Cell-Biomaterial Interface Cues Reinstate Declining Stemness in Aging Adult Stem Cells

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Statement of Purpose: Human mesenchymal stem cells (**hMSCs**) offer great therapeutic potential for clinical applications, but exhibit a decline in overall health when isolated from aging patients or serially expanded *in vitro*. Cell "stemness" refers to the ability to retain self-renewal capacity and differentiation potential in stem cell populations, and is controlled by specific transcription factors such as Nanog and SOX2[1]. hMSCs have been shown to originate from pericytes, an elusive extravascular cell type, in numerous tissues, but dissection and clarification of this relationship is absent in the literature[2]. The expression of stemness proteins declines with *in vitro* expansion[1], and this correlates with increasing "developmental" distance from the pericyte phenotype, suggesting that rejuvenation of hMSCs might result in phenotypic reversion to yield bona fide pericytes.

Although matrix-derived physicochemical cues have been shown to regulate cell fate decisions, alterations in stemness in response to changing substrate properties remain unknown. Growing evidence indicates that material properties, such as the spacing of adhesion proteins[3], mediate outside-in signaling for cell-biomaterial interactions at the interface. Therefore, the aims of this study are to elucidate i) the influence of multiplex biomaterial matrix cues in modulating hMSC stemness, ii) how this modulation affects the hMSC/pericyte phenotype, and iii) the pathway(s) responsible. The overarching goal is to develop methods for reinstating the therapeutic potential of aging hMSCs.

Methods Spin-coated copolymers composed of three subunits were used in this study: poly(ε-caprolactone) (**PCL**), poly(ethylene glycol) (**PEG**), and carboxyl-PCL (**cPCL**). Copolymers are identified as *x*%PEG-*y*%PCL-*z*%cPCL (x, y, and z: molar ratio). Extensive material characterization was first performed, followed by in-depth biological analyses of cellular function, including flow cytometry, qRT-PCR, immunofluorescence staining, and functional assays. Aging hMSCs (≥ passage 11) were cultured on tissue culture polystyrene (**TCPS**) or test polymers, and compared against passage (P)8 hMSCs on TCPS (control).

Results The copolymer substrates exhibited significant differences in physicochemical properties, including protein adsorption, hydrophilicity, roughness, and stiffness (data not shown due to space restraints). The cellular response was heavily influenced by copolymer composition, including gene-level expression of *Nanog* (**Fig. 1A**) and *SOX2* (**Fig. 1B**). Specifically, 4%PEG-96%PCL (referred to as 4%PEG) stimulated the most significant increase in the expression of both *Nanog* and *SOX2*, which coincided with the lowest degree of osteogenic differentiation (not shown). Interestingly, hMSCs cultured upon 4%PEG, but not TCPS, expressed all three proteins of pericyte markers, including neuron-glial antigen 2 (NG2p), platelet endothelial cell adhesion molecule (PECAM), and α-smooth muscle actin (αSMA) (**Fig. 1C**). As a further confirmation, hMSCs on 4%PEG expressed these same markers at the gene level, along with additional pericyte-specific markers[2], including CD146 and PDGFR-β (not shown). Since the spacing of adhesion proteins has been shown to regulate cell fate and differentiation[3], the presentation of adsorbed proteins was analyzed on the two surfaces. The adsorption of FITC-labeled collagen in the absence of cells revealed a sub-micron, porous pattern of protein presentation on 4%PEG, as opposed to the homogeneous coverage of collagen on TCPS (**Fig. 1D**), likely contributing to the observed cellular response. Additional data not listed here include improved differentiation capacity of hMSCs when moved from culture upon 4%PEG to TCPS, *in vitro* stabilization of endothelial tubes (i.e. functional pericyte behavior) by hMSCs cultured upon 4%PEG, and a TGF-β-mediated pathway by which the adsorbed proteins are driving the increased stemness and "phenotypic switch" toward a pericyte cell type.

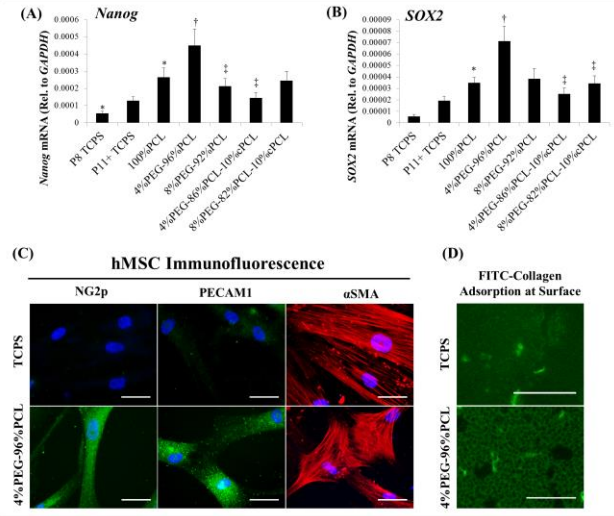

Figure 1: **Copolymer composition regulates cell function as indicated by: (A,B)** Quantitative real-time PCR (qRT-PCR) and **(C)** Immunofluorescence (scale = 50μm). These effects are likely modulated by **(D)** protein presentation at the material surface (scale = 20μm). **p*<0.05, †*p*<0.01 versus P11+ TCPS; ‡*p*<0.05 versus 4%PEG.

Conclusions: This study is the first of its kind to investigate how cues from synthetic culture substrates reinstate hMSC stemness and aims to identify the biological mechanisms responsible for this outside-in signaling. Furthermore, our data provide the first *in vitro* evidence that hMSCs are, in fact, pericytes and that the phenotype can undergo a transient switch in response to biomaterial cues. The findings from this study will be exploited for maintaining/reinstating the therapeutic efficacy of aging hMSCs for basic science, engineering, and clinical applications. **References:** [1]Tsai C-C *Mol Cell* 2012 (47:169-182) [2]Crisan M *Cell Stem Cell* 2008 (3:301-313) [3]Trappmann B *Nat Mater* 2012 (11:642-649)