Microribbon-like Hydrogels with Diverse Biological Cues for Forming 3D Scaffolds: a "Lego-building" approach

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Statement of Purpose: Hydrogels have been widely used for fabricating tissue engineering scaffolds due to their biocompatibility, injectability as well as tunable chemical and physical properties. Both synthetic and natural polymers have been used to construct hydrogels as cell niche including polyethylene glycol (PEG), gelatin, hyaluronic acid (HA) and chondroitin sulfate (CS). However, most hydrogels are nanoporous, whereas macroporosity is desirable for efficient nutrient diffusion, cell proliferation and matrix deposition. Second, few hydrogels developed-to-date allow independent tuning of niche cues such as macroporosity, biochemical ligand density and mechanical stiffness. This makes it difficult to decipher complex cell-niche interactions. To overcome these limitations, we have recently reported a method to fabricate gelatin hydrogels into microribbon-like structures, which can subsequently crosslinked into 3D macroporous scaffolds for direct cell encapsulation. The goal of this study is to develop a universal method for fabricating microribbon-like hydrogels based on various extracellular matrix-mimicking biological cues including PEG, gelatin, HA and CS. We further seek to validate the feasibility to align these microribbons to provide topographical cues to guide cell alignment in 3D.

Methods: To fabricate hydrogels first into microribbon-like structures, we first wet-spin maleimide functionalized polymer precursors with various chemical compositions (gelatin, PEG, HA and CS) into isopropanol bath. Dithiothreitol (DTT) was added in the bath to crosslink the as-spun microribbons. The stiffness of microribbons were tuned by changing the maleimide functionality. To allow second crosslinking of formed microribbons to form 3D scaffolds, the polymer precursor solution was pre-treated with cysteamine to introduce amine group before the wet-spinning. The formed microribbons were then treated with aminoethyl methacrylate to allow photocrosslinking among the microribbons to form 3D macroporous network. Additional biochemical ligands could be added onto microribbon surface via maleimide groups of polymer precursors before wet-spinning or co-polymerized with methacrylate groups. The macroporosity, biochemical and mechanical properties of resulting scaffold that cells are sensing were independently tuned by varying microribbon density, choice of biochemical ligands (cysteine or peptide CRGDS), and microribbon rigidity. Outcomes were analyzed using scanning electron microscope (SEM), cell proliferation, immunostaining and confocal microscopy.

Results: Our fabrication process led to successful formation of microribbon-like hydrogels using various polymer precursors including gelatin, PEG, HA and CS (**Fig. 1A**). The mechanical stiffness of the microribbons and the formed scaffolds could be tuned independently. Increasing hydrogel crosslinking density increased the microscopic stiffness of individual PEG microribbon, while increasing the density of microribbons could increase the stiffness of the macroscopic stiffness of the as-formed scaffolds (**Fig. 1B**). Unlike conventional hydrogels, these microribbon-based scaffolds exhibit markedly enhanced flexibility and could sustain up to 50% cyclic deformation without failing (data not shown). When encapsulated in HA-based microribbon scaffolds, human adipose-derived stem cells showed robust cell adhesion, spreading and proliferation in 3D over 7 days of culture **(Fig. 1C**). We also successfully achieved aligning microribbons in 3D, which guided alignment of smooth muscle cells when seeded in 3D (**Fig. 1D**).

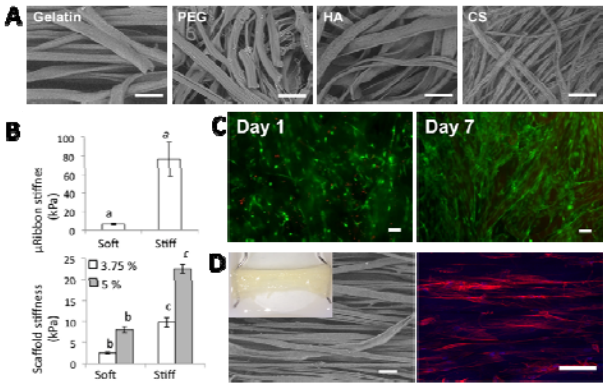

Figure 1. (A) Representitve SEM images of microribbons fabricated from different materials. **(B)** Mechanical stiffness of individual microribbon and formed scaffolds tuned by changing internal crosslinking density and microribbon density. **(C)** Robust cell spreading and proliferation in microribbon scaffolds (green, calcein AM). **(D)** Enhanced cell alignment in microribbons scaffolds (red, F-actin; blue, nuclei).

Conclusions: Here, we report the design and synthesis of crosslinkable microribbons from various polymer precursors as building blocks for fabricating a cell-laden, macroporous scaffold. Unlike conventional hydrogels, these microribbons allow independently tunable niche properties (biochemical, mechanical, and topographical cues). Such microribbon-based hydrogels can serve as novel building blocks for engineering 3D tissues with customizable niche properties. We envision these microribbons-based biomaterials could provide a valuable tool for facilitating the analyses of how the interaction of multi-factorial niche signaling influences cell fate in 3D. Furthermore, the microribbons can support cell alignment in 3, which can be useful for guiding regeneration of linear tissues such as nerves, tendons and muscles etc.