A Synthetic Non-degradable Polyethylene Glycol Hydrogel Retards Adverse Post-infarct Left Ventricular Remodeling

STEPHAN DOBNER, MD, DEON BEZUIDENHOUT, PhD, PADMINI GOVENDER, MSc,
PETER ZILLA, MD, PhD, AND NEIL DAVIES, PhD

Cape Town, South Africa

ABSTRACT

Background: Left ventricular remodeling after myocardial infarction is a key component of heart failure and it has long been postulated that it may result from increased wall stress. It has recently been suggested that an injectable, non-degradable polymer may limit pathological remodeling in a manner analogous to that of cardiac support devices. We have tested a non-degradable polyethylene glycol (PEG) gel in a rat infarction model.

Methods and Results: After permanent ligation of the left anterior descending artery in male Wistar rats, PEG gel reagents were injected into the infarcted region and polymerized in situ. At 4 weeks, fractional shortening and infarct volume were unchanged relative to a saline injected control, but the infarct-induced left ventricular end-diastolic diameter (LVEDD) increase was substantially reduced (43%, $P < .05$) and wall thinning was completely prevented. At 13 weeks, the LVEDD were similar for both saline- and PEG-injected hearts. The non-degradable PEG gels did elicit a macrophage-based inflammatory reaction.

Conclusions: The injection of non-degradable synthetic gel was effective in ameliorating pathological remodeling in the immediate postinfarction healing phase, but was unable to prevent the dilation that occurred at later stages in the healed heart. (*J Cardiac Fail 2009;15:629−636*)

Key Words: Myocardial infarction biomaterial.

In 2005, 16 million people suffered from coronary heart disease, 8.1 million of whom were recuperating from a myocardial infarction (MI).[1] Up to one third of MI patients develop heart failure, a condition currently affecting 5.3 million people, making it the most common cause thereof.

Considering that 30% to 40% of patients die from heart failure within 1 year after being diagnosed[2]—indeed, even with optimal modern therapy the annual mortality rate is around 10%[3]—alternative therapies are urgently needed.

Over the last 10 years, heart dimensions have emerged as a meaningful surrogate marker for morbidity and mortality and have also proven their value in monitoring disease progression. Cardiac remodeling after a myocardial infarct is independently associated with a poor prognosis,[4] and even fairly minor increases in ventricular volume are associated with a major independent increase in the risk of death in those patients.[5]

The attenuation of left ventricular (LV) remodeling, as measured by changes in end-systolic and end-diastolic LV volumes, is among the primary effects of the current standard medical treatment (angiotensin-converting enzyme inhibitors, β-blockers, angiotensin receptor blockers), showing a strong negative correlation with the development of heart failure and mortality rates.[6−14]

Among the mechanisms responsible for pathological remodeling is a shift in the balance of matrix metalloproteinases—tissue inhibitors of matrix metalloproteinases toward matrix metalloproteinases leading to an increased extracellular matrix turnover, which directly contributes to progressive LV dilation.[15,16] Others are the enhanced formation of reactive oxygen species and the activation of the neurohumoral pathway, which further increases the risk of cardiomyocyte death—in part via angiotensin II−mediated water retention and increased volume load on the heart with more stretch-induced abnormalities.[17] Eventually, the combined volume and pressure load on non-infarcted areas of the myocardium leads to heart failure.[18−21]

With the high prevalence of heart failure, substantial research has been devoted toward developing new treatments with an emphasis on cell delivery. Questions have arisen as to whether the improved LV pump function observed in many cell delivery approaches has been solely a result of cellular paracrine signaling, causing myocardial regeneration, neovascularization,[22] or decreased apoptosis,[23] or whether changes found in ventricular function may purely or in part be a mechanical consequence of increasing the thickness of the LV wall.[24,25]

Therefore, the potential of biomaterial delivery into the infarcted heart to tackle post-infarct remodeling and progression into heart failure have begun to be explored.

Collagen, fibrin, alginate, and matrigel are among the materials used thus far and initial results have given reason for cautious optimism. Increased wall/scar thickness,[26−28] attenuation of adverse LV remodeling,[28] decreased infarct size,[29] and improvement of functional parameters[26−28] have been demonstrated.

In a recent publication, Wall and colleagues have used a finite element modeling approach in an attempt to shed light on some of the mechanisms responsible for LV improvement after material/cell injections into the heart.[25] They have shown that the injections help to normalize elevated wall stresses with the stiffest materials showing the greatest benefit.

