Delivering Regenerative Cues to the Heart: Cardiac Drug Delivery by Microspheres and Peptide Nanofibers

Jay C. Sy · Michael E. Davis

Abstract Our understanding of signaling pathways and cues vital for cardiac regeneration is being refined by laboratories worldwide. As various mechanisms enabling cardiac regeneration are becoming elucidated, delivery vehicles suited for these potential therapeutics must also be developed. This review focuses on advances in two technologies, novel degradable microspheres for controlled release systems and self-assembling peptide nanofibers for cell and factor delivery. Polyketals, a new class of resorbable polymers, are well suited for treating inflammatory diseases due to biocompatible degradation products. Polyketals have been used to deliver small molecule inhibitors and antioxidant proteins to rat models of myocardial infarction with notable improvements in cardiac function. Self-assembling peptide nanofibers are a class of hydrogels that are well-defined scaffolds made up of 99% water and amenable to incorporation of a variety of bioactive cues. Work done by our laboratory and others have demonstrated functional improvements using these hydrogels as both a drug delivery vehicle for proteins as well as a defined microenvironment for transplanted cells. Combining non-inflammatory polymer microspheres for sustained release of drugs with self-assembling nanofibers yields multifunctional scaffolds that may soon drive the body's healing response following myocardial infarction towards cardiac regeneration.

Keywords Cardiac Regeneration · Drug Delivery · Cell Scaffold · Polyketals · Peptide Nanofibers · Myocardial Infarction

Introduction

Cardiovascular disease remains a leading problem in the USA with over a million Americans expected to suffer a myocardial infarction this year [1]. Improved therapies such as percutaneous coronary intervention and antithrombotic drugs have reduced the mortality rate of myocardial infarctions (MI); however, cardiac dysfunction remains an issue due to inadequate healing of the heart following ischemia. Much research has implicated the inflammatory response following MI as one of the key regulators of cardiac dysfunction [2–8]; it is part of the body's healing response to the acute injury. However, the endogenous healing response to the injury is insufficient, leading to cellular hypertrophy, non-contractile scar formation and eventual heart failure, with transplantation being the only definitive cure. Recent findings have challenged the dogma that the heart is a terminally differentiated organ with no regenerative capacity [9–11] and have identified cells and cues that are central to the regenerative response. The biology behind achieving cardiac regeneration is a rapidly evolving area of research with potential treatments—ranging from cell therapy to paracrine factors—being tested in trials worldwide. Though studies show promise in some instances, one common shortcoming is the lack of suitable delivery vehicles for the treatment, which can drastically

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improve the efficacy of the various therapies. Micro-particles have been used as controlled drug delivery vehicles for a variety of diseases; however, in the cardiac field, most microparticle work has focused on using microparticles as platforms for detection of myocardial infarction biomarkers, such as troponin and other markers of cellular damage [12–16].

Micro- and Nanoparticle-Mediated Drug Delivery

Systemic, intravenous, or intracoronary delivery of small molecules and proteins remains popular forms of drug delivery for treating MI due to minimal invasiveness. These delivery methods require frequent dosing to achieve relevant concentrations in the infarct zone for significant periods of time. This greatly increases the chances for potential side effects and toxicity. More localized delivery of therapeutics may be achieved by specialized surgical tools for direct intramyocardial injection [17]. This approach is effective for cell therapy, where the "therapeutic" ultimately engrafts in the site of injection. However, for pharmaceutical interventions, localized injections are quickly washed into bloodstream, both diluting the therapeutic and negating any local delivery effects. Two alternative delivery strategies that circumvent these issues are systemically delivered targeted drug delivery vehicles and locally delivered sustained release formulations. Targeting the myocardium has primarily focused on using liposomal and antibody-based approaches. Antibodies directed against intracellular proteins exposed following infarction and inflammatory proteins that are upregulated following MI have been the focus of infarct-specific delivery [18–20]. However, some research suggests that the infarcted heart inherently has enhanced permeability and retention property similar to that seen in tumors, which may passively enhance accumulation of nanoparticles in the infarct [21]. While these liposomal formulations require modifications for serum stability [22], they offer the advantages of excellent size control at the submicron scale and the unique potential to "plug and seal" membranes [23]. This phenomenon was investigated by Verma et al. using liposomal formulations to deliver adenosine triphosphate [24] to rabbit models of MI, reducing the volume of at-risk myocardium as measured by nitroblue tetrazolium. Other groups have utilized the mechano-acoustic properties of liposomes in order to achieve on-demand release of encapsulated therapeutics. Liposomes can be acoustically disrupted by ultrasound, a common imaging modality for cardiovascular applications, resulting in localized delivery. A variety of compounds have been delivered to the heart including gene therapy constructs that encode vascular endothelial growth factor (VEGF) for therapeutic vascularization and tissue plasminogen activator for clearance of thrombi [24–28].

