Injectable shear-thinning hydrogels used to deliver endothelial progenitor cells, enhance cell engraftment, and improve ischemic myocardium

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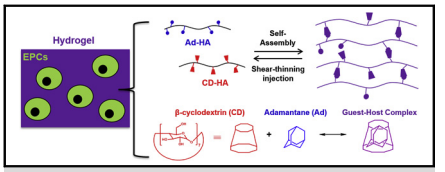
ABSTRACT

Objectives: The clinical translation of cell-based therapies for ischemic heart disease has been limited because of low cell retention (<1%) within, and poor targeting to, ischemic myocardium. To address these issues, we developed an injectable hyaluronic acid (HA) shear-thinning hydrogel (STG) and endothelial progenitor cell (EPC) construct (STG-EPC). The STG assembles as a result of interactions of adamantine- and β-cyclodextrin–modified HA. It is shear-thinning to permit delivery via a syringe, and self-heals upon injection within the ischemic myocardium. This directed therapy to the ischemic myocardial border zone enables direct cell delivery to address adverse remodeling after myocardial infarction. We hypothesize that this system will enhance vasculogenesis to improve myocardial stabilization in the context of a clinically translatable therapy.

Methods: Endothelial progenitor cells (DiLDL$^+$ VEGFR2$^+$ CD34$^+$) were harvested from adult male rats, cultured, and suspended in the STG. In vitro viability was quantified using a live-dead stain of EPCs. The STG-EPC constructs were injected at the border zone of ischemic rat myocardium after acute myocardial infarction (left anterior descending coronary artery ligation). The migration of the enhanced green fluorescent proteins from the construct to ischemic myocardium was analyzed using fluorescent microscopy. Vasculogenesis, myocardial remodeling, and hemodynamic function were analyzed in 4 groups: control (phosphate buffered saline injection); intramyocardial injection of EPCs alone; injection of the STG alone; and treatment with the STG-EPC construct. Hemodynamics and ventricular geometry were quantified using echocardiography and Doppler flow analysis.

Results: Endothelial progenitor cells demonstrated viability within the STG. A marked increase in EPC engraftment was observed 1-week postinjection within the treated myocardium with gel delivery, compared with EPC injection alone (17.2 $\pm$ 0.8 cells per high power field (HPF) vs 3.5 cells $\pm$ 1.3 cells per HPF, $P = .0002$). A statistically significant increase in vasculogenesis was noted with the STG-EPC construct (15.3 $\pm$ 5.8 vessels per HPF), compared with the control ($P < .0001$), EPC ($P < .0001$), and STG ($P < .0001$) groups. Statistically significant improvements in ventricular function, scar fraction, and geometry were noted after STG-EPC treatment compared with the control.



Overview of hydrogel composition; representation of STG-containing EPCs.

Central Message

A shear-thinning injectable hydrogel provides improved delivery and retention of EPCs for ischemic myocardium.

Perspective

A shear-thinning injectable hydrogel provides improved delivery and enhanced retention of EPCs for ischemic myocardium. We have investigated the mechanisms of angiogenesis associated with this therapy and the reduction in scar formation. Our data demonstrate a very significant angiogenic response and associated retention of myocardial biomechanical functioning with this therapy.

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Abbreviations and Acronyms

Ad-HA = adamantane-modified HA
CD = β-cyclodextrin
CD-HA = β-cyclodextrin–modified HA
DAPI = 4′,6-diamidino-2-phenylindole
EPC = endothelial progenitor cell
HA = hyaluronic acid
HPF = high power field
LAD = left anterior descending
STG = shear-thinning gel
STG-EPC = STG-EPC construct
TBA = tetrabutylammonium hydroxide

Conclusions: A novel injectable shear-thinning HA hydrogel seeded with EPCs enhanced cell retention and vasculogenesis after delivery to ischemic myocardium. This therapy limited adverse myocardial remodeling while preserving contractility. (J Thorac Cardiovasc Surg 2015;150:1268-77)

