Reversible and irreversible activations of YAP/TAZ in hMSCs on phototunable hydrogels

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Statement of Purpose: The underlying concepts of this study are founded on the principle of cellular mechanotransduction: the hypothesis that cells sense and integrate epigenetic, physical signals to influence cell fate decisions. Interestingly, Dupont et al.[1] reported the first critical link between the extracellular environment and intracellular signaling, namely that the Hippo-related transcription factors Yes-associated protein (YAP) translates mechanical information into protein expression by localizing in the nucleus and regulating mRNA expression. Our study presents the notion that stem cells are not only influenced by current matrix mechanics, but that they are influenced by past microenvironmental cues and this biophysical history influences future fate. Specifically, we investigate YAP nuclear versus cytoplasmic localization in hMSCs when cultured on a materials system that allows in situ regulation of elasticity.

Methods: Synthesis of hydrogel components. Polyethylene glycol di-photodegradable acrylate (PEGdiPDA) was synthesized and characterized as previously described[2]. The adhesive peptide OOGRGDSG (diethylene glycol-diethylene glycol-glycine-arginine-glycine-aspartic acid-serine-glycine) was synthesized (Protein Technologies Tribute peptide synthesizer) through Fmoc solid-phase methodology and HATU activation. Acrylic acid was coupled on resin to the N-terminal amine with HATU to synthesize Acryl-OOGRGDSG.

Fabrication of photodegradable hydrogels for cell seeding. The preparation of PEGdiPDA, photodegradable hydrogels was adapted from previously described protocols[2]. Gel solutions were prepared with 2.5 wt% PEGdiPDA, 10 wt% PEGA, 5 mM Acryl-OOGRGDSG, 0.2 M ammonium persulfate (APS), and 0.1 M tetramethylethylenediamine (TEMED). Gels were formed on acrylated cover glass with diameter of 18 or 22 mm and a thickness of 100 mm. Soft hydrogels (~ 2 kPa) were prepared by irradiating the initial photodegradable hydrogels (~ 10 kPa) with UV light (l = 365nm; I_0 = 10 mW/cm^2) for 360s.

Mechanical dosing on photodegradable hydrogels. hMSCs were seeded on stiff hydrogels at 1000 cells/cm^2 cultured in growth media. Hydrogels were subsequently softened *in situ* with light (λ = 365nm; I_0 = 10 mW/cm^2, 6 min) at 1, 7, or 10 days after seeding. After in situ softening, the cell-laden constructs were analyzed at 1, 3, and 5 days via immunostaining.

Results: hMSCs were cultured on an activating hydrogel substrate (10 kPa) where activation was defined as YAP-localization to the nucleus. After 1 day of culture, the gel was softened to a de-activating modulus (2kPa). Interestingly, YAP and RUNX2 were activated in the nucleus, but changed in response to softening of the substrate. This transient effect can be observed by noting that after just 3 days on the softened substrate, the transcription factors re-located to the cytoplasm with basal nuclear expression (DSt1-So3). De-activation of the transcription factors persisted 5 days after softening (Dst1-So5), which indicated that the initial activation of hMSCs was fully reversible at this mechanical dose (Fig. A). hMSCs were then cultured on activating hydrogels for 10 days prior to softening (Fig. B). Interestingly, under these conditions of longer culture on a stiff substrate, YAP and RUNX2 remained activated in the nucleus even 10 days after softening.

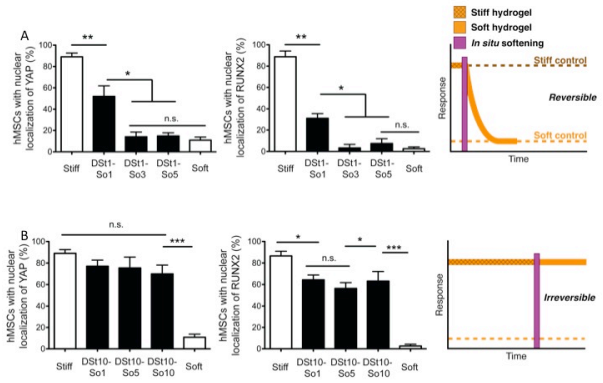

Figure A: YAP and RUNX2 response to *in situ* softening after 1 day of mechanical dosing on stiff hydrogels. Figure B: YAP and RUNX2 response to *in situ* softening after 10 days of mechanical dosing on stiff hydrogels.

Conclusions: Based on the presenting data, we conclude that mechanical dosing can cause reversible and irreversible activation of YAP and RUNX2; a threshold mechanical dose resulted in constitutive activation of the transcription factors.

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