Another broad class of drug delivery particles is based on naturally derived hydrogels. Extracellular matrix proteins—such as collagen, gelatin, and fibrin—have been used as matrices for sustained release of therapeutic compounds. These materials are often supplemented with synthetic hydrogels, such as poly(ethylene glycol), in order to improve mechanical properties or impart specific functionalities [29]. However, while these materials are typically delivered as an injectable hydrogel or tissue engineered patch, some have been formulated as microparticle drug delivery vehicles. Gelatin microparticles have been used to encapsulate angiogenic and anti-fibrotic factors. These include plasmid DNA, proteins such as basic fibroblast growth factor, and chemical agonists [30–33]. While potentially biocompatible, there are concerns regarding the ability to control degradation profiles, especially with potential upregulation of gelatinases and other proteases following MI [34].

Biodegradable, hydrophobic polymers have been widely used in sustained release formulations both in the clinic and in research laboratories. Synthetic polymers are often better characterized than naturally derived materials and can survive a variety of processing techniques. The most common class of biodegradable polymers used in medicine is polyesters, such as poly (lactic-co-glycolic acid) (PLGA). These polymers rely on the water-cleavable ester linkage (Fig. 1a) and degrade into low molecular weight carboxylic acids, which can be metabolized or cleared by the body. While not widely used for drug delivery directly to the myocardium, PLGA has been used for other cardiovascular applications such as stent coating [35, 36] and cardiac tissue engineering [37]. PLGA and the related polymer poly(glycolic acid) has been widely used in tissue engineering, particularly as a controlled release platform to temporally control growth factor release for neovascularization of constructs [38]. There has been one report of a potential myocardial application of PLGA to deliver heat shock protein-27 in a controlled manner [39]. In this study, the treatment was performed on cultured cardiomyocytes only, though it was able to rescue apoptosis.

Our laboratory has focused on the use of a new class of biodegradable polymers called polyketals [40]. Polymers based on the ketal linkage provide key advantages over the more widely used polyesters. The ketal linkage is an acid-sensitive, hydrolyzable bond that degrades to produce acetone and a diol (Fig. 1b–d) and can be tuned to degrade over time spans ranging from days to months [41, 42]. The two main advantages with using polyketals over more
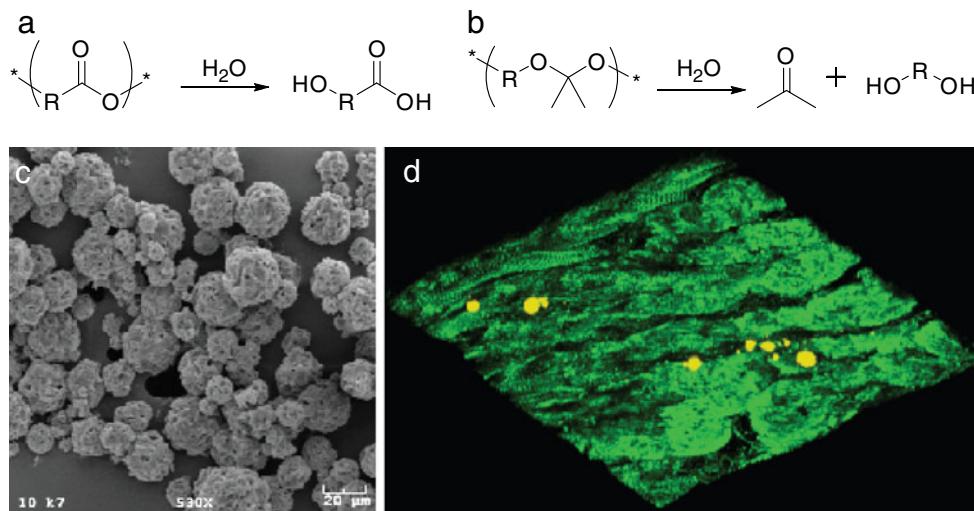
Fig. 1 Degradable polymer microparticles. **a** Widely used degradable polymers, such as PLGA, are based off ester linkages that degrade into carboxylic acids. **b** Polyketals are based on the ketal linkage, which degrades into acetone and a diol. Both of these degradation byproducts are uncharged and do not affect local pH. **c** Scanning electron micrograph of PCADK microparticles produced through the single emulsion-solvent evaporation procedure (*scale bar* 20 μm). **d** PCADK microparticles, loaded with fluorescent dye, are retained in the myocardium. Particles were visualized using laser scanning confocal microscope, 160 μm×160 μm×15 μm Z-stack. *Green* alpha-myosin heavy chain, *yellow* rubrene-loaded microparticles

