Regulation of Cardiomyocyte Hypertrophy by Scaffold Stiffness, Ligand Presentation, and Co-culture

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Introduction: The limited regenerative capacity of cardiac muscle inhibits the reversal of damage caused by a myocardial infarction. Unfortunately, the hypertrophy which occurs when adult cardiomyocytes (CMs) try to recover the mechanical loading capabilities lost after a myocardial infarction is both maladaptive and pathological (1). The most notable phenotypic alteration associated with pathological adult hypertrophy is CM expression of a "fetal gene motif" (2). Several factors are known to induce hypertrophy, and cardiac cells themselves produce many pro-hypertrophic molecules, including angiotensin-II (AngII). Many studies have focused on designing tissue engineered constructs for restoring cardiac function (3), a goal which will ultimately involve implantation of these neo-tissues in the hypertrophic environment of the infarcted heart. In this work, we investigate how physical and biological properties of the biomaterial scaffold, as well as co-culture with other cardiac cell types, impacts the response of CMs to a native disease-inducing factor, AngII.

Methods: Cells from an immortalized murine cardiomyocyte cell line (HL-1s) were encapsulated within 3-D photocrosslinked polyethylene glycol (PEG)-based scaffolds of varying stiffness (20 kPa, 7.75 kPa and 1.32 kPa). PEG scaffolds were covalently modified with either the RGD or YIGSR adhesive peptide sequence, or left unmodified (control). These cell-seeded scaffolds were then cultured for four days in transwell suspension plates in the presence or absence of murine endothelial cells (ECs) grown on the transwell inserts. Hypertrophy was stimulated through the addition of 5 nM AngII to the culture medium every two days. The impact of scaffold environment and co-culture on hypertrophy was measured via qRT-PCR for markers of fetal gene expression (i.e., upregulation of α-skeletal actin (αSkA), α-smooth muscle actin (αSMA), atrial natriuretic protein (ANP) and β-myosin heavy chain (βMHC)).

Results: In the absence of AngII and ECs, cardiomyocytes cultured in 3-D scaffolds exhibited roughly the same levels of hypertrophic marker expression across all scaffold stiffnesses and peptide presentations. The expression of one representative hypertrophic gene, ANP, is shown in Figure 1. However, some scaffold-specific trends did emerge upon treatment with AngII. Addition of AngII differentially impacted ANP expression across the scaffold conditions. Namely, the greatest hypertrophy occurred in the stiffest scaffold, and the YIGSR peptide supported the greatest hypertrophy within the softer scaffolds.

Co-culture with ECs yielded substantially different hypertrophic outcomes, both in the presence and absence of AngII (Figure 2). In the absence of AngII, expression of hypertrophic markers greatly varied across the scaffold and peptide conditions, and was generally higher than in the monoculture. Addition of AngII to CMs in 3-D scaffolds co-cultured with ECs resulted in an upregulation of hypertrophy that was consistent with monoculture results; namely, the CMs in scaffolds containing YIGSR exhibited the largest increases in ANP expression. Similar trends were found for the four other hypertrophic markers tested.

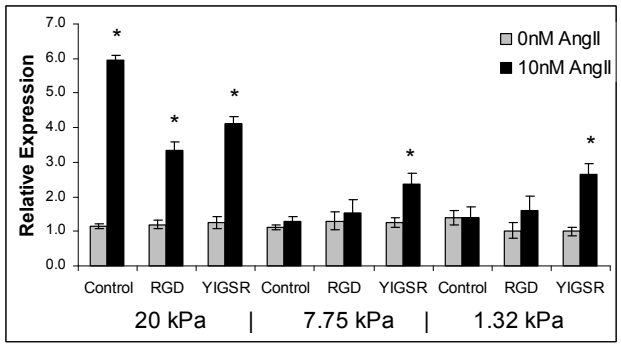

Figure 1. ANP expression by cardiomyocytes cultured in 3-D PEG scaffolds. * $p<0.05$ vs. untreated control.

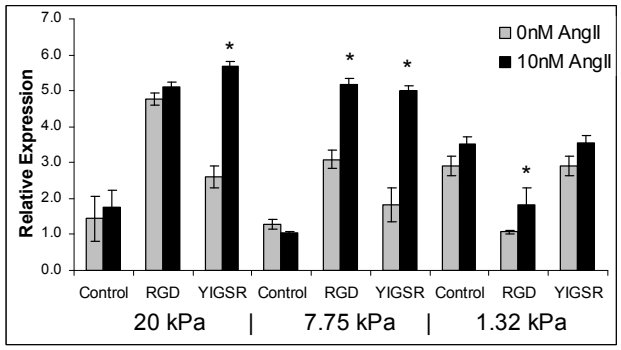

Figure 2. ANP expression by cardiomyocytes in 3-D scaffolds cultured in the presence of endothelial cells. *$p<0.05$ vs. untreated control.

Conclusions: Cells are highly sensitive to changes in the biomaterial environment, and the composition of this environment can regulate the cellular response to not only growth factors and cytokines, but also native disease-inducing biomolecules. Ultimately, engineered tissue replacements will often be implanted in organs that are rich in such disease-inducing factors, such as the post-infarct hypertrophied heart. Our findings reveal differences between scaffold conditions that would not have been seen had the experiments been executed solely in healthy culture conditions. By characterizing how markers of cell dysfunction are affected by conditions that will be experienced in diseased tissue, a more accurate model for developing better tissue engineered scaffolds can be created.

References:

(1) Wagner M, Ann NY Acad Sci 1999;874: 1-10.
(2) Schaub MC. J Mol Med 1997;75: 901-20.
(3) Papadaki M. Am J Physiol Heart Circ Physiol 2001;280: H168-78.