Nascent protein secretion directs cell mechanosensing and function in three-dimensional hydrogels

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Statement of Purpose: Synthetic hydrogels are engineered with a range of biochemical and biophysical properties for applications such as tissue engineering and as platforms to investigate how properties of the extracellular matrix (ECM) impact cell behavior[1]. However, the role of nascent proteins that cells quickly secrete and assemble within the pericellular space is typically overlooked in hydrogel design and interpretation of these experiments (Fig. 1). Thus, we designed a metabolic labeling technique to visualize nascent proteins and investigated their role in directing cell function in degradable hyaluronic acid (HA) hydrogels.

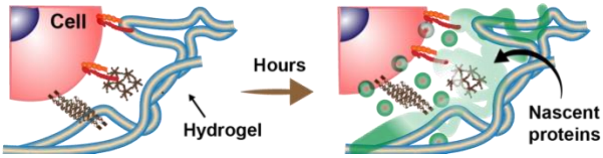

Figure 1: Cells interact with a 3D hydrogel for short periods before depositing nascent proteins that mask the engineered hydrogel environment.

Methods: Hydrogels were synthesized through norbornene-modified HA mixed with 1 mM thiolated RGD and crosslinked with proteolytically (MMP) degradable crosslinkers via a light-mediated thiol-ene reaction[2]. Nascent proteins were labeled through strain-promoted cycloaddition of incorporated azidohomoalanine (AHA) and DBCO-488 (Fig. 2A) and immunofluorescence for fibronectin (Fn). Encapsulated human mesenchymal stromal cell (hMSC) spreading and osteogenic and adipogenic differentiation were quantified by confocal microscopy and immunofluorescence of osteocalcin (osteo) and fatty acid binding protein (FABP, adipo), including with perturbation of cell-nascent protein adhesion (monoclonal antibody anti-human Fn, HFN7.1)[3].

Results: Staining of nascent proteins over 6 days (growth media, supplemented with AHA) showed networks of extracellular proteins around encapsulated hMSCs (Fig. 2B). Cells spread as indicated by increased aspect ratios at day 6 coincident with increased nascent protein thickness (Fig. 2C). AHA labeling colocalized with specific ECM proteins such as cellular Fn (Fig. 2D), as well as laminin, types I, IV collagen (not shown). Perturbation of cell adhesion to the central integrin binding domain of Fn through HFN7.1 over 6 days resulted in significantly reduced spreading (Fig. 2E). Blocking cell adhesion to secreted Fn also reduced YAP/TAZ nuclear localization at 6 days (not shown) and osteogenic differentiation at 14 days (Fig. 2F). Perturbing adhesion to RGD through soluble RGD or secreted collagen through a monoclonal antibody against the Integrin α2 domain also resulted in similar cellular outcomes (not shown).

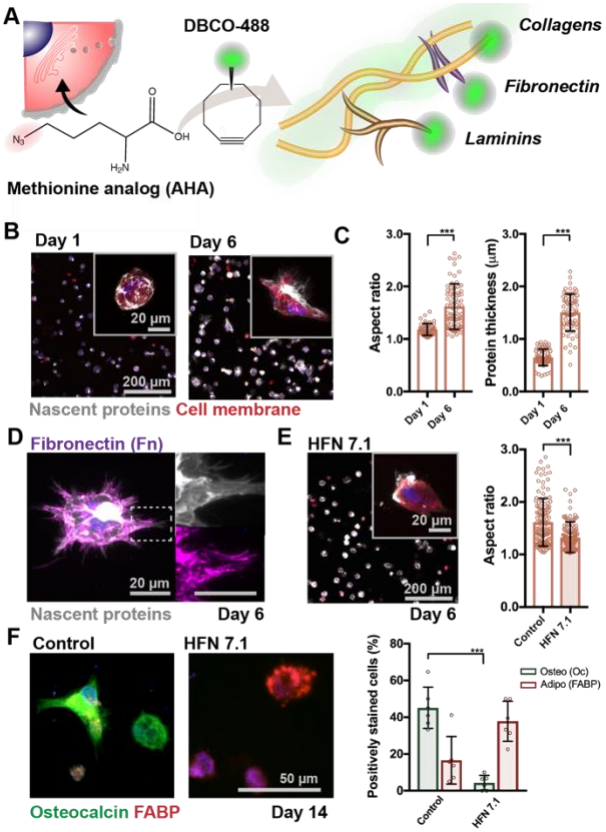

Figure 2. A Schematic of nascent protein labeling. The methionine analog azidohomoalanine (AHA) is incorporated into nascent proteins during synthesis. **B** Representative images and **C** quantification of cell spreading and nascent protein thickness of hMSCs encapsulated in proteolytically degradable hydrogels (9 kPa). **D** Representative immunofluorescence of fibronectin (Fn) of hMSCs at day 6. **E** Cell spreading of HFN7.1 treated hMSCs at 6 days. **F** Quantification of positively stained cells (%) for osteocalcin and FABP after 14 days (mean ±SD, n > 150 cells per group, 6 gels per group for differentiation, *** $p \leq 0.001$).

Conclusions: Metabolic labeling with AHA enabled the visualization of nascent proteins and their impact on spreading and mechanosensing of encapsulated cells. Our results indicate that cellular functions in synthetic hydrogels are not only influenced by the initial engineered interface presented to the cell, but also by the deposition and adhesion to nascent ECM proteins. Ongoing work is to better understand these cell-nascent protein interactions and utility for tissue engineering and repair.

References: [1](Guvendiren M. et al Curr Opin Biotech 2013;841-846), [2](Gramlich WM. et al Biomaterials 2013;34, 9803−11), [3](Keselowsky BG. et al PNAS 2005;17,5953-57).