Biomaterials to Enhance Stem Cell Function in the Heart

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Abstract: —Transplantation of stem cells into the heart can improve cardiac function after myocardial infarction and in chronic heart failure, but the extent of benefit and of reproducibility of this approach are insufficient. Survival of transplanted cells into myocardium is poor, and new strategies are needed to enhance stem cell differentiation and survival in vivo. In this review, we describe how biomaterials can enhance stem cell function in the heart. Biomaterials can mimic or include naturally occurring extracellular matrix and also instruct stem cell function in different ways. Biomaterials can promote angiogenesis, enhance engraftment and differentiation of stem cells, and accelerate electromechanical integration of transplanted stem cells. Biomaterials can also be used to deliver proteins, genes, or small RNAs together with stem cells. Furthermore, recent evidence indicates that the biophysical environment of stem cells is crucial for their proliferation and differentiation, as well as their electromechanical integration. Many approaches in regenerative medicine will likely ultimately require integration of molecularly designed biomaterials and stem cell biology to develop stable tissue regeneration. (Circ Res. 2011;109:910-922.)

Key Words: myocardial infarction ■ stem cell therapy ■ biomaterials ■ tissue engineering ■ protein delivery
Healthy myocardial tissue is more than just a collection of myocytes, endothelial cells, smooth muscle cells and fibroblasts. The different cell types comprising adult myocardium interact to form a highly organized and dynamic contractile tissue. These cells also interact with the extracellular matrix to form a highly organized and dynamic network of cells and matrix. The extracellular matrix contains inotropic stimuli and growth signals for adult myocytes, as well as signals for mitosis, growth, differentiation, and migration of stem and progenitor cells. When cardiac tissue is damaged by a myocardial infarction (MI), cells die in the infarcted region and the normal highly organized architecture of extracellular matrix disappears with the cells.

Different populations of resident cardiac stem cells have been isolated from adult myocardium, including human myocardium. However, cardiac stem cells present in the myocardium seem to be too low in number, have inadequate differentiation potential, or receive insufficient instructions to complete cardiac regeneration after MI. In the natural healing process of MI in mammals, myocytes are replaced primarily by scar tissue. Generation of a limited number of new myocytes after MI occurs in the border region of the infarct but not in the infarct zone itself. This zonal selectivity could be explained by the proximity of surviving capillaries and preserved perfusion, but another explanation is that the surrounding viable myocardium and extracellular matrix contain crucial signals for recruitment, activation, or maturation of stem cells in the heart. This concept could challenge attempts to inject a population of stem cells in the center of the infarct zone because these cells may not lead to formation of mature myocytes, a finding supported by a number of studies. A solution to this problem could be delivery of differentiation and maturation signals at the same time of stem cell delivery; these signals could be soluble proteins, extracellular matrix, or signals transmitted through cell–cell contact. Biomaterials are the likely means to deliver these signals.

Recently, significant progress has been made in the field of biomaterials and the field of cardiac regeneration using stem cells. In this review, we primarily discuss new findings at the intersection of both fields. More specifically, we discuss biomaterials used for stem cell transplantation and methods for controlling the biochemical and biophysical microenvironment of transplanted stem cells in the heart. This review focuses on research published in the past 5 years; for reviews of earlier published work, we refer to references. We also discuss methods in biomaterials research to enhance engraftment and differentiation of stem cells, to increase angiogenesis, and to enhance electric and mechanical integration of newly formed cardiomyocytes. We preferentially cite studies performed in the heart but use examples of studies in other tissues as well if promising new biomaterials technologies have not yet been applied in the heart. For review articles covering cardiac regeneration using stem cells, we refer to other sources. For review articles covering myocardial tissue engineering, we refer to other sources. For a review on the effects of the extracellular matrix on cell signaling, we refer the readers to other sources.

**Cardiac Regeneration Using Stem Cells**

To repair the damaged heart, many different stem cells have been used in preclinical and clinical studies. Many types of cells injected in the heart have been shown to improve cardiac function but at the same time, in almost all studies, injected cells have a low survival rate after transplantation. Plausible explanations for this improved cardiac function despite a lack of survival and transdifferentiation into myocytes are secretion of paracrine signals that act on surrounding myocardium or stimulation of endogenous repair. Here, we assume that the ultimate goal of stem cell transplantation is formation of new and functional myocardial tissue, and thus we focus on enhancement of the function of stem cells that have high potential for differentiation into cardiomyocytes. However, many studies on the effects of biomaterials on stem cells have used bone marrow-derived mesenchymal stem cells (MSCs) because of their availability and relatively easy culture. Although the differentiation potential of MSCs into cardiomyocytes is limited, in some places we cite studies describing promising new biomaterials using MSCs if studies have not yet been performed with pluripotent stem cells. For the same reason, in some places we cite studies using bone marrow-derived hematopoietic stem cells or endothelial progenitor cells (EPCs). For a detailed description of stem cells used for cardiac regeneration, we refer to other reviews.

