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Spatiotemporal control of cell fate and cardiac differentiation

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Abstract— Congestive heart failure is a leading cause of morbidity and mortality in the United States and worldwide [1]. Although not the only cause of congestive heart failure, loss of myocardium due to obstructive coronary artery disease is a major contributor to this condition [2,3]. *The loss of myocardium is mainly regional and, therefore, localized therapy holds the most promise*. In recent years, many clinical studies have been initiated to deliver localized therapy in the form of various cell types for reconstitution of the myocardium [4-6]. However, there is much debate on the optimal cell type, whether or not stem cells can differentiate into functional myocardium and the long-term effects of these non-myocytes. In addition to exogenous cell delivery, paracrine effects arising from delivery of angiogenic factors and other biochemical agents suggest that the myocardium retains the ability to remodel and heal [7,8]. Understandably, there has been tremendous focus on both growth factor- and gene therapy-based therapeutics. While a source of great promise, direct growth factor delivery to the myocardium will most likely be inefficient as several studies have noted that many of these small proteins are carried away in the highly vascularized cardiac tissue [9,10]. Gene therapy, while providing an excellent analytical tool, has not met with enthusiasm clinically, mostly due to the inability to quantify delivery and nonspecific targeting *in vivo*. This body of work supports two crucial conclusions: (1) *appropriate biological cues that act locally on the myocardium can improve functional outcomes*, and (2) *spatiotemporal control over the delivery and presentation of these cues remains challenging and inefficient.*

The current body of work on cardiac drug delivery and stem cell therapy support two crucial conclusions: (1) *appropriate biological cues that act locally on the myocardium can improve functional outcomes*, and (2) *spatiotemporal control over the delivery and presentation of these cues remains challenging and inefficient.* Using specialized biomaterials, we are able to address this. Our laboratory has used functional nanoparticles to deliver regenerative cues with both spatial and temporal control. By adding biomimetic signals to the surface of the nanoparticles, we are able to enhance nonphagocytic cell uptake, achieve cell-specific targeting, and deliver molecules in a time-controlled manner. Furthermore, by combining our nanoparticles with self-assembling peptide nanofibers, we can achieve some of these in 3D while retaining implanted cells.

Following infarction, and during cell differentiation and survival, there are unique sequences of events that contribute to the pathophysiology of the processes. By enhanced understanding of these signals, and the spatiotemporal control needed to deliver them, it will help design better therapeutics for cardiac regeneration.

Using high-throughput screening, we have identified several carbohydrates that bind with high affinity to both cardiomyocytes and cardiac progenitor cells. For example, n-acetylglucasmine (GlcNAc) and related sugars bound with high affinity to both of these important cell types. Modification of the sugars with phospholipds allowed incorporation of into hydrophobic nanoparticles with high fidelity. When delivered to cardiomyocytes or cardiac progenitor cells, these biologically-modified nanoparticles enhanced uptake by several fold. In addition to using sugars, we also used metal affinity chromatography chemistry to link Nickel-NTA to the outside of nanoparticles. This not only allowed for conjugation of Histidine-tagged proteins to the outside for temporal delivery, but also allowed for conjugation of cell-specific targeting agents as well. Indeed, when His-VE-Cadherin was used as a model targeting agent, binding and uptake by endothelial cells increased significantly.

For cell therapy, incorporation of proper signals to the micoenvironment is critical. One such signal that greatly impacts differentiation is the Notch1 pathway. We were able to immobilize Jagged1, the ligand of Notch1, on to self-assembling peptide nanofibers. Inclusion of this bioactive signal significantly enhanced cardiogenic differentiation. Morever, by including time release nanoparticles, we were able to improve differentiation and survival significantly more, underscoring the need to spatiotemporal control of drug delivery for enhancing cell therapy.

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