Restoration of left ventricular geometry and improvement of left ventricular function in a rodent model of chronic ischemic cardiomyopathy

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Objectives: Various approaches to myocardial reconstruction have been developed for the treatment of congestive heart failure resulting from ischemic cardiomyopathy.

Methods: In this study we determined whether in situ application of polymers could reshape left ventricular geometry in a chronic rodent model of ischemic cardiomyopathy.

Results: We demonstrate that alginate and fibrin can augment left ventricular wall thickness, resulting in reconstruction of left ventricular geometry and improvement of cardiac function. Echocardiographic results at 5 weeks after injection of alginate demonstrated persistent improvement of left ventricular fractional shortening and prevention of a continued enlargement of left ventricular dimensions, whereas fibrin glue demonstrated no progression of left ventricular negative remodeling. There was increased arteriogenesis in both the alginate and fibrin glue groups compared with that seen in the phosphate-buffered saline control group. Infarct size was significantly reduced in the fibrin group ($P < .05$), and there was a trend toward a smaller myocardial infarction in the alginate group.

Conclusion: Intramyocardially injected polymers can be used to reshape the aneurysmal left ventricle and might therefore be an approach for myocardial reconstruction and a potential option in treating chronic heart failure in human subjects.

Heart failure is a major health problem in the world. Currently, therapeutic approaches in treating patients with chronic heart failure include pharmacologic therapies, mechanical devices, and surgical intervention,[1,2] whereas heart transplantation remains the only viable solution for end-stage congestive heart failure.[3]

Cardiac tissue engineering aims to repair damaged myocardium by combing cell biology, material science, and engineering principles.[4,5] Myocardial infarction (MI) results in left ventricular (LV) dilation, wall thinning, fibrosis, and reduced cardiac performance. One major concept of tissue engineering is to take into account the effect of the extracellular matrix (ECM). ECM scaffolds not only provide mechanical support for tissue but also regulate cell function.[6] Engineered cardiac constructs composed of scaffold materials alone or in combination with cells or growth factors have been widely investigated.[4] A range of materials from synthetic materials, such as polyglycolic acid, polylactic acid, or polyethylene glycol, to biologic materials, such as gelatin, collagen, or Matrigel (BD Biosciences, Franklin Lakes, NJ), have been used for myocardial repair.[4] Recently, polymers have been shown to be effective as an in situ tissue-engineering approach for preserving cardiac function after an acute MI.[4] Compared with a bioengineered cardiac graft, the injectable scaffolds remain in liquid form until after injection. After solidification in vivo, the engineered biopolymer matrices have the potential to provide mechanical support and promote cell migration and proliferation and angiogenesis.[6-9]

The use of tissue-engineering techniques for myocardial repair has focused on tissue repair after an acute MI. In this study we investigated and compared the therapeutic effects of fibrin and alginate in a rodent model of chronic ischemic cardiomyopathy. We demonstrated that fibrin and alginate alone could reconstruct a LV aneurysm, improve cardiac function, reduce infarct scar percentage, and stimulate neovascularization. All these findings underline the potential of injectable biopolymers in cardiac tissue engineering for myocardial repair.

MATERIALS AND METHODS

Biopolymers

Fibrin glue (CROSSEAL Fibrin Sealant; Omrix Biopharmaceuticals, Inc, New York, NY) consists of 2 components: biologic active components and thrombin. Biologic active components consist mainly of human fibrinogen, and thrombin is a highly specific protease that transforms the

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Abbreviations and Acronyms

ECM = extracellular matrix
LV = left ventricular
LVID = left ventricular internal dimension
MI = myocardial infarction
MRI = magnetic resonance imaging
PBS = phosphate-buffered saline
RGD = arginine-glycine-asparagine

fibrinogen into fibrin. Alginate solution (1.5%) was made from dissolving a high mannuronic acid (M units) alginate (ProNova LVM; FMC BioPolymer, Haugesund, Norway) in 0.9% NaCl. Alginate gel formation was based on the addition of the cross-linker solution, 102 mmol/L $CaCl_2$.[10]

Rat Chronic MI Model

The animal protocol was approved by the Committee for Animal Research of the University of California San Francisco and was performed in accordance with the recommendations of the American Association for Accreditation of Laboratory Animal Care. This ischemia–reperfusion model has been described extensively in our previous studies.[9,11] Female Sprague–Dawley rats (225–250 g) underwent 20 minutes of left anterior descending artery occlusion, followed by reperfusion. The chest was then closed, and the animal was allowed to recover for 5 weeks to allow for the development of a LV aneurysm.[9]