Cells with stem cell properties have been isolated from the adult myocardium, including human. At least some types of cardiac stem cells can form cells with properties of all major cardiac lineages, including myocytes, endothelial...
cells, and fibroblasts, both in vitro and in vivo.\textsuperscript{5,23} Potential cells for transplantation include induced pluripotent stem cells (iPS),\textsuperscript{26–29} which most commonly are skin fibroblasts that have been transformed into pluripotent cells. The iPS cells are potentially one step closer to the “ideal” stem cell for cardiac regeneration because they have the differentiation potential of embryonic stem cells (ESCs),\textsuperscript{27} but potentially with less risk of teratogenicity\textsuperscript{30} and fewer ethical concerns.\textsuperscript{31} However, iPS cells could be biased toward differentiation into certain lineages because of epigenetic memory, in which iPS cells retain information of the parental source and remnants of the reprogramming process.\textsuperscript{32} The ideal stem cell should be easy to isolate, should have a high survival rate after delivery, and should form contractile and nonarrhythmogenic cardiac tissue. Furthermore, cell preparation methods must be low-cost, robust, scalable, and use chemically defined materials.\textsuperscript{33} Of note, a recent study indicated that induction of complete pluripotency for iPS cells might not be necessary to generate beating cardiomyocytes.\textsuperscript{34–36}

In this review, we focus on delivery of multipotent and pluripotent stem cells and not on delivery of their differentiated progeny. Major drawbacks of the injection of pluripotent stem cells are the potential for teratogenicity and for misdirected differentiation into unwanted lineages.\textsuperscript{37,38} One method to overcome these drawbacks is differentiation of ESCs into cardiomyocytes in vitro before transplantation. Differentiation protocols for ESCs have been optimized and show robust differentiation into myocytes in vitro.\textsuperscript{39,40} ESC-derived cardiomyocytes have been incorporated in different biomaterials for transplantation in the heart.\textsuperscript{41–43}

Even if the ideal cardiac stem cell exists or could be designed, infarcted myocardium may not provide the temporal and spatial signals present in viable myocardium that are necessary for adequate survival, proliferation, differentiation, and functional incorporation into organized myocardial tissue. Engineered biomaterials likely will be necessary to deliver these signals to transplanted stem cells.

**Biomaterials Used for Stem Cell Transplantation in the Heart**

Many different definitions exist for biomaterials, with one broad definition being materials used as a medical device for implantation.\textsuperscript{10} Historically, most research on biomaterials focused on orthopedics and development of prosthetics. With more sophisticated medical devices, the emphasis on biomaterials moved toward biocompatibility. More recently, novel molecularly designed biomaterials have been developed that can deliver growth factors and control the environment of transplanted cells.

Biomaterials injected on their own—without inclusion of cells—can decrease remodeling after MI by increasing thickness of the infarct, resulting in decreased wall stress on surviving myocardium.\textsuperscript{15} This effect is more pronounced when the passive mechanical properties of the biomaterial are similar to those of healthy myocardium.\textsuperscript{15} For instance, an injectable alginate hydrogel has been developed for intramyocardial\textsuperscript{44} or intracoronary\textsuperscript{45} injection after MI that increases the wall thickness in the infarcted area. Injection of this alginate hydrogel results in a larger myocardial mass and a decreased end-diastolic volume.\textsuperscript{44,45} However, the effects of biomaterials without stem cells might be temporary; a recent study in which a polyethylene glycol hydrogel was injected in infarcted rat hearts showed a decrease in end-diastolic volume 4 weeks after MI,\textsuperscript{46} but benefits disappeared after 3 months. This study suggests that the positive effects on wall stress after injection of biomaterials without cells might disappear when the biomaterials are degraded.

Biomaterials used for cardiac regeneration ideally will have properties of myocardial extracellular matrix, including similar passive mechanical properties, signals for cellular attachment, and signals for proliferation and differentiation. In addition, biomaterials that are no longer needed will preferably degrade without toxic metabolites and with a controlled degradation rate. Biomaterials should also result in limited foreign body reaction and limited inflammatory response. Foreign body reaction with giant cells can occur with inert biomaterials like polyethylene glycol (PEG).\textsuperscript{48} Myocardial biomaterials should last long enough to guide integration of transplanted cells, but not long enough to interfere with the essential physiological coupling between differentiated cells.\textsuperscript{10} Furthermore, viscosity of injectable biomaterials preferably will be low enough to allow injection through long and narrow catheters, but at the same time solidification should be fast enough to prevent washout of the injected cells. Different injectable biomaterials have been used to deliver stem cells to the heart (Tables 1 and 2), including matrigel,\textsuperscript{47} collagen,\textsuperscript{48–51} fibrin,\textsuperscript{50,52} Polylactic-co-glycolic acid (PLGA),\textsuperscript{53} self-assembling peptides,\textsuperscript{54–57} and alginate.\textsuperscript{44,45} In this section, we provide an overview of biomaterials most commonly used for stem cell delivery in preclinical studies.

**Matrigel**

Matrigel is a solubilized protein preparation extracted from Engelbreth-Holm-Swarm mouse sarcoma, a tumor rich in basement membrane proteins.\textsuperscript{58} The major component of matrigel is laminin, followed by collagen IV, heparan sulfate proteoglycan, and entactin.\textsuperscript{58} Matrigel is a liquid at low temperatures but forms a hydrogel within minutes at 37°C. Therefore, matrigel can be used as an injectable hydrogel for delivery of stem cells to cardiac tissue. It has been shown that matrigel increases the retention of ESCs when injected after MI, and a combination of matrigel with ESCs improves cardiac function after MI.\textsuperscript{47} Because matrigel is a material secreted by living cells, it contains many growth factors that vary from batch to batch, and this could have a variable impact on growth and differentiation of stem cells. Furthermore, matrigel is derived from a mouse tumor and therefore is not appropriate for clinical translation.