established degradable polymers lies in biocompatibility and tunability. Polyketals have the unique property of degrading into neutral compounds rather than acidic byproducts. As a result, degradation of polyketals in vivo does not affect the local pH in the surrounding tissue, which may happen with polyester degradation [43–46]. This is a potential advantage when treating diseases where inflammation plays a large role, such as cardiac dysfunction following MI, as a local drop in pH could further exacerbate inflammation.

Our lab has published work on delivering of the p38 MAPK inhibitor SB239063 via microparticles fabricated from poly(cyclohexane-1,4-diylacetone ketone) (PCADK), a polyketal polymer [47]. The p38 MAPK is a common target for post-MI therapy due to its role in cardiac myocyte apoptosis and activating the inflammatory response [48, 49]. Several clinical trials using similar p38 MAPK inhibitors have been conducted or are currently ongoing for heart disease and a number of other inflammatory diseases such as arthritis [50]. Rats in the study were subjected to permanent occlusion of the left anterior descending coronary artery and microparticles injected directly into the infarcted myocardium. Microparticles were fabricated with large diameters (10–20 μm) so that they would lodge into the tissue rather than be carried into the bloodstream. These microparticles survived the mechanical stresses of the beating heart and avoided being carried in the microcirculation (Fig. 1d). Activation of p38 MAPK and downstream effects were reduced as early as 3 days following infarction. However,

no significant improvement in cardiac function was seen at 7 days. Fractional shortening was improved by more than 10% between days 7 and 21 with PCADK microparticles with comparable PLGA controls showing a decrease in heart function. Histological sections showed reduced fibrosis in animals treated with the polyketal encapsulated inhibitor, suggesting that the released inhibitor slowed or prevented collagen deposition and adverse remodeling of the left ventricle.

Similar studies have been conducted with encapsulated superoxide dismutase (SOD) in PCADK microparticles [51, 52]. SOD is an antioxidant enzyme that scavenges the superoxide radical ($O_2^{\cdot-}$). Following MI, there is a large increase in superoxide levels, followed with a downregulation of native SOD [53]. While overexpression studies show a promising role for enhanced SOD expression improving function after MI, protein delivery studies show little effect, if any [54–56]. PCADK microparticles containing SOD were assessed in an ischemia–reperfusion model of myocardial infarction in rats [57]. Animals treated with a single intramyocardial injection of polyketal microparticles loaded with SOD (PK-SOD, 80 U per rat) showed significantly less superoxide in the border zone as compared with controls, including a free SOD injection. This resulted in reduced myocyte apoptosis and ultimately improved acute cardiac function at 3 days with a trend of improved cardiac function approaching statistical significance at 21 days. Given that the SOD therapy alone may not be sufficient for long-term outcomes, animals were treated with both

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PK-SOD- and SB239063-loaded microparticles; cardiac function was further improved over PK-SOD and other controls. This result suggests that there is no single "magic bullet" for preventing and perhaps reversing cardiac dysfunction; future treatments must consider the time course of disease progression when delivering therapeutics. Our novel approach to achieving temporal control of drug delivery is discussed below.