Injection Operations

Sprague–Dawley rats underwent left coronary artery occlusion for 20 minutes followed by reperfusion to test the hypothesis that a fibrin or alginate scaffold thickens the thinned wall of the aneurysmal left ventricle and reshapes LV geometry. Five weeks after infarction, at which time the remodeling process is largely complete, injections of either the control (0.5% bovine serum albumin in phosphate-buffered saline [PBS], n = 10), fibrin (n = 10), or alginate (n = 10) were made directly into the infarcted myocardium. Biopolymers were delivered as 2 components with a Duploject applicator (Baxter, Deerfield, Ill), which holds the 2 components fibrin and thrombin for the fibrin group and sodium alginate and calcium chloride for the alginate group in separate syringes and provides simultaneous mixing and delivery. All injections were made through 27-gauge needles into the infarcted area of the left ventricle. The infarct area was identified by a darker region of the LV wall with reduced contractility, mostly within the anterior wall. Three animals from each group (n = 3) were killed 24 hours after injection to examine the location and structural effect of the polymer injections compared with control results. Animals were perfused with 3 mol/L KCl before death to arrest the hearts in the diastolic phase of contraction.

To test the effects of in situ application of biopolymers in a chronic aneurysmal MI, the remainder of the animals (PBS, n = 7; fibrin, n = 7; and alginate, n = 7) were allowed to recover and survived for an additional 5 weeks after the injection (10-week-old MI) instead of dying at 24 hours after injection.

Echocardiography

Transthoracic echocardiographic analysis was performed on all animals after achievement of anesthesia with isoflurane (2 L/min) 5 weeks after MI as a baseline echocardiogram. Follow-up echocardiograms were obtained 2 days and 5 weeks after injection (10 weeks after MI). The methodology of echocardiography used has been previously described and has demonstrated reproducibility and accuracy in a rat MI model.[9,12] Fractional shortening (FS) as a measure of systolic function was calculated as follows:

$$FS\ (\%) = [(LVIDd–LVIDs)/LVIDd]\times 100\%,$$

where *LVID* represents the LV internal dimension, *d* represents diastole, and *s* represents systole. An echocardiographer blinded to the treatment group acquired the images and performed the data analysis.

Histology

After the echocardiogram at 10 weeks after MI, the rats were killed with a pentobarbital overdose (200 mg/kg). The hearts were rapidly excised and fresh frozen in O.C.T. freezing medium (Sakura Finetek, Torrance, Calif) and were then sectioned into 10-μm slices. Ten representative slides were stained with hematoxylin and eosin and Masson's trichrome stain (Accustain; Sigma, St Louis, Mo). Morphologic assessment of infarct size and LV wall thickness was quantified based on planimetry on Masson's trichrome–stained slides with SPOT 4.0.5 software (Diagnostic Instruments, Sterling Heights, Mich), as previously described.[13] The infarcted area and left ventricle were traced. The infarct size was determined by the scar area divided by the LV area and was reported as a percentage of the total left ventricle. The segmental distribution of wall thickness was determined by measuring the anterior, lateral, posterior, and septal walls of each individual slide, taking the average of 10 slides for each heart.

Immunohistochemistry and Immunofluorescence

Five slides equally distributed through the infarct area were selected to examine the extent of the inflammatory response. The slides were fixed in acetone for 15 minutes and incubated in avidin, biotin, Sniper, and Eraser blocking solutions (Biocare Medical, Concord, Calif). The slides were stained with mouse anti-CD68 monocyte/macrophage monoclonal antibody (dilution 1:50; Chemicon International, Temecula, Calif) by using immunohistochemistry and diaminobenzidine stain (Animal Research Kit; DAKO, Glostrup, Denmark). The degree of inflammatory response was semiquantified by using a scoring method with scores of 0 to 4 (0, nothing; 1, few; 2, moderate; 3, much; 4, most). Another 5 slides were taken to examine angiogenesis in the infarct area. The slides were fixed in 1.5% formaldehyde and then blocked with staining buffer (0.3% Triton X-100 and 2% normal goat serum in PBS). Sections were stained with anti-α smooth muscle actin monoclonal antibody (dilution 1:400, Sigma) and goat anti-mouse IgG2a (dilution 1:1000; Molecular Probes, Eugene, Ore). Positive smooth muscle staining with a visible lumen of a diameter 10 to 100 μm within the infarct was used as the criterion to calculate arteriole density.[13]

Statistical Analysis

Data were reported as the mean ± standard deviation. A paired *t* test was used for comparing baseline and postinjection echocardiographic data for animals serving as internal controls. Echocardiographic data across groups, histologic assessment, and immunostaining were compared by using 1-way analysis of variance with Holm's adjustment.

RESULTS

Acute Injection of Biopolymers Reshapes the Aneurysmal LV Wall

In those animals killed 24 hours after injection, fibrin glue and alginate were observed within the infarct wall. At the location of injection, both fibrin glue and alginate markedly altered the LV geometry, resulting in an LV cavity geometry more resembling the control geometry. The reshaping of the LV wall was not seen with the PBS control (Figure 1).

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