**Collagen**

Collagen is a ubiquitous extracellular matrix protein that provides tensile strength to tissues. Turnover of collagen is an important factor in the formation of scar tissue in the infarct zone and in the remodeling of cardiac tissue in the remote zone after MI. Collagen also can be used as a substrate for three-dimensional in vitro cell culture and for in vivo tissue engineering applications. It is an inexpensive, nontoxic, nonimmunogenic, and biodegradable compound. Because of
its viscosity, collagen prevents redistribution of MSCs injected into the infarcted myocardium to remote organs.\textsuperscript{48,50} Collagen gels have been used as a supporting matrix in tissue-engineered constructs of EPCs.\textsuperscript{49} These constructs then have been implanted epicardially in rats after MI and have been shown to improve cardiac function by increasing vascular density.\textsuperscript{49} Using a percutaneous approach, collagen gels also have been used to deliver MSCs or viral particles into the pericardial space in large animals.\textsuperscript{59}

**Table 1. Biomaterials Used to Enhance Stem Cell Function in Preclinical Models of Myocardial Infarction**

<table>
<thead>
<tr>
<th>Biomaterial</th>
<th>Stem Cells</th>
<th>Factor</th>
<th>Animal</th>
<th>Functional Benefit</th>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrigel</td>
<td>ES cells</td>
<td>NA</td>
<td>Mouse</td>
<td>FS + 15.2%</td>
<td>NA</td>
<td>47</td>
</tr>
<tr>
<td>Collagen</td>
<td>MSCs</td>
<td>NA</td>
<td>Rat</td>
<td>NA</td>
<td>Retention</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>MSCs</td>
<td>NA</td>
<td>Rat</td>
<td>+ dP/dT + 1800 mm Hg/s</td>
<td>Retention</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>MSCs</td>
<td>NA</td>
<td>Rat</td>
<td>FS + 6%</td>
<td>NA</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>EPCs</td>
<td>SDF-1</td>
<td>Rat</td>
<td>EF + 28%</td>
<td>Angiogenesis</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>ESC-derived CM</td>
<td>NA</td>
<td>Rat</td>
<td>+ dP/dT + 800 mm Hg/s</td>
<td>Retention</td>
<td>50</td>
</tr>
<tr>
<td>Fibrin</td>
<td>MSCs</td>
<td>NA</td>
<td>Rat</td>
<td>EF + 24%</td>
<td>Retention</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>MSCs</td>
<td>NA</td>
<td>Pig</td>
<td>NA</td>
<td>Angiogenesis</td>
<td>52</td>
</tr>
<tr>
<td>Self-assembling peptides</td>
<td>MNCs</td>
<td>NA</td>
<td>Pig</td>
<td>EF + 24%</td>
<td>Retention</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>ES cells</td>
<td>FGF-10</td>
<td>Mouse</td>
<td>NA</td>
<td>Differentiation</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>CSCs</td>
<td>IGF-1</td>
<td>Rat</td>
<td>EF + 8.4%</td>
<td>Maturation</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>CSCs</td>
<td>NA</td>
<td>Mouse</td>
<td>FS + 9.7%</td>
<td>Angiogenesis</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>MSCs</td>
<td>RGD</td>
<td>Rat</td>
<td>EF + 24.4%</td>
<td>Retention</td>
<td>68</td>
</tr>
<tr>
<td>PLCL</td>
<td>MSCs</td>
<td>NA</td>
<td>Rat</td>
<td>EF + 23%</td>
<td>Infarct size</td>
<td>120</td>
</tr>
</tbody>
</table>

CSC indicates cardiac stem cell; dP/dT, rate of rise of left ventricular pressure; EF, ejection fraction; EPC, endothelial progenitor cell; ES cells, embryonic stem cells; FGF-10, fibroblast growth factor-10; FS, fractional shortening; IGF-1, insulin-like growth factor-1; MNC, mononuclear cell; MSC, mesenchymal stem cell; NA, not applicable; PLCL, polylactide-co-caprolactone; RGD, arginine–glycine–aspartic acid; SDF-1, stromal cell-derived factor-1.

**Fibrin**

Fibrin is a fibrous protein that plays an essential role in blood clotting. A fibrin matrix is formed within seconds when fibrinogen is mixed with thrombin, a method on which commercially available fibrin glue is based.\textsuperscript{60} A major advantage of fibrin as a biomaterial is its availability of a Food and Drug Administration-approved product. Similar to collagen, two main strategies have been pursued to deliver stem cells to the heart using fibrin: construction of a fibrin patch for epicardial application\textsuperscript{52} or the use of fibrin as an injectable biomatrix together with stem cells.\textsuperscript{50} A third strategy is epicardial application of fibrin glue immediately after and at the site of injection.\textsuperscript{61} When cells are injected through the epicardium during surgery, application of fibrin glue increases retention of injected stem cells.\textsuperscript{61} Fibrin also has been used to deliver proteins, plasmids, and viral vectors for tissue regeneration.

