VARIABLE OXYGEN CONDITIONS AND CARDIOMYOCYTE STRUCTURE AND FUNCTION IN NOVEL IMMUNO-HEART CHIP

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Introduction

The immune system is known to play a multi-functional role in the recovery process after hypoxic cardiac injury. This process consists of an acute inflammatory response, followed by inflammation resolution, and then repair of the tissue [1]. Macrophages of certain functional phenotypes (M1: pro-inflammatory; M2: pro-healing) play different roles in each phase of this recovery process. Cardiac tissue is also populated by resident cardiac macrophages, which may also play a role in normal healthy function of the heart [2]. Shifts in macrophage functional phenotype may be required for optimal post-hypoxia remodeling of the heart [3].

As all previous *in vitro* cardiac experiments have been done without the presence of macrophages or their factors, this study is essential for the design of a novel platform that will establish a baseline of how macrophages and immunomodulatory factors influence cardiomyocyte morphology and function. Furthermore, the results will elucidate the synergistic effect of macrophages on cardiac function such as enhanced electrical connectivity–which may lead to stronger and more coherent force production.

Methods

Experiments involving the culture of both primary neonatal rat ventricular myocytes (NRVMs) and bone marrow derived macrophages (BMDMs) were carried out to examine the juxtacrine and paracrine effects they have on each other. Hypoxia was induced in the cardiomyocytes via a hypoxia chamber, at varying severities and durations. Cardiomyocyte structure was evaluated using custom ImageJ and Matlab codes that quantify architectural changes in cardiomyocyte cytoskeletons and tissue quality [4]. Different media compositions were used to induce M1 and M2 macrophage phenotypes. Macrophage functional activation was evaluated based on measurements of paracrine factor secretion via ELISA.

Results

Co-culture Common Media Formulation A common co-culture media was identified as having no significant effect on NRVM morphology (cell aspect ratio and area) and no significant effect on macrophage cytokine secretion. M1 and M2 stimulated media significantly reduced some cardiomyocyte architecture metrics.

Juxtacrine Co-culture When NRVMs and BMDMs were cultured together (**Fig. 1**), the presence of macrophages caused a significant reduction in NRVM architecture metrics (Z-line OOP, Actin OOP, mean continuous Z-line length (MCZL)) and tissue quality (Z-line fraction). Additionally, M1 and M2 media stimulations further exacerbated the decrease in Actin OOP and MCZL. In the presence of NRVMs, M1 and M2 macrophages significantly increased their secretion of TNF-α and IL-10 respectively.

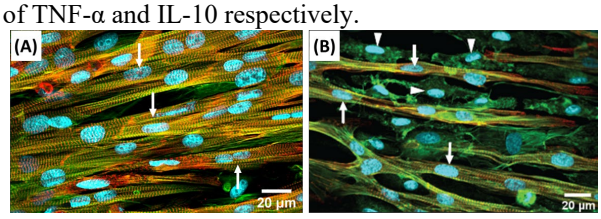

Figure 1 (A) NRVM Monoculture and (B) NRVM-BMDM Co-culture. *Stains: Nuclei (cyan), Z-lines (red), Actin (green). Triangles indicate macrophages and arrows indicate cardiomyocytes seeded in the same culture.*

Paracrine Co-culture NRVMs and BMDMs were then cultured on separate coverslips where they could only communicate through paracrine factors. The same decreases in cardiac architecture and tissue quality observed previously were not observed under paracrine conditions, suggesting that physical contact is necessary to yield the structural changes. M1 macrophage secretion of TNF-α was no longer increased in paracrine co-culture, suggesting that cell-cell contact is required to induce the increased TNF-α secretion. M2 macrophages kept the increased secretion of IL-10, suggesting that NRVMs are secreting a paracrine factor that upregulates IL-10 secretion.

Discussion

Cardiomyocyte and macrophage responses to hypoxia have been studied separately, but the emergent properties that arise from the reciprocal interactions between these two cell types remain poorly understood due to challenges of such investigations *in vivo*. Moreover, these emergent properties are not necessarily the sum of the individual responses. The objective of this work was to develop a novel immuno-heart chip in order to elucidate the relationship between hypoxia, cardiac structure and biomechanics, and cardiomyocyte-macrophage interactions. The next step in this study is to evaluate the co-cultured tissue's contractility (systolic, diastolic, and active stress) via our lab's muscular thin film based "heart-on-a-chip" [5].

References

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