directly study the effect of chemical environment on cell fate without the complication of interactions with the substrate. hMSCs were seeded on the arrays and monitored via immunostaining and *in situ* hybridization techniques coupled with a chip-reader (Bio-Rad VersArray ChipWriter Pro System) for evaluation of proliferation and differentiation. Subsequent evaluation of the effect of surface concentration of EGMP on osteogenic differentiation was performed.

Results: By combining the versatility of standard printing processes with on-chip immunostaining and *in situ* hybridization analyses, significant differences in hMSC proliferation and differentiation were found based on variations in surface functionality. hMSCs on EGMP-containing hydrogels exhibited the greatest increases in proliferation, as assessed through immunostaining, greater than all other hydrogel compositions, and up to 1.7-fold greater than control surfaces at days 4 and 10 of the study. In addition, *in situ* hybridization revealed that methacrylic acid-functionalized surfaces promoted 2-fold aggrecan expression, an indicator of chondrogenic differentiation, compared with control hydrogels after 10 days. EGMP-functionalized hydrogel spots increased hMSC CBFA1 gene expression to about 4-fold greater than expression on control spots. Both t-butyl- and fluoro-functionalized spots increased hMSC PPARG gene expression to 3-fold and 2-fold greater than controls by day 10, supporting the hypothesis that hydrophobic, lipid-like moieties induce adipogenic differentiation. Most promising, all culture was performed without any external soluble signals, relying completely on material chemistry to evoke differentiation. Finally, the concentration dependence of osteogenic differentiation was ascertained for EGMP, and all concentrations greater than 1 mM were capable of inducing increased CBFA1 gene expression by hMSCs. However, the greatest induction of CBFA1 was found with the 5 mM EGMP concentration. Since 50 mM EGMP was found to promote proliferation to a great extent, it is possible that cells seeded on higher concentrations of EGMP exhibit retarded osteogenic differentiation due to increased proliferation.

Conclusions: A high-throughput method was utilized to study hMSC proliferation and differentiation induced by different chemical moieties. Various methacrylated monomers were combined with PEG and copolymerized to yield arrays of chemical functionalities on a standard microscope slide, which allowed for numerous conditions to be tested simultaneously. Proliferation of hMSCs was greatly affected by EGMP-functionalized hydrogels, where dose-dependent effects were found. Interestingly, differentiation, as analyzed by *in situ* hybridization, was profoundly impacted by chemical functionality: methacrylic acid functionalized gels induced the greatest aggrecan expression, an indicator of chondrogenic differentiation; phosphate-functionalized gels induced the greatest osteogenic differentiation, monitored by core binding factor alpha 1 (CBFA1) gene expression; and t-butyl- and fluoro-functionalized gels induced the greatest adipogenic differentiation, as measured by peroxisome proliferative activated receptor gamma gene expression. Finally, concentration-dependent effects were assessed by monitoring hMSC CBFA1 gene expression on gels containing 1-50 mM EGMP. All concentrations greater than 1 mM increased CBFA1 gene expression compared to controls, while the 5 mM concentration had the greatest effect.

References:

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