Furthermore, it has recently been proposed that an injectable polymer that is non-degradable may be needed for long-term beneficial effects on heart remodeling.[30] However, because there is a stronger possibility of eliciting an inflammatory response when using a non-degradable scaffold, the chosen material's degree of inertness may prove to be critical.

In the present study, we show for the first time that the injection of a synthetic non-degradable hydrogel into the heart is feasible. The clinical use of natural polymers, such as collagen, is somewhat limited by handling problems, the difficulty of engineering its properties, and potential immunogenicity.[31]

We have chosen a non-degradable, polyethylene glycol (PEG)-based hydrogel for its mechanical properties as it ranks among the stiffest materials mentioned in Wall's paper. Also, PEG remains amongst the most inert synthetic biomaterials to date.[32]

We have determined the short- and long-term effects a permanent mechanical reinforcement has on post-infarction ventricular remodeling in an attempt to answer whether the presence of a biomaterial does produce and maintain some of the same benefits as cell transplantation, namely wall thickening, beneficial LV remodeling, and functional improvement.

Methods

PEG Derivatization

Vinyl sulfone derivatized PEG was prepared by methods similar to those employed by Lutolf et al.[33] Briefly, a 5% PEG (20 kDa, 8-arm, hydroxyl-terminated: 20PEG-8OH, Shearwater/Nektar) solution in dry dichloromethane was reacted with $5\times$ molar excess of sodium hydride followed by $50\times$ molar excess of divinyl sulfone under inert atmosphere for 48 hours. After neutralization of the remaining sodium hydride with glacial acetic acid and removal of the precipitated sodium acetate salt through centrifugation and vacuum filtration, reduction of the volume by rotary evaporation, and precipitation in $10\times$ excess cold diethyl ether, the product was dried (room temperature, 24 hours, reduced pressure). Purification of the product (20PEG-8VS) was achieved through $3\times$ re-precipitation from dichloromethane in diethyl ether and drying. PEG-$[O-(CH_2)_2-SO_2-CH_a = CH_{cis}H_{trans}]_8$. (yield, 65%; ^{1}H NMR; 400 MHz; CDCl$_3$; δH 6.1 [H_{cis}]; 6.4 [H_{trans}]; 6.8 [H_a]). Complete conversion was shown by the absence of the $-CH_2-OH$ peak in the ^{13}C spectrum of the derivatized product (62 parts/min in the unmodified PEG-OH) and a 97% conversion calculated from integration of the ^{1}H peaks.

PEG Hydrogel Labeling and Formation

Forty nanograms of Alexa Fluor 660 C2 maleimide (Invitrogen, Carlsbad, CA) was added to 50 μL of 12 mg/mL dithiothreitol (Sigma-Aldrich, Steinheim, Germany) in phosphate-buffered saline (0.15 M, pH = 7.4) and reacted for 30 minutes at room temperature. Gels of 10% (m/v) nominal concentration were prepared by dissolving 10 mg of 20PEG-8VS in 50 μL phosphate-buffered saline, and then adding 50 μL of the above labeled dithiothreitol solution, vortex mixing, immediately aspirating the admixture into a syringe, and injecting the contents into the myocardium.

Induction of MI and Injection of PEG Hydrogels

MI was produced in male Wistar rats weighing approximately 180 to 220 g, as previously described.[34] Briefly, rats were anesthetized with a 5% isoflurane (Safeline Pharmaceuticals (PTY) LTD, Johannesburg, RSA)/oxygen mix and, after tracheal intubation with a 16 G intravenous indwelling cannula (Braun, Melsungen, Germany), placed on an heated Deltaphase operating board (Braintree Scientific Inc, Braintree, MA). During the surgery, animals were ventilated at 110 beats/min with a small animal ventilator (Harvard Apparatus, Holliston, MA) and anaesthesia was maintained with 1.5% isoflurane/oxygen. The hearts were exposed via left thoracotomy and, after a pericardiotomy, a MI was induced by permanently ligating the left anterior descending artery 2 to 3 mm below the left atrial appendix with a 6-0 Prolene suture. MI was confirmed by electrocardiogram, color change, and dyskinesis of the LV wall. After successful ligation, animals were randomized to either receive 100 μL injections of saline or vinyl sulfone (PEG-VS). For the sham operation only, a thoracotomy and pericardiotomy were performed.

Via 2 to 3 injections, a total of 100 μL of PEG hydrogel or saline was delivered to the infarct area within 2 minutes of coronary artery ligation.

For analgesia, intramuscular buprenorphine (Temgesic, Schering-Plough LTD, Woodmead, RSA) was given during the first 48 hours after surgery.

