Effects of crosslinking density on viability and maintenance of human embryonic stem cell-derived cardiomyocytes in elastin-like hydrogels

Cindy Chung[1,2], Melanie Marchand[3], Beth Pruitt[2], and Sarah Heilshorn[1]
[1]Materials Science and Engineering, [2]Mechanical Engineering, [3]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA

Statement of Purpose: Cardiovascular disease affects approximately 80 million Americans each year[1]. After a myocardial infarction, blood supply to the heart is interrupted, resulting in ischemia, oxygen shortage, and cell death. The loss of functioning cardiomyocytes (CMs), which have a limited ability for self repair, can ultimately lead to heart failure. Recently, stem cell-based technologies have emerged as a promising alternative for cardiac repair and regeneration[2]. Using recombinant protein technology, we have synthesized a family of elastin-like biomaterials that allow for the independent tuning of cell adhesion, modulus, and degradation[3]. In this study, we explore the effects of crosslinking density on the viability and maintenance of cardiac differentiated human embryoid bodies in 3D elastin-like hydrogels.

Methods: Elastin-like protein was synthesized using recombinant protein technology[3]. The modular protein design consists of 4 alternating repeats of a fibronectin-derived RGD sequence and a structural elastin-like domain. Protein strands are crosslinked through lysine residues using β-[tris (hydroxymethyl) phosphino] propionic acid (THPP), where hydrogel crosslinking density is tuned by varying THPP:protein reactive group stoichiometry (4:1, 2:1, 1:1 and 0.5:1). Human embryonic stem cells (H9) were spontaneously differentiated into cardiomyocytes through embryoid body (EB) formation. Beating embryoid bodies were mechanically dissected and encapsulated in 5 wt% hydrogels (5 μl) with one EB per gel. Viability was determined by metabolic activity via AlamarBlue cell proliferation assay. Phenotype and functionality was characterized by the onset of spontaneous contractility, beat rate, electrical pacing, and immunohistochemical staining for cardiac markers.

Results: Human EBs remained viable in RGD elastin-like hydrogels for up to 2 weeks of culture as indicated by metabolic activity (Figure 1A). Increased metabolic activity was observed for 2:1, and 1:1 groups over time, while metabolic activity was maintained for the 4:1 group. Here, increased crosslinking density may limit cell proliferation within the hydrogel. Positive immunohistochemical staining for cardiac markers confirms cardiomyocyte differentiation and phenotype (Figure 1B). The onset of spontaneous contraction in the hydrogels was quantified by dividing the number of contracting hydrogels by the total number of metabolically active hydrogels in each group (Figure 1C). Increased crosslinking density resulted in delayed onset of spontaneous contraction despite no significant differences ($p > 0.05$) in beat rate among groups after day 1 (Figure 1D). This delayed onset of contractility may reflect an adaptation period needed for cardiomyocytes to adjust to their microenvironment, where increased crosslinking density results in increased hydrogel moduli. When electrically paced, contractility of encapsulated EBs responded to pacing frequencies of 0.5, 1.0, and 2.0 Hz demonstrating retention of cell function.

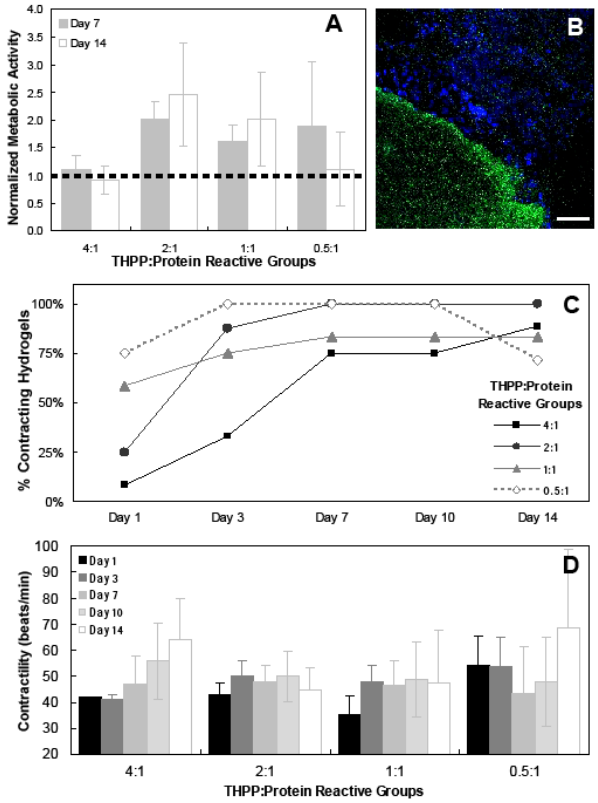

Figure 1. (A) Metabolic activity normalized to day 1, (B) immunohistochemical staining for cardiac troponin T (green), scale bar = 100 μm, (C) percentage of spontaneously contracting hydrogels, and (D) beat rates of human EBs in elastin-like hydrogels for 1, 3, 7, 10 and 14 days.

Conclusions: Understanding cell-microenvironment interactions is important for the development of cardiac scaffolds. Here, we have shown that human ESC-CMs remain viable in elastin-like hydrogels, where the phenotype and function of these cells is maintained for up to 2 weeks. Additionally, increased crosslinking density has been shown to delay the onset but not the rate of spontaneous contraction within these hydrogels.

Acknowledgements: The authors acknowledge funding support from NSF EFRI-CBE-0735551, Stanford Cardiovascular Institute, NSF DMR-0846363.

References: (1) Lloyd-Jones *et al*., *Circulation* 2010; 121:e1-e170 (2) Laflamme *et al*., *Nature Biotech* 2007 ; 25 :1015-1024; (3) Straley and Heilshorn, *Soft Matter* 2009; 5:114-124.