**Table 2. Properties of Selected Biomaterials for Tissue Regeneration**

<table>
<thead>
<tr>
<th>Biomaterial</th>
<th>Degradation Products</th>
<th>Degradation Time</th>
<th>Inflammatory Response/Toxicity</th>
<th>Cell Viability and Proliferation</th>
<th>Cell Differentiation Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrigel</td>
<td>Amino acids\textsuperscript{58}</td>
<td>Low N of neutrophils\textsuperscript{121}</td>
<td>↑ Retention\textsuperscript{47}</td>
<td>↑ Differentiation\textsuperscript{48}</td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td>Amino acids</td>
<td></td>
<td>↑ Retention\textsuperscript{48,50} ↑ Survival\textsuperscript{122}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrin</td>
<td>Amino acids</td>
<td></td>
<td>↑ Retention\textsuperscript{50}</td>
<td>↑ Vasculature\textsuperscript{123}</td>
<td></td>
</tr>
<tr>
<td>Alginate</td>
<td>Saccharides\textsuperscript{44}</td>
<td>4–6 wk\textsuperscript{44}</td>
<td>↑ Viability\textsuperscript{124}</td>
<td>↑ Attachment\textsuperscript{124}</td>
<td></td>
</tr>
<tr>
<td>Synthetic Biomaterials</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self-assembling peptides</td>
<td>Amino acids</td>
<td>2 wk\textsuperscript{67}</td>
<td>↑ Retention\textsuperscript{14}</td>
<td>↑ Differentiation with IGF-1\textsuperscript{174}</td>
<td></td>
</tr>
<tr>
<td>PGA</td>
<td>Glycolic acid\textsuperscript{125}</td>
<td>3 wk\textsuperscript{125}</td>
<td>Dose dependent toxicity\textsuperscript{126}</td>
<td>↑ attachment 127</td>
<td></td>
</tr>
<tr>
<td>PLLA</td>
<td>Lactic acid\textsuperscript{126}</td>
<td>Depending on MW\textsuperscript{128}</td>
<td>Dose dependent toxicity\textsuperscript{126}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLGA</td>
<td>Lactic acid,\textsuperscript{53} glycolic acid</td>
<td>&gt; 3 wk\textsuperscript{125}</td>
<td>Macrophages,\textsuperscript{46} foreign body\textsuperscript{46}</td>
<td>↑ Cell aggregation\textsuperscript{129}</td>
<td></td>
</tr>
<tr>
<td>PEG</td>
<td>Nondegradable\textsuperscript{46}</td>
<td>&gt; 13 wk\textsuperscript{46}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLCL</td>
<td>Lactic acid,\textsuperscript{120} caprolactone</td>
<td>2 wk\textsuperscript{131}</td>
<td>Dose dependent toxicity\textsuperscript{126}</td>
<td>↑ Attachment\textsuperscript{132}</td>
<td></td>
</tr>
<tr>
<td>PCL</td>
<td>Caprolactone\textsuperscript{131}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{1}IGF indicate insulin-like growth factor; MW, molecular weight; PCL, poly-e-caprolactone; PEG, polyethylene glycol; PGA, polyglycolic acid; PLCL, polylactide-co-e-caprolactone; PLGA, polyactic-coglycolic acid; PLLA, poly-L-lactic acid.
applications in many different tissues and provides sustained release for up to 2 weeks.\textsuperscript{60} When used to deliver MSCs to the heart after MI, fibrin increases retention of MSCs\textsuperscript{60} and increases angiogenesis.\textsuperscript{52}

**Decellularized Extracellular Matrix**

Decellularized porcine heart valves are now commonly used in clinical practice, but decellularized whole organs or tissue constructs are a relatively new research area. Complete hearts can be decellularized by perfusion with detergents and used as a scaffold for implantation of myocytes and endothelial cells.\textsuperscript{62} These decellularized constructs show contractile function, although limited.\textsuperscript{62} Ultimately, this strategy could lead to transplantation of in vitro engineered hearts using decellularized exogenous matrix and autologous stem cells. An alternative approach is lyophilization and solubilization of decellularized extracellular matrix,\textsuperscript{53,64} a method that results in a soluble and injectable biomaterial similar to native extracellular matrix. This myocardial matrix could be used to deliver stem cells and it is possible that it contains cues for cardiac lineage commitment.

**Polylactic-coglycolic Acid**

PLGA is a copolymer used in manufacturing of biodegradable sutures and has been approved by the Food and Drug Administration for drug delivery approaches.\textsuperscript{53} This polymer is degraded to nontoxic lactic and glycolic acid moieties, and the degradation rate can be modified by adjusting the ratio of lactic to glycolic acid.\textsuperscript{53} PLGA is used for the manufacturing of microparticles for drug delivery. Commonly used methods for PLGA microparticle formation are single-emulsion solvent evaporation or double-emulsion solvent evaporation. The single-emulsion method uses an emulsification of oil in water, whereas the double-emulsion process involves emulsification of water in oil in water in which small water droplets are incorporated into the organic phase during manufacturing.\textsuperscript{53} Oil-in-water emulsions allow for delivery of hydrophobic drugs,\textsuperscript{65} whereas water-in-oil-in-water emulsions allow for delivery of hydrophilic molecules, including DNA and proteins.\textsuperscript{53,65} However, polymerization of PLGA is a harsh chemical process that can lead to denaturation of proteins and that restricts seeding of cells after polymerization has been completed.\textsuperscript{66} Therefore, PLGA has been used primarily to generate tissue engineered scaffolds. A major advantage of PLGA scaffolds is that they can be used for many different applications; an example is printing of a pattern of 20-\(\mu\)m-wide fibronectin strips on PLGA sheets.\textsuperscript{67} This pattern stimulates a more elongated morphology of MSCs instead of their classical flattened growth and leads to myogenic differentiation.\textsuperscript{57} Thus, control of cell shape with biomaterials could be a novel method to guide differentiation of stem cells into myocytes.

Besides PLGA, other synthetic biomaterials like PEG\textsuperscript{46} or poly-N-isopropylacrylamide (PNIPAAm)\textsuperscript{63} have been shown to be injectable in the myocardium. However, delivery of stem cells in the myocardium has not been studied yet. Furthermore, many synthetic materials like poly-L-lactic acid (PLLA)\textsuperscript{41} have been used to culture stem cells or cardiomyocytes in vitro but have not been used in vivo yet.

