Engineering Functional Cardiac Tissues Using Micropatterned Hydrogels

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Statement of Purpose:

Heart failure is a major international health issue. Myocardial mass loss and lack of contractility are precursors to heart failure. Surgical demand for effective myocardial repair is tempered by a paucity of appropriate biological materials. These materials should conveniently replicate natural human tissue components, convey persistent elasticity, promote cell attachment, growth and conformability to direct cell orientation and functional performance. In this study, microfabrication techniques were combined with recombinant human tropoelastin to generate a highly elastic photocrosslinked protein-based hydrogel containing well-defined micropatterns. These elastic substrates were then used to engineer biomimetic cardiac tissue constructs. The micropatterned hydrogels, produced through photocrosslinking of methacrylated tropoelastin (MeTro), promoted the attachment, spreading, alignment, function, and intercellular communication of cardiomyocytes by providing an elastic mechanical support that mimics their dynamic mechanical properties in vivo. The fabricated MeTro hydrogels also support the synchronous beating of cardiomyocytes in response to electrical field stimulation. These novel engineered micropatterned elastic gels are designed to be amenable to 3D modular assembly and establish a versatile, adaptable foundation for the modeling and regeneration of functional cardiac tissue with potential for application to other elastic tissues including blood vessels, skin and heart valves.

Methods: Tropoelastin was purified from bacteria on a multi-gram scale as previously described[1]. The protein was then methacrylated by the addition of methacrylate anhydride to a tropoelastin solution. Various mirofabrication techniques, including micromolding and photomasking, were then used in combination with photocrosslinking to generate microfabricated highly elastic MeTro gels. For example, MeTro hydrogels containing microchannels were fabricated by using a polydimethylsiloxane-based membrane with 20 µm x 20 µm channel width and spacing and a photomask layout (designed by AutoCAD software) was used to engineer patterned MeTro gels. The fabricated hydrogels were used for 3D cell encapsulation and 2D surface attachment, proliferation and alignment of neonatal rat heart cardiomyocytes (CMs). Cellular attachment and spreading on MeTro gels were assessed by staining the cells with DAPI and rhodamine-phalloidin to visualize cell nuclei and F-actin filaments, respectively. Immunostaining was also performed to investigate the expression of CM proteins (e.g. sarcomeric α-actinin and connexin-43) on the gels.

Results: Highly elastic MeTro gels could be formed by UV crosslinking of methacrylated tropoelastin prepolymer in an aqueous solution within 30 sec[2]. MeTro gels exhibit high extensibility (up to 400% before rupture) and have superior mechanical properties that outperform other photocrosslinkable hydrogels. MeTro gels allow for cell encapsulation in a flexible 3D environment and the manufacture of highly elastic 2D films for cell attachment, growth, and proliferation. We also microfabricated micropatterned MeTro gels with high pattern fidelity (**Fig 1A, B**)[3]. The microfabricated gels supported the adhesion and spreading of the CMs seeded on the surfaces of the hydrogels (**Fig 1C**). In addition, these microfabricated MeTro hydrogels successfully promoted all the characteristics of CMs including attachment, spreading, alignment, phenotype (**Fig 1D**) and synchronized beating (**Fig 1E**), which ultimately led to the formation of highly functionalized cardiac tissues.

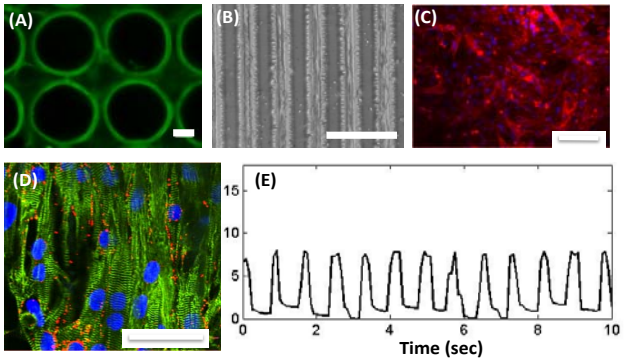

Fig 1. Methacrylated tropoelastin hydrogel. (A, B) Formation of patterns with various geometries on MeTro gel by using different microfabrication techniques, (B) Rhodamine-labeled phalloidin/DAPI staining for F-actin/cell nuclei of CM seeded on microfabricated MeTro gel on day 8 of culture, (C) immunostaining of CM markers on MeTro gel on day 8 of culture, gel stained for sarcomeric α-actinin (green)/connexin-43 (red)/nuclei (blue) (scale bar = 50 µm), (D) beating behavior of CMs on micropatterned MeTro gel.

References:

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