Polymer microspheres have been used to deliver growth factors and other proteins, but in general, large amounts of protein are required due to low encapsulation efficiencies and protein denaturation. Organic solvents that are typically used to process polymers can reduce protein activity. Previous work with PLGA microparticles and other materials have functionalized surfaces with heparin [58–60]. This approach relies on heparin-binding domains found on many growth factors. The affinity between heparin and various growth factors allows for local retention and sustained delivery. Our laboratory recently adopted a similar approach using immobilized metal affinity strategies for rapid delivery of histidine-tagged proteins. Using the metal affinity system, we are able to bind a variety of proteins including those lacking a heparin-binding domain. Using a nitrilotriacetic acid (NTA)–lipid conjugate, we functionalized the surface of polyketal microparticles in order to non-covalently bind proteins bearing the His_6-tag [61]. NTA is widely used as a chelator for Ni^{+2}-ions in metal affinity columns commonly used for recombinant protein purification [62, 63]. Loading proteins onto the surface of the microparticle has several advantages over traditional microencapsulation strategies; proteins are loaded with high efficiency using dilute solutions with no risk of protein denaturation due to exposure to organic solvent. Our studies showed rapid release of His_6-tagged proteins, with 50% of tethered protein being released after 2 h while maintaining the sustained release profiles of hydrophobic compounds from the core of the microparticle. This tethering strategy is highly flexible, as the surface modification strategy is not polymer specific and can be adapted to most microparticle formulations. Furthermore, NTA-Ni/His_6 is widely used for recombinant protein purification, making a large library of proteins readily available. Our data suggest that bioactive proteins can be used to activate signaling pathways (i.e., VEGF delivery) or for microparticle targeting of cell surface receptors (VE-cadherin dimerization). This gives the potential to deliver a "one-two punch" of therapeutics from a single microparticle formulation. Multiple types of proteins tethered to the surface of the microparticle can provide both targeting capabilities as well as rapidly acting factors to rescue at risk myocytes, while more slowly released hydrophobic drugs encapsulated in the core of the microparticle can exert effects over the course of weeks or months.

Hydrogel-Mediated Drug Delivery

Sustained release of proteins and other hydrophilic drugs has primarily been accomplished by the use of hydrogels [39, 64, 65]. Hydrogels have the added advantage of being well suited for use as a cell scaffold for cell therapy applications due to their three-dimensional structure. Cells have the potential to improve cardiac function following MI by contributing to vascularization of the infarcted tissue, improving cell survival via paracrine signaling, or by directly contributing to contractile function of the tissue. The various cell types tested and outcomes have been reviewed extensively elsewhere [66–68]. One common element with current clinical trials is the delivery vehicle-free transplantation procedure; delivery methods range from simple intravenous delivery, direct myocardial injection of the cells suspended in buffer, to more sophisticated methods such as engineering cell sheets [69] or delivery cells with biological hydrogels [70–72]. In most cell therapy approaches, few transplanted cells engraft into the damaged heart tissue due to inadequate delivery strategies and thus can benefit from effective design of delivery vehicles. One of the overarching concerns with tissue engineering and cell therapy is the local microenviroment of the transplanted cells. The microenvironment directly affects the levels of cell engraftment, proliferation, and differentiation following transplantation. In the case of the injured myocardium, transplanted cells are exposed to a hypoxic environment with high levels of oxidative stress and pro-inflammatory cytokines. These factors combine to severely reduce cell viability and efficacy of cell therapy. It is estimated that less than 1–20% of transplanted cells survive and engraft into the host heart, depending on cell type and delivery method [73–75].

Cell scaffolds provide a platform for delivery of bioactive molecules as well as a physical environment to allow for cell attachment thus preventing cells from being carried away. A variety of materials have been used as cell scaffolds including synthetic polymers and hydrogels [76], native extracellular matrix (ECM) proteins [77], and processed native ECM [78]. Naturally derived materials are typically used in ex vivo tissue engineering approaches and have been extensively reviewed elsewhere [79, 80].In all cases, nanoscale topology, similar to what cells see in their native microenviroment, has proved to be an important feature. De novo engineering of peptide sequences can form well-defined nanofibers [81] under physiological conditions. These synthesized peptides may have regulatory benefits as the cell scaffold is more chemically defined and

