Engineering Interpenetrating Network Hydrogels as Biomimetic Cell Niche with Independently Tunable Biochemical and Mechanical Properties

Xinming Tong[1], Fan Yang[1,2].
[1]Department of Orthopedic; [2]Department of Bioengineering, Stanford University, Stanford, CA.

Statement of Purpose: To facilitate elucidating cell-niche interactions, hydrogels have been widely used as artificial cell niche given their tissue-like water content as well as tunable chemical and physical properties. However, few hydrogels developed to-date allow independent tuning of niche properties such as biochemical signals and mechanical stiffness. And hydrogel degradation often results in simultaneous change of biochemical ligand density. This makes it difficult, if not impossible, to interpret the contribution of various niche cues to the observed cellular responses. To allow independent tuning of niche properties, here we report the development of a novel method for constructing stable, homogeneous interpenetrating network (IPN) hydrogel as stem cell niche with independently tunable niche properties (biochemical, mechanical and degradation) using cell-friendly processes.

Methods: We chose poly(ethylene-glycol) (PEG) as the backbone structure due to its "blank slate" structure and amenability to chemical modification. To crosslink the two networks simultaneously, two distinct mechanisms were utilized. Biochemical precursors were crosslinked by amine-NHS coupling, while mechanical precursors were crosslinked by thiol-ene radical addition. To make the IPN hydrogel degradable, enzymatically cleavable peptide CGPQGIWGQC cysteine residuals could be incorporated into mechanical network. Compressive mechanical testing was performed to determine the effects of varying the concentration of biochemical or mechanical blocks on the hydrogel stiffness. Human adipose-derived stem cells (hADSCs) were cultured on IPN hydrogel substrates with independently tunable biochemical cues and matrix stiffness, and outcomes were analyzed by cell morphology and osteogenic differentiation.

Results: The properties of the as-formed IPN hydrogels were confirmed by monitoring the stability of incorporated bioactive ligands over time, cell viability, enzymatic-mediated degradation, and ability to support cell spreading in 3D. Increasing the concentration of mechanical precursors from 2.5-10% (w/v) resulted significant increase in the stiffness of the IPN hydrogels, covering a broad range from brain tissue-like stiffness (0.9 kPa), muscle tissue-like stiffness (18.6 kPa) to stiffness range known to promote musculoskeletal tissue differentiation (54 or 91 kPa) (**Fig. 1a**, $p < 0.0001$). In contrast, increasing biochemical ligand (RGD) concentration from 0 to 2.5 mM resulted in negligible changes in the compressive modulus of IPNs (**Fig. 1b**). Increasing hydrogel stiffness and increasing RGD density both resulted in faster cell spreading and more cell proliferation. While keeping the RGD density constant at 2.5 mM, increasing IPN stiffness from 0.9 kPa to 54.6 kPa led to 6-fold increase in ALP gene expression (**Fig. 1c**). Further increase of IPN stiffness from 54.6 kPa to 91.3 kPa did not lead to significant changes in ALP expression. Keeping the hydrogel stiffness constant (54 kPa), increasing RGD density from 0.25 mM to 2.5 mM also resulted in a dose-dependent increase in ALP gene expression.

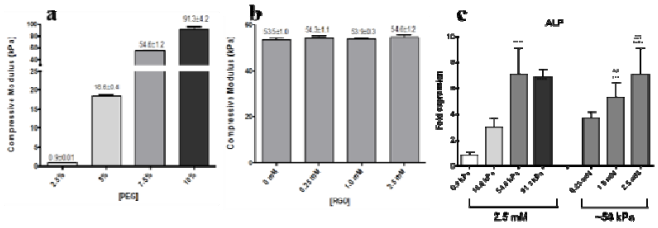

Figure 1. Modulation on mechanical stiffness by (**a**) concentration of mechanical precursor and (**b**) concentration of biochemical ligands. (**c**) Quantitative gene expression of osteogenic markers ALP of hADSCs cultured for 14 days in osteogenic medium.

Conclusions: Here we report a novel IPN hydrogel platform with independently tunable biochemical and mechanical properties, which can be used as biomimetic cell niche. This IPN hydrogel allows homogeneous and stable presentation of biochemical ligands, and varying biochemical ligand concentration will not significantly change the matrix stiffness. Unlike previous IPN strategies, our method is cell-friendly and allows simultaneous and independent formation of the biochemical and mechanical network. While we demonstrate the application of IPN hydrogels for directing stem cell differentiation as an example, the IPN hydrogels may also be utilized for studying other cell behavior such as cancer cell-niche interactions. Given the ability to decouple niche properties in such IPN hydrogel platforms, it also provides a robust material platform for developing combinatorial studies to help elucidate how complex niche signaling interact together to influence cell fate in 3D, and may also hold great promise for rapidly identifying optimal scaffold compositions for promoting desirable cellular processes and tissue formation.