Ischemic heart failure is a major global health concern. Within the United States, a myocardial infarction occurs every 34 seconds.[1] Current therapies for heart failure, including both medical and surgical treatments, are palliative only and result in slight improvements in length and quality of life. Overall, a reduction in mortality from acute myocardial infarctions has occurred, but treatment strategies for those patients who develop ventricular dysfunction are still lacking. As a result, the number of patients who progress to heart failure has not improved, and both the incidence and prevalence of heart failure have increased.[2-5]

Cell-based therapies for treating ischemic myocardium are emerging as a treatment option for this ever-growing public health challenge. To date, numerous cell types have been used in experimental models, including fetal myocytes, embryonic stem cell–derived myocytes, skeletal myoblasts, endothelial progenitor cells (EPCs), and mesenchymal stem cells.[6-9] Our lab and others have demonstrated that EPCs are easily expanded in vitro and have the ability to engraft and improve cardiac function and attenuate ventricular remodeling when delivered after infarction.[10-14]

Therapy with EPCs involves postnatal vasculogenesis that can be used to revascularize ischemic myocardium; they are pluripotent, bone marrow–derived stem cells with the ability to differentiate into de novo vasculature.[15] Initial work was focused on cytokine therapies to locally recruit EPCs to the ischemic myocardium, because EPCs are known to promote vasculogenesis.[12] Unfortunately, translatable outcomes were marginal at best. Further studies were focused on directly delivering syngeneic EPCs locally to the damaged and hibernating myocardium.[6,16,17] Both small and large animal studies showed significant vasculogenic responses in ischemic myocardium. However, the benefit has not been translatable to a sustainable clinical model.

One of the major challenges for the therapeutic application of EPCs has been optimizing cell delivery, dispersal, and engraftment. After direct cell injection into the myocardium, <1% of the cells are retained, as shown by cell-tracking studies.[18-21] Numerous factors contribute to the poor cell retention, including exposure of the cells to ischemia and inflammation, mechanical washout of cells from the beating myocardium and coronary vasculature, and leaking of the cell suspension from the targeted injection site.[22-24] Overall, most cell death occurs within the first few days of cell delivery. Consequently, a delivery and stabilization mechanism is likely to promote longer cell retention and engraftment.[24]

One possible approach involves the use of biomaterials to provide a surrogate extracellular matrix to localize cells at the delivery site. Hydrogels in particular may provide an environment for protection from the insults of inflammation and ischemia to reduce immediate cell death upon exposure.[12,25] Hydrogels are biocompatible materials that have gained increasing interest, owing to their ability to enhance cell and biomolecule delivery.[26-30]

We developed a shear-thinning injectable hydrogel to deliver cells to ischemic myocardium. The hydrogel was designed to flow through a syringe with application of shear force and then reassemble at the injection site.[30] Tuning the properties of the hydrogel to the targeted delivery site reduces mechanical damage[31,32] and increases ability to control for cell release through gel degradation.[31]

Our shear-thinning gel (STG) is based on hyaluronic acid (HA) and assembles by the guest-host interactions of adamantane-modified HA (guest-macromer; Ad-HA) and β-cyclodextrin–modified HA (host-macromer; CD-HA).[30] As a biomaterial, HA is particularly attractive, because it is found abundantly in tissue and used in biomedical applications.[31] Furthermore, derivatives of adamantane are used in pharmaceuticals, and cyclodextrans are recognized by the US Food and Drug Administration as safe and approved for use in pharmaceuticals, cosmetic products, and food products.[30] We have chosen to use EPCs for this study because they are readily available, highly vasculogenic, and have demonstrated clinical potential. We hypothesize that the construct of the STG with incorporation of EPCs will allow for higher cellular retention rates within the ischemic myocardium, with subsequent robust vasculogenesis and minimization of adverse ventricular remodeling.

METHODS

Animal Care and Biosafety

Male adult Wistar rats (250-300 g) were obtained from Charles River Laboratories, Inc (Boston, Mass). Food and water were provided ad libitum. This investigation adheres to the National Institutes of Health guidelines on animal care and use, conforms to institutional ethical review, and has been approved

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