**Self-assembling Peptides**

Self-assembling peptides are small peptides typically consisting of 8 to 16 amino acids in a sequence with alternating hydrophobic and hydrophilic residues.\textsuperscript{56} They are soluble in water and at low pH but form a stable hydrogel of nanofibers within minutes at physiological pH and salt concentrations.\textsuperscript{56} This rapid responsiveness to physiological conditions makes them an attractive biomaterial for injection into tissues. Other major advantages of self-assembling peptides are that they can be manufactured easily by standard chemical peptide synthesis and that they are degraded into natural amino acids over a time period of weeks.\textsuperscript{57} Self-assembling peptide nanofibers can be used for three-dimensional cell culture and are nontoxic.\textsuperscript{56}

Self-assembling peptide nanofibers improve retention of bone marrow mononuclear cells 4 weeks after transplantation when injected intramyocardially in pigs with MI.\textsuperscript{54} Furthermore, peptide nanofibers also stimulate differentiation of bone marrow cells into vascular endothelial and smooth muscle cells and can improve both systolic and diastolic function.\textsuperscript{54} As described, self-assembling peptides can be engineered for protein delivery\textsuperscript{56} and adhesion signals can be incorporated into the peptide sequence. For instance, it has been reported that incorporation of the common adhesion motif RGD (arginine–glycine–aspartic acid) in the sequence of self-assembling peptides improves survival of MSCs after injection in infarcted rat hearts.\textsuperscript{68} This study also suggested that the RGD motif increased differentiation of MSCs in cardiomyocytes.\textsuperscript{68} Furthermore, incorporation of cell-specific adhesion signals in hydrogels also can direct differentiation into certain lineages. For example, incorporation of the neurite-promoting laminin epitope peptide sequence of isoleucine-lysine-valine-alanine-valine in a hydrogel of self-assembling peptide nanofibers guides differentiation of neural progenitors selectively toward neurons instead of astrocytes.\textsuperscript{69,70}

**Controlling the Biochemical Microenvironment of Stem Cells**

**Protein Delivery**

Biomaterials can be used to deliver small and large molecules, including proteins, to tissues (Figure).\textsuperscript{50} Many proteins diffuse rapidly from the injection site or are degraded rapidly by proteases present in inflammatory environments like infarcted tissue. Furthermore, control of temporal and spatial distribution of delivered proteins may be critical for effective responses by target cells.\textsuperscript{10} For instance, spatial distribution is important for proteins with chemotactic properties; these chemokines need to be delivered in such a way that a chemotactic gradient can be formed.\textsuperscript{56} When a gradient of a chemokine is too steep, cells can be repelled instead of attracted, which is a phenomenon called fugetaxis.\textsuperscript{71} Temporal distribution is particularly important for differentiation factors; expression of a specific differentiation factor at the right time and place is crucial for normal cardiogenesis.\textsuperscript{1,72}

During embryonic development, stem cells are highly sensitive to a fine-tuned temporal orchestration of Wnt, fibroblast growth factor (FGF), BMP, Hedgehog, and Notch signaling...
pathways. A given signal might promote differentiation at a certain stage during development but might inhibit differentiation at another stage. Considering the number of proteins participating and the delicate temporal and spatial orchestration of these signals, controlled delivery of all signaling molecules that promote cardiac differentiation with a single injection of a biomaterial is a daunting challenge. Identification of the key signaling factors will be crucial to define a limited number of proteins that can be engineered for delivery approaches with stem cells.

Different strategies have been developed for local delivery of proteins and to prevent rapid diffusion from the site of injection. Timed release systems generally contain an aqueous phase with soluble protein embedded in a degradable phase, which is most commonly an organic polymer. An example is PLGA, in which small aqueous particles containing the protein are released over time by hydrolysis of the surrounding PLGA solid phase. Degradation properties and thus protein release kinetics can be altered by changing the ratio of lactic to glycolic residues. A drawback of this technology is that a number of proteins are degraded by the chemical process during polymerization. An alternative approach is delivery of proteins by tethering them to hydrogels. For example, hydrogels consisting of self-assembling peptide nanofibers can be used for delivery of proteins like insulin-like growth factor-1 or stromal cell-derived factor-1 (SDF-1). Biotinylated insulin-like growth factor-1 has been tethered to biotinylated self-assembling peptides using streptavidin as a linker, which allows delivery of insulin-like growth factor-1 to the heart for more than 1 month. It has been shown that delivery of insulin-like growth factor-1 in this manner, together with cardiac stem cells, leads to a greater improvement of cardiac function after MI compared to cells alone and to a development of more mature myocytes. Instead of using a biotin–streptavidin linker, another approach is to make fusion proteins of the protein to be delivered and the 16-amino acid sequence of self-assembling peptides. For instance, a recombinant fusion protein of SDF-1 and the sequence of self-assembling peptides has been stably incorporated in a hydrogel of self-assembling peptide nanofibers. This approach led to increased homing of EPCs to the heart, increased angiogenesis, and improved cardiac function. Another system that has been designed for delivery of paracrine factors uses heparin-presenting nanofibers. This system can be used to deliver heparin-binding proteins like vascular endothelial growth factor or basic fibroblast growth factor.

**Gene Delivery**

Local delivery of plasmids or viruses in myocardium could potentially enhance stem cell therapy. Gene therapy currently lacks precise quantitative control of expression, but some applications may tolerate broad ranges of overexpression. Biomaterials can sustain delivery, protect plasmid DNA, and increase transfection efficiency. Different biomaterials have been used to deliver plasmids and viral vectors, including fibrin, water-soluble lipopolymers, or RGD-conjugated bioreducible polymers. Because transplanted cells can be transfected more efficiently in vitro before injection, the combination of DNA delivery by biomaterials with stem cell delivery has little promise; a potential application could be delivery of proangiogenic plasmids with stem cells to increase survival and successful engraftment.