At the end of the study period (day 28 or 3 months), animals were sacrificed by injecting 1 mL saturated KCl (Sigma-Aldrich) into the left ventricle, thereby arresting the heart in diastole.

The animal study protocol had been approved in writing by University of Cape Town Animal Ethics Committee. All animal studies were performed in accordance with the National Institutes of Health (NIH, Bethesda, MD) guidelines.

Echocardiography

A 5% isoflurane/oxygen mix was used for the induction of the anaesthesia. The rats were placed on a Deltaphase operating board in a left lateral decubitus position. Anaesthesia was maintained with mixture of 1.5% isoflurane/oxygen. Images acquired with a Siemens Acuson Sequoia 512 Ultrasound system and a 15L8 transducer (Siemens, Berlin, Germany) were analyzed at the time of acquisition. Short-axis 2-dimensional and m-mode measurements of the LV diameters were taken at the level of the papillary muscle and all measurements were averaged over 3 consecutive cardiac cycles.

LV fractional shortening (in percent) was calculated as following: (EDD-ESD)/EDD * 100, where EDD is end-diastolic diameter and ESD end-systolic diameter.

Echocardiography was performed at 14 days, 28 days, and 3 months by an examiner blinded to the treatment groups.

Infarct Size Measurement

Explanted hearts were flushed with saline, fixed in 4% paraformaldehyde (Sigma-Aldrich) for 24 hours, cut into 4 equally sized parts, and embedded in paraffin. After obtaining a 3-μm section, the 4 parts were trimmed and a second 3-μm section was cut 250 μm deeper. All 8 sections were stained with Massońs trichrome stain (see the following section), and then captured using a Nikon E1000 M (Nikon, Tokyo, Japan) with a 0.5× magnification lens. The infarct size derived from midline length measurements was calculated by dividing the sum of midline infarct lengths, acquired with Visiopharm Integrator Systems (Visiopharm, Hørsholm, Denmark), from all sections by the sum of midline circumferences from all sections and multiplying by 100, as previously described.[35]

Scar Thickness

Using all 8 Masson's trichrome sections, scar thickness measurements were taken at 1-mm intervals within the region where the scar occupied greater than 50% of the left ventricle wall. The measurements were then added and divided by the number of measurements taken for every single sample to calculate the average thickness of the scar. For sham-operated animals, the thickness of the wall was measured in the corresponding region and quantified in similar fashion.

Histology

Masson's Trichrome Stain. A Masson's trichrome stain was done to detect collagen in the scarred region of infarcted hearts. After fixation in 4% paraformaldehyde (Sigma-Aldrich), all samples were processed through graded alcohol and then embedded in paraffin wax. Tissue sections (3 μm) were heat-fixed on a hotplate at 60°C, dewaxed with xylene, and taken through alcohol followed by a wash in running tap water. The sections were then flooded with 0.5% acid fuchsin (Merk, Gauteng, South Africa), followed by 1% phosphomolybdic acid (Sigma-Aldrich) to remove excess acid fuchsin. A 2% light green (Sigma-Aldrich) solution was used as a counterstain.

ED1 (Anti-rat cd68). Macrophages were identified with an anti rat ED1 antibody that recognizes a 90 to 110 kDa glycosylated protein expressed on rat cytoplasmic granules in macrophages. Myocardial tissue sections (3 μm) were dewaxed, hydrated, and pretreated with a ready to use proteinase k solution (Dako, CA). Sections were then incubated with a 1:100 dilution of mouse anti rat ED1/CD68 primary antibody (Serotec, Kidlington, Oxford, UK) in 1% bovine serum albumin (Jackson Immunoresearch, West Grove, PA) in phosphate-buffered saline. The primary antibody was then detected with 1:1000 of a CY3 donkey anti-mouse IgG antibody (Jackson Immuno research Lab). Slides were finally counterstained and mounted with DAPI (Vector laboratories, Burlingame, CA). Images were acquired with a Nikon 90i fluorescence microscope (Nikon Corp, Tokyo, Japan)

PEG-Alexa Fluor 660 Detection. The distribution of polyethylene hydrogel in heart tissue was determined by detection of the covalently bound Alexa fluor 660 maleimide marker. Myocardial tissue sections (3 μm) were dewaxed, hydrated, and mounted with DAPI. The infarcted region of the section was determined from an adjacent serial section that had been stained with Masson's trichrome. Stitched images were acquired with a Nikon 90i fluorescence microscope for both serial sections, the images were overlayed in Adobe Photoshop (Adobe Systems Inc, San Jose, CA), the infarcted area outlined on the fluorescent image, and the stitched Masson's trichrome image discarded to allow the labeled PEG to be clearly discerned.

