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Small peptide functionalized thiol–ene hydrogels as culture substrates for understanding valvular interstitial cell activation and *de novo* tissue deposition

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ABSTRACT

A thiol–ene polymerization platform was used to synthesize peptide functionalized poly(ethylene glycol) hydrogels, which were initially characterized and compared to theoretical predictions of Young's modulus via a theoretical crosslinking density equation presented herein. After thorough characterization, this material system's utility for answering specific biological hypotheses was demonstrated with the culture and observation of aortic valvular interstitial cells (VICs). Specifically, these materials were used to better understand the role of substrate elasticity and biochemical functionality on VIC α-smooth muscle (αSMA) expression and secretory properties (i.e. *de novo* extracellular matrix (ECM)). The Young's moduli of the hydrogels varied from 28 kPa (activating, 90% myofibroblasts) to 4 kPa (non-activating, 15% myofibroblast), and the biochemical functionality was tailored by incorporating three small adhesive peptide sequences, RGDS, VGVAPG and P15. To promote VIC adhesion, a basal [RGDS] of 0.8 mM was used in all formulations, while the [VGVAPG] or [P15] were varied to be lower than, equal to or higher than 0.8 mM. The substrates with 1.2 mM VGVAPG and all gels with P15 led to significantly higher αSMA expression for both stiff and soft substrates, as compared to 0.8 mM RGDS alone. Importantly, all gel conditions αSMA expression were significantly lower than tissue culture poly(styrene) (TCPS; ~4- to 10-fold difference). The ECM produced decreased significantly as the total integrin-binding peptide concentration increased, but was significantly higher than that produced on TCPS. This easily tailored material system provides a useful culture platform to improve the fundamental understanding of VIC biology through isolating specific biological cues and observing VIC function.

1. Introduction

Valvular interstitial cells (VICs) are the main cell population in heart valves and are responsible for maintaining valve homeostasis [1]. In healthy valves, VICs typically reside in a quiescent fibroblast state, but can be activated to form myofibroblasts upon injury to the valve [2]. In this activated state, they are known to be able to contract [1–5], proliferate [2,3,6] and express a host of different proteins, including cytokines [2,5,6], matrix metalloproteinases (MMPs) [1,2,6,7] and extracellular matrix (ECM) molecules [2,3,6–9], all of which help maintain proper valve function. However, this delicate balance of VIC activation and secretory properties can go awry if misregulation and repeated injuries occur. For example, the prolonged activation and elevated ECM expression of VICs can lead to calcific aortic stenosis [6,10–13].

The microenvironment of the valve has an acute influence on VIC phenotype and function, and researchers are only beginning to understand how valve attributes and conditions direct VIC behavior. Activation of VICs to the myofibroblast phenotype is known to depend on the culture substrate stiffness [2,6–9,14,15] and to occur in response to specific proteins presented on culture substrates [1,9,11,12,16,17]. This increase in activation due to physical or biochemical cues has also been linked to subsequent increases in ECM production [1,17,18]. Understanding the complex interplay between cellular cues and matrix interactions, and how they influence VIC secretory properties, especially the deposition of ECM, would be extremely advantageous, when trying to design bioactive culture platforms to probe and eventually direct VIC function. Todate, few studies have reported on how VIC-material interactions influence the composition and deposition of ECM produced by VICs [12,17,19]. This is further complicated by the fact that, when VICs are isolated from valve tissue and cultured on traditional tissue culture plasticware, all of the VICs become activated myofibroblasts in less than 48 h, which causes potentially non-physiologically relevant cellular responses [1].

To achieve precise control of VIC culture conditions, a thiol–ene step-growth photopolymerization was employed to fabricate a highly defined culture substrate. These materials were fabricated

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