**MicroRNA Delivery**

MicroRNAs (miRNAs) are emerging as critical modulators of cardiovascular development and disease. MiRNAs are single-stranded RNAs of 22-nucleotides that inhibit the expression of specific mRNA targets through base pairing between the miRNA and sequences located in the untranslated regions of the target mRNAs. It is estimated that the human genome encodes up to 1000 miRNAs. An important role of miRNAs seems to be the “fine-tuning” of gene expression during tissue development and tissue homeostasis. Targeting miRNAs raises exciting new possibilities to enhance stem cell function. For instance, formation of new blood vessels is regulated by miRNAs, both by proangiogenic and antiangiogenic miRNAs. Delivery of antagonists of antiangiogenic miRNAs could be a novel angiogenic therapy
in the cardiovascular system. An example of a highly expressed antiangiogenic miRNA in endothelial cells is miR-92a. Administration of an antagonim against miR-92a increased angiogenesis in a model of hind limb ischemia. This antagonim potentially could be used to enhance stem cell function after MI by decreasing tissue ischemia.

For experimental RNA delivery, two different approaches are currently pursued. One approach involves delivery of synthetically produced oligonucleotides into the desired cells by way of targeting agents, chemical modifications, or direct administration in the organ. Another approach is delivery of genetic material into the nucleus using viral vectors to overexpress small RNA precursors using the cell’s gene transcription machinery. Viral vectors have some advantages over oligonucleotides: stronger levels of “knockdown” of gene expression, the treatment needs to be performed only once because levels are maintained by transcription, and the dose remains constant even in dividing cells. As with gene therapy approaches, however, expression levels are difficult to control with viral vectors. Therefore, most therapeutic programs targeting miRNAs are working on synthetic oligonucleotides, which may have more predictable pharmacokinetics. However, getting synthetic RNAs into the cytoplasm is challenging because of their size and charge. Furthermore, unmodified RNA is cleaved rapidly by nucleases in the plasma, which raises the need for modifications.

Similarly to protein therapy and gene therapy, the pharmacokinetics of miRNA-based therapies remain a hurdle, because targeting of expressed miRNAs raises possibilities for effects in other organs. This might necessitate the use of local delivery methods developed for protein or gene delivery or the use of targeted delivery strategies. A recent example of a biomaterial used for targeted delivery of small RNAs is the use of nanoparticles consisting of a cyclodextrin-based polymer, PEG as a stabilizer, and a ligand to a transferrin receptor found on the surface of the cancer cells. This strategy is currently used in a clinical trial targeting melanoma cells. In another study, PCL nanofibers have been used to create a sustained release vehicle delivering small RNAs for at least 28 days.

**Cell-Conjugated Nanoparticles**

A recent study described a promising new strategy to deliver drugs or proteins together with transplanted cells. Synthetic drug carrier nanoparticles were coupled to the surface of T-lymphocytes using free thiol on cell surface proteins. Liposome-like synthetic nanoparticles of 100 to 300 nm in diameter with a drug-loaded core and phospholipid surface layer were covalently linked to free surface thiols using thiol-reactive maleimide head groups incorporated in the lipid bilayer of the nanoparticles. Particle conjugation was achieved by a two-step process in which donor cells were first incubated with nanoparticles to permit maleimide-thiol coupling, followed by PEGylation to quench residual reactive groups of the particles. Conjugation of these drug-loaded nanoparticles to the surface of T-cells did not cause toxicity to the cells, did not interfere with cell function, and led to sustained drug release in proximity of the donor cells. Although this approach has not yet been used in the heart, this novel strategy could be an efficient tool for delivery of small molecule drugs, siRNAs, and proteins together with transplanted cells. The strategy has several advantages, such as active compounds are released over time and in close proximity of the transplanted cells, high local concentrations of the compounds, and less off-site effects.

**Controlling the Physical Microenvironment of Stem Cells**

Most research on stem cell differentiation has been dedicated to identification and modification of soluble biochemical factors in the microenvironment. These biochemical factors include proteins that bind to cell surface receptors and act as growth and differentiation signals. However, the physical microenvironment of stem cells also plays an important role for differentiation, growth, and integration of stem cells. Stem cells, including cardiac stem cells, have been shown to cluster in stem cell niches at specific anatomic locations and with a specific physical microenvironment. Surprisingly little is known about which physical characteristics of the extracellular matrix in these niches are important for stem cell proliferation and differentiation. The characteristics of the physical microenvironment that have been shown to modulate stem cell fate in different tissues include extracellular matrix stiffness, interactions with nanoscale features, cyclic stretching, and cell shape (Figure). Importantly, most studies examining the effects of the physical microenvironment on stem cell fate have been performed on adult stem cells such as MSCs, which are not pluripotent and have less cardiomyogenic potential compared to ESCs.