reproducible compared with purified ECM or recombinantly expressed proteins. Furthermore, as these are just peptides, specific functionalities can be engineered into the scaffolds as needed.
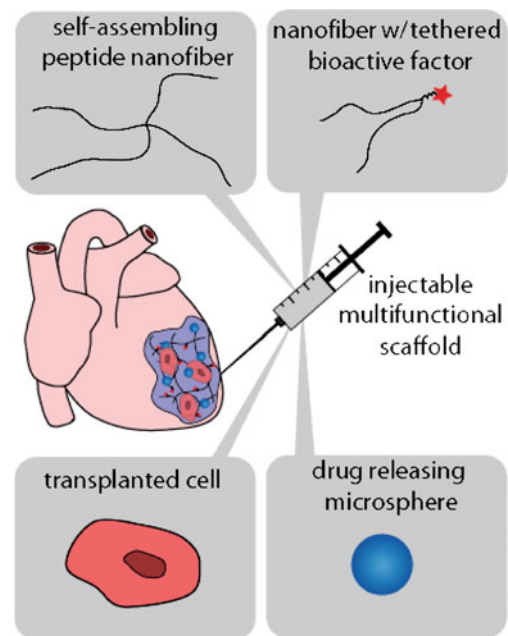
Self-assembling peptide nanofibers are peptide repeats (example: (RARADADA)$_2$) that have both hydrophillic and hydrophobic components and alternating charges, allowing them undergo self-assembly in physiological solutions. The hydrophobic moieties form helices, exposing the charged residues that come together via ionic interactions [82]. Self-assembling peptides have been used with success in animal models as sustained release drug delivery vehicles in order to exert a local effect. This is particularly useful for cytokines that can have detrimental systemic effects or for proteins that are rapidly degraded and cleared in the circulation. Delivery of platelet-derived growth factor-BB (PDGF-BB) was sustained in vivo by embedding the factor within self-assembling peptide nanofibers [83]. In comparison with free PDGF-BB, sustained delivery improved angiogenesis, cardiomyocyte survival, and long-term function. Another key example of this strategy is the delivery of stromal cell-derived factor-1 (SDF-1) to the infarct [84]. SDF-1 is a chemoattractant for stem cells and may play a vital role in recruiting progenitor cells into the infarct. SDF-1 has not reached its therapeutic potential as protease activity can cleave SDF-1 into neurotoxins implicated in certain forms of dementia. In this work, SDF was expressed with a protease-resistant signal, followed by a self-assembling peptide sequence allowing it to be incorporated in to a native self-assembling peptide scaffold. Local, sustained delivery of the active factor resulted in increased stem cell recruitment, capillary density, and ultimately improved cardiac function. As the biology of adult stem cells and resident progenitor cells is better understood, self-assembling peptide scaffolds provide a platform to deliver migratory and differentiating cues to drive regeneration.

Protein delivery with self-assembling peptide nanofibers can also be used to enhance cell therapy following infarction. Delivery of insulin-like growth factor-1 (IGF-1) enhanced both neonatal myocyte, as well as cardiac progenitor cell (CPC) efficacy [85, 86]. IGF-1 is a small growth factor that is bound by IGF-binding proteins in serum; thus, delivery of the free protein is highly inefficient. As CPCs are being used in human clinical trials, developing strategies to improve their growth and survival following transplantation is critical. Additionally, PDGF was used to attempt to improve skeletal myoblast efficacy in a pig model of MI [87]. While no statistical improvement was seen with the treatment groups, the PDGF group did improve angiogenesis. These data demonstrate that while controlled delivery of bioactive factors can supplement implanted cell therapy, more studies need

to be performed to properly match the cell type and specific factor(s) delivered.

Conclusion

The ultimate goal, in our laboratory, is to combine the self-assembling peptides with polyketal microspheres in a multifunctional cell delivery vehicle (Fig. 2). Given the flexibility of the two technologies, the multifunctional scaffold can contain bioactive molecules—ranging from small molecule inhibitors to large antioxidant proteins and growth factors—released over specific time courses. As cardiac dysfunction following MI is a progressive disease, the therapeutic drug delivery must follow suit. What is beneficial at one point in the disease may be harmful at another. Using biomaterials, we have the ability to orchestrate the body's response following MI in order to reduce inflammation, to promote cellular survival, and to recruit and differentiate progenitor cells towards cardiac lineages. With spatial and temporal control afforded by multifunctional scaffold design, combined with novel data that are generated from basic mechanistic studies, more options will be available for biomaterial-based drug delivery for treating MI.

Fig. 2 Multifunctional cell scaffolds. Self-assembling peptide nanofibers can be formulated in an injectable form. Mixtures of self-assembling peptides, fibers functionalized with bioactive compounds, drug releasing microspheres, and cells can be injected into the myocardium. Upon gelation, the multifunctional scaffold provides a microenvironment better suited for cell engraftment and releases cues vital for cardiac regeneration

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