Surface Properties Modulate Integrins, Pluripotent Markers, and Morphogens in Embryonic and Adult Stem Cells

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Statement of Purpose: Tissue engineering and regenerative medicine fields have established the use of stem cells due to the ability of these cells to differentiate into different cell types. Multipotent adult stem cells (MSCs) have the ability to differentiate into cells that produce mesenchymal tissues such as bone, cartilage, fat, muscle, and ligaments. MSCs can be isolated from several tissues, most commonly bone marrow or fat. Embryonic stem cells (ESCs) are pluripotent cells isolated from the blastocyst inner cell mass and can differentiate into all somatic cell types. Induced pluripotent stem cells (iPSCs) are somatic cells reprogrammed by ectopic expression of specific transcription factors, resulting in an ESC phenotype.

Most protocols supplement culture media with chemical and biological factors to direct stem cell fate. However, effects of substrate properties on stem cell fate have only been partially examined in adult stem cells and remain poorly understood in pluripotent stem cells. Biomaterials have been used to control MSC differentiation into chondrocytes, osteoblasts, and adipocytes. Inherent surface properties such as roughness and chemistry modulate MSC osteoblastogenesis. However, little is known whether these physical properties can impact ESC and iPSC phenotypes. We hypothesize that surface properties can induce differentiation and control ESC fate. We also hypothesize that surface properties modulate conserved developmental signaling pathways such as Wnt signaling. The aim of this study was to examine if differences in surface roughness or chemistry can regulate integrins, transcription factors, and growth factors in embryonic and adult stem cells.

Methods: Human bone marrow-derived MSCs (MSCs), human adipose-derived MSCs (ASCs), human embryonic stem clone H9 (ESCs), embryonic stem cells differentiated to mesenchymal stem cells (ESCs-MSCs), or induced pluripotent stem cells (iPSCs) were plated on TCPS or Ti surfaces with different surface modifications (smooth (PT) [Sa<0.2μm], submicron roughness (A) [Sa=0.6μm], micro-roughness (GB) [Sa=3.2μm], submicron/micron roughness (SLA) [Sa=2.5μm], or hydrophilic SLA (modSLA) [Sa=2.5μm]). Cells were cultured for 7 days in media without addition of exogenous differentiation factors. After incubation, real-time qPCR analysis was performed for integrins (ITGA1, ITGA2, ITGA3, ITGA5, ITGA6, ITGAV, ITGB1, ITGB3), lineage transcription factors (SOX2, POU5F1, GATA4, and NANOG), and growth factors (WNT1, WNT3A, WNT5A, BMP2, BMP4, TGFB1, VEGFA, FGF2). Data are mean±SEM for n=6 independent cultures/variable (ANOVA, post hoc Bonferroni's modification of Student's t-test).

Results: Integrin expression was up-regulated by surface roughness. SLA and modSLA surfaces induced the highest ITGA1, ITGA2, ITGAV, and ITGB1 levels in MSCs and ASCs. ESCs, ESCs-MSCs, and iPSCs exhibited similar response, increasing ITGA1, ITGA2, and ITGB1 on A and SLA modifications. ITGA6 was upregulated in all cells tested on A, SLA and modSLA. Interestingly, surface roughness modulated pluripotent marker expression in adult stem cells. MSCs and ASCs up-regulated NANOG on A, SLA, and modSLA; POU5F1 was up-regulated on GB, and down-regulated on A, SLA, and modSLA; and GATA4 was up-regulated in comparison to PT and A on GB, SLA, and modSLA. SOX2 was not detected on MSCs or ASCs. A-surfaces induced the highest SOX2, GATA4, POU5F1, and NANOG in ESCs-MSCs. ESCs and iPSCs had highest POU5F1 mRNA on GB and highest NANOG on acid-etched surfaces. Expression of important morphogens such as Wnts, BMPs, and TGF-β1 were modulated by surface roughness. WNT5A, BMP2, BMP4, TGFB1, VEGF, and FGF2 were highest on acid etched surfaces in all cell types examined. WNT3A was up-regulated in pluripotent stem cells (ESCs, ESCs-MSCs, and iPSCs) only on GB modifications.

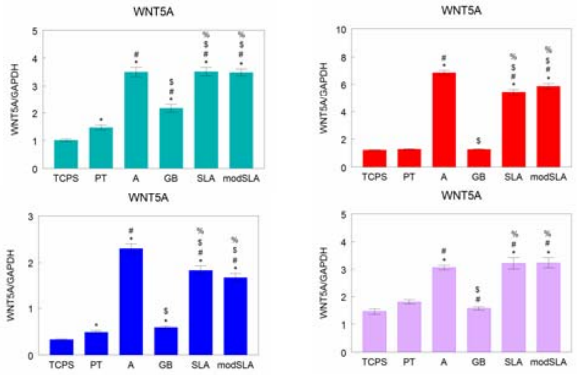

Figure 1. WNT5A expression in MSCs (A), ESCs (B), iPSCs (C), and ESCs-MSCs (D). Cells cultured on TCPS, PT, A, GB, SLA, or modSLA were incubated for 7 days without addition of exogenous factors. *,p<0.05 v. TCPS; #,p<0.05 v. PT; $,p<0.05 v. A; %,p<0.05 v. GB.

Conclusions: Biomaterial surface properties such as surface energy and submicron-/micron-scale topographies regulate mRNA expression of integrins, lineage specification transcription factors, and morphogens in both embryonic and adult stem cells. ESCs regulated integrins in response to physical cues, suggesting that these cells are able to "sense" topographical cues from their microenvironment. Genes associated with pluripotency and linage determination were up-regulated in adult stem cells suggesting an important role of these genes on adult stem cell differentiation. The results indicate that physical cues can be used to direct stem cell fate without the addition of exogenous factors. Future studies will tailor physical cues to control differentiation into specific lineages depending on cell type used.