**Extracellular Matrix Stiffness**

Extracellular matrix stiffness and elasticity can be important factors determining stem cell fate. For example, the physical effects of matrix elasticity are important for expansion and proliferation of hematopoietic stem cells. Culturing primitive hematopoietic cells on tropoelastin, which is an elastic biomaterial, leads to a two-fold to three-fold expansion of undifferentiated cells in vitro compared to culture on non-elastic biomaterials. Furthermore, differentiation of MSCs into different lineages is sensitive to the elasticity or stiffness of the extracellular matrix. Soft matrices mimicking brain tissue are neurogenic, stiffer matrices mimicking muscle tissue are myogenic, and rigid matrices mimicking bone are osteogenic. When MSCs are cultured in vitro, reprogramming is possible with addition of soluble factors during the first week of culture, but after several weeks MSCs commit to the lineage specified by matrix elasticity and not by soluble factors present. MSCs sense matrix elasticity by pulling against the matrix with nonmuscle myosin II isoforms that are part of the cellular motors. This information affects gene expression by signaling through cellular mechanotransducers. For the purpose of cardiac regeneration after MI, biomaterials could be designed with the optimal stiffness for a particular stem cell, for instance, by varying the number of cross-links between polymers.

**Nanotopography**

Cells respond to topographical properties of the extracellular matrix on a nanometer scale by adhesion molecules on their
cell surface. Nanotopography can be important in the design of new tissue engineering materials because nanoscale dimensions of the surface are capable of modulating cellular responses. Nanotopography can influence cell morphology, adhesion, motility, proliferation, protein expression, and gene regulation. Nanotopography also contains important cues for the differentiation of stem cells. It has been shown that both the cell bodies and nuclei of MSCs become elongated when cultured on nanopatterns with gratings of 350 nm width. Muscular and neuronal gene markers were significantly upregulated in MSCs cultured on these nanopatterns. These results indicate that differences in molecular conformation, surface topography or roughness, fiber diameter, or other nanoscopic modifications of biomaterials can guide differentiation of stem cells into myocytes. Furthermore, electric properties like action potential, conduction velocity, and the expression of a cell–cell coupling protein are sensitive to differences in the nanoscale features of the surrounding extracellular matrix. For example, tissue constructs of neonatal rat ventricular myocytes on a hydrogel guided by nanoscale mechanical cues display anisotropic action potential propagation and contractility characteristic of the native tissue. Some features like surface topography or roughness are more easily modified in solid compared to liquid biomaterials, whereas other features like fiber diameter are more easily modified in hydrogels.

Cell shape can be modified by printing patterns of adhesion signals like fibronectin on biomaterials in the micrometer range. Printing of a pattern of 20-μm-wide fibronectin strips on PLGA sheets stimulates a more elongated adhesion of MSCs and leads to myogenic differentiation. Shape-dependent control of lineage commitment has been shown to be mediated by RhoA activity via ROCK-mediated cytoskeletal tension. Gene expression in cells also can be modified by cell shape distortion with micropatterns on biomaterials. Manipulating cellular shape may be a promising method to guide differentiation of stem cells into myocytes.

Cyclic Strain

Cells in the myocardium and arteries are subjected to constant cyclic strain, and cyclic stretch might guide differentiation of stem cells into myocytes or smooth muscle cells. MSCs commit to the smooth muscle lineage when they are subjected to cyclic strain in one direction (uniaxial), but not when they are subjected to strain in two directions (equiaxial). Uniaxial strain better-mimics circumferential strain experienced by smooth muscle cells in arteries. Biomaterials could be designed in such a manner that they are stiffer in one direction, allowing control of the direction of strain transmitted to the transplanted cells and thus guiding differentiation. ESCs are also responsive to cyclic stress; cyclic stress transduced on ESCs via focally attached magnetic beads induces spreading and differentiation in mouse ESCs, but not in more differentiated cells. This response of ESCs is dictated by the cell softness, suggesting that less differentiated (softer) ESCs are more responsive to focal cyclic stress. The finding that softness of mouse ESCs makes them sensitive to local cyclic stress suggests that small local forces might play important roles in embryogenesis and in stem cell differentiation in adult animals.

Biomaterials to Enhance Stem Cell Engraftment and Angiogenesis in the Heart

Most stem cells are small cells, and when they are injected in the myocardium a substantial proportion leaves the heart within minutes. Because many injectable biomaterials increase viscosity of the injected cell suspension, they can also increase retention of cells. This is an easy but effective way by which biomaterials can increase efficacy of stem cell transplantation. Some biomaterials, like self-assembling peptides or matrigel, are liquid in solution but rapidly form a hydrogel when injected in physiological salt concentrations or temperatures. This adaptive property increases retention of injected cells without dramatically increasing viscosity of the injectate.

Long-term survival of stem cells is another important goal of stem cell therapy but is more challenging to improve than early cell retention. Infarcted myocardium is an ischemic and inflammatory environment hostile to most cells. Biomaterials with anti-inflammatory properties can enhance survival of cells. Some biomaterials enhance angiogenesis and decrease ischemia in the time frame of a few weeks. Because formation of functional vessels tends to be a slow process, successful stimulation of angiogenesis might be a viable strategy to increase stem cell engraftment. In the adult myocardium, endothelial cells outnumber myocytes by 3:1 and are in close proximity of every myocyte. Endothelial cells not only form blood vessels and provide oxygen and nutrients to the myocytes but also exert trophic and inotropic effects on myocytes. Despite the fact that endothelial cells are a necessary part of myocardium, clinical trials with angiogenic proteins like vascular endothelial growth factor and FGF-2 have not resulted in an angiogenic therapy that improves cardiac function. Biomaterials have the potential to promote angiogenesis and to support other types of transplanted cells.

