Development of a novel microfabricated cell-laden bioelastomer

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Statement of Purpose: Hydrogels are used as scaffolds for tissue engineering applications because of their biocompatibility and high water content, which resemble the natural tissue microenvironment. However, the scope of their application is often limited by their low mechanical properties and limited extensibility, particularly in the regeneration of elastic tissues such as cardiovascular tissues[1]. Most hydrogels are brittle and rupture easily when stretched. Synthetic elastomers such as polysiloxanes, polyurethanes, polyhydroxyalkanoates, poly(diol citrates), poly(glycerol-sebacate) are highly elastic but they are poor substrates for three dimensional (3D) cell encapsulation and growth[2,3]. Here, we report the synthesis of a novel substantially elastic, biologically compatible, human protein-based elastomer, methacrylated tropoelastin (MeTro), which can be used in both 3D elastic environments for cell encapsulation and 2D microfabricated gels for cellular alignment. This new, versatile class of highly elastic hydrogel is produced through photocrosslinking of methacrylated recombinant human tropoelastin, the resilience-imparting protein found in all elastic human tissues, and can be finely tuned to desirable elasticity and stiffness, which facilitates broad applications.

Methods: Tropoelastin was purified from bacteria on a multi-gram scale as previously described[4]. The protein was then methacrylated. Various microfabrication 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 both 3D cell encapsulation and 2D surface attachment, proliferation and alignment of neonatal rat heart cardiomyocytes. Cell viability was determined by using a Live/Dead assay Kit (Invitrogen). Cellular alignment and spreading on MeTro gels were assessed by staining the cells with rhodamine-phalloidin and DAPI to visualize F-actin filaments and cell nuclei, respectively. Immunostaining was also performed to investigate the expression of cardiomyocytes proteins on the gels.

Results: We demonstrated that highly elastic MeTro gels could be formed rapidly, within a few seconds, by UV crosslinking of methacrylated tropoelastin prepolymer in an aqueous solution. MeTro gels exhibited superior mechanical properties compared to other photocrosslinkable gels, including substantial extensibility up to 400% before rupture, reversible deformation with low energy loss, and high resilience upon stretching. These properties make MeTro gels suitable for the engineering of elastic tissues such as cardiac and skin where matrix elasticity plays an important role in maintaining native structural configurations of tissues. Using microfabrication techniques, we generated micropatterned MeTro gels with high pattern fidelity (**Fig 1a-b**). The microfabricated gels supported the adhesion and spreading of 3T3 fibroblasts seeded on the surfaces of the hydrogels (**Fig 1c**). We also demonstrated that MeTro gels can be successfully used to encapsulate cells and support their viable persistence. As shown in **Fig 1d-e**, fibroblasts encapsulated in a MeTro gel exhibited > 90% viability for at least 7 days in culture. In addition, MeTro gels containing microchannels were used to align cardiomyocytes. Cardiomyocytes seeded on patterned MeTro substrates oriented with an elongated morphology typical of differentiated cells along the direction of microchannels and expressed well-differentiated sarcomeres and diffuse gap junctions, similar to those of native adult rat ventricular myocardium (**Fig 1f**). These novel engineered elastic gels can be considered for the modeling and regeneration of a range of functional elastic tissues.

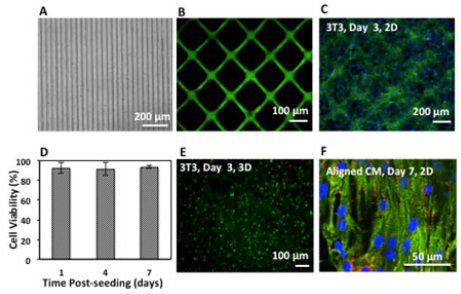

Fig 1. Representative images of microfabricated MeTro gel (A, B); Rhodamine-labeled phalloidin/DAPI staining for F-actin/cell nuclei of fibroblasts seeded on microfabricated MeTro gel (C); Quantification of cell viability 1, 4, and 7 days after encapsulation (D); Live/Dead images from fibroblasts encapsulated within 10% (w/v) MeTro gels (E); Immunostaining staining and expression of cardiomyocyte proteins on gels, sarcomeric α-actinin (green), connexin-43 (red), DAPI (blue) (F).

References: (1) Sun J.-Y., Nature, 2012, 489, 133-136; (2) Engelmayr G.C.Jr, Nat Mater 2008, 7, 1003-10; (3) Wang Y, Nat Biotech 2002, 20, 602-6; (4) Baldock C, Proc Natl Acad Sci USA 2011, 108, 4322-27.