Ideally, transplanted stem or progenitor cells will form new and functional myocytes. Thus far, in at least some reports using bone marrow stem cells, the primary mechanism might be an increase in capillary density. An explanation for this improved cardiac function in these trials could be paracrine signaling from newly formed capillaries. When the transplanted stem cell type mainly differentiates into endothelial cells, additional angiogenic stimuli might be less useful. However, when the transplanted cell type mainly differentiates into myocytes, biomaterials could play a supportive role by delivering angiogenic factors and stimulating angiogenesis. For instance, self-assembling peptide nanofibers have been used to deliver SDF-1, a chemotactic protein for EPCs. Delivery of SDF-1 not only increased capillary density but also improved cardiac function after MI. This technology could be delivered to infarcted myocardium together with stem cells with myogenic potential, resulting in new myocyte formation supported by angiogenesis provided by self-assembling peptide nanofibers.
Enhancing Differentiation
During development and in the adult organism, the natural extracellular matrix contains cues for self-renewal and differentiation of stem cells. Only recently, however, biotechnological applications have been developed using these cues for cell proliferation and differentiation. For example, a system has been described for culturing iPSCs on recombinant human laminin-511, a component of the natural niche of ESCs. When small clumps of human ESCs or iPSCs are plated on laminin-511, cells spread out in a monolayer and maintain cellular homogeneity. This homogeneous monolayer of iPSCs provides controllable conditions for study and design of differentiation methods.

During embryonic development, transitioning from proliferative progenitors to differentiated cardiomyocytes requires both downregulation of proliferation signals and upregulation of differentiation signals. Delivery of differentiation factors together with ESCs or iPSCs is a promising but challenging approach because the specific temporal and spatial window in which these cells are responsive to various differentiation factors. Chan et al recently showed that FGF-10 doubles the amount of cardiomyocytes formed in vitro. Furthermore, when FGF-10 was delivered with self-assembling peptide nanofibers together with ESCs in the infarct zone in mice, the number of troponin-positive newly formed cells doubled compared to the group without FGF-10. Although these results are promising, this research area is in its infancy and more knowledge on differentiation factors is needed before a significant number of mature myocytes are formed with this approach.

Enhancing Electric and Mechanical Integration in the Heart
During development, rounded cardiomyocyte precursors become elongated and align themselves end-to-end in a pattern of parallel organized bundles. Electric conduction parallel to the bundles of myocytes is three-times faster than conduction perpendicular to the bundles. MI followed by scar formation disrupts normal conduction with increased arrhythmogenicity, which can be further aggravated by transplanting stem cells that do not differentiate into mature myocytes or into myocytes that do not fully integrate with surviving myocardium. In most preclinical and clinical studies, stem cells have been injected either intracoronary or intramyocardially. The expectation of these studies is that stem cells will differentiate into cardiomyocytes and would then align themselves with surviving myocardium, with full electromechanical integration. As our knowledge on elongation and alignment processes during development increases, new strategies for driving this integration may exploit biomaterials. A crucial part of successful tissue engineering approaches is graft/host integration of transplanted constructs. These constructs not only need to be connected electrically and mechanically to surviving myocardium but also need a connection to the vasculature. Therefore, proangiogenic scaffolds are promising materials for tissue engineering approaches.

Development of tissue engineering strategies that promote alignment of newly formed myocytes with existing myocytes could decrease the arrhythmogenicity of stem cell transplantation and at the same time increase mechanical coupling. As described, nanotopography of biomaterials can influence cell morphology and electromechanical integration. Another strategy to increase adhesion is incorporation of the common integrin adhesion peptide sequence RGD. For instance, RGD-labeled PEG and poly-lactic acid copolymers increase endothelial cell spreading in the polymer. Modification of polyethylene urethane (PEU) surfaces with RGD moieties increased endothelial cell adhesion and viability when compared with PEU. However, RGD sequences are not cell-specific and can enhance fibroblast adhesion as well. Careful spacing and orientation of RGD ligands are also important for control of cell spreading and proliferation; it has been shown that variations in nanoscale organization of RGD in alginate gel altered the adhesion of preosteoblasts.

Recently, a new concept of interaction between adult cardiomyocytes and progenitor cells has been proposed, microRNA signaling. Mircrorna signaling entails diffusion of miRNA molecules through gap junctions between adjacent cells. The first described example is diffusion of miR-499 from adult cardiomyocytes, which have high expression levels of miR-499, to neighboring cells. Theoretically, microRNA signaling could lead to coordination of gene expression patterns between neighboring cells and a better electromechanical integration.

Cell Sheets and Engineered Cardiac Tissue Constructs
An alternative approach to injectable biomaterials is the epicardial implantation of in vitro engineered cardiac tissue constructs. Patches are constructed in vitro by combining an artificial extracellular matrix with myocytes derived from stem cells. A major advantage of this approach is that cells are evenly distributed throughout the matrix and differentiation can occur in a controlled in vitro environment. Major disadvantages are the need for surgical implantation and the need for connection to the host vascular system for survival of the graft. Tissue-engineered constructs have been made with various biomaterials, including matrigel and collagen. We refer readers interested in this exciting research area to more detailed reviews.

Similarly to tissue-engineered patches, cells sheets of one or more layers of in vitro-grown cells can be implanted epicardially after MI. The major difference between both approaches is that cell sheets have an extracellular matrix produced by the cells themselves. Cell sheets of MSCs improve cardiac function after MI by reversing cardiac wall thinning and improve animal survival after MI. In these transplanted MSCs, cell growth was observed, as was differentiation of a number of cells into cardiomyocytes and blood vessels. The approach of cell sheet transplantation works only for cells, like MSCs, that produce a strong collagenous matrix. More immature stem cells with higher differentiation potential do not produce enough extracellular matrix material for successful epicardial implantation.

Future Directions
Biomaterials can enhance stem function by many different mechanisms. In the near future, our knowledge of biomaterial
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