Statistics

Two-tailed Student t-tests were used to assess differences between two groups. $P \leq .05$ was considered significant. Data are expressed as mean values $\pm$ SEM.

Results

Survival

A total of 91 rats were used in this study. The survival in the 4-week experiment was 100% (42 rats, replicates [sham group: 10; saline and PEG groups: 11]). In the 13-week study, 1 rat died from fibrillation immediately after the thoracotomy was performed. The remaining 48 animals survived (97.96%; 48 rats, replicates (sham group: 10; saline and PEG groups: 14)). The overall survival rate for the study was 98.9%.

Durability of PEG Hydrogel in Rat Hearts

Distribution of PEG hydrogel was assessed at 4 and 13 weeks by detection through fluorescent microscopy of a covalently attached far-red fluorescent label. The label was easily detected at both time points and the hydrogels had very similar signal intensities (Fig. 1). PEG hydrogel was found distributed throughout the infarcted region and its border zones at 4 and 13 weeks with the amount of gel appearing to remain constant.

Echocardiographic Analysis

The fractional shortening was not significantly different between saline and PEG hydrogel injected hearts at 2, 4 and 13 weeks (Table 1). The dimensions of the PEG hydrogel injected hearts were significantly reduced at end diastole at 2 and 4 weeks as compared with the saline controls (Table 1, Fig. 2). The increase in heart size that was induced by infarction was reduced by 33% at 2 weeks and 43% at 4 weeks ($P \leq .05$) for the PEG hydrogel−injected heart relative to the saline control. The dimension changes at end-systole showed
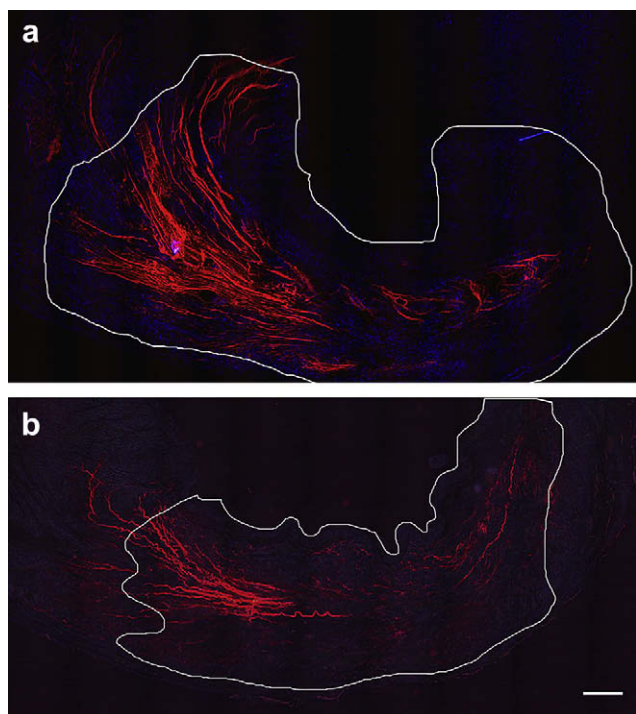
Fig. 1. Assessment of polyethylene glycol (PEG) gel distribution in an infarcted rat heart. Representative micrographs of Alexa Fluor 660 labeled PEG (red) at (a) 4 and (b) 13 weeks. The white line delineates the infarcted regions. Nuclei are stained blue with DAPI. Bar represents 500 μm.

a similar but nonsignificant trend, whereby the infarction-induced increase was reduced by 22% and 23% in the PEG gel injected hearts at 2 and 4 weeks, respectively ($P = .25$ and .19).

The initial partial preservation of heart dimensions obtained by synthetic hydrogel injection was, however, lost at 13 weeks, with the ESD and EDD being equivalent for both saline and PEG hydrogel–injected hearts (Table 1, Fig. 2).

The injection of PEG hydrogel into sham-operated hearts did not affect fractional shortening at 2 weeks, but there was a small but significant reduction at 4 weeks relative to sham-operated hearts (Table 1). By 13 weeks, there was a further marked deterioration in fractional shortening

for this group relative to the sham group. At 2 weeks, both EDD and ESD were significantly increased relative to the sham for PEG gel–injected hearts, but by 4 weeks only the ESD was significantly increased; this difference was amplified at 13 weeks.

Postmortem Infarct Size and Scar Thickness

Infarct size at 4 weeks in the saline group was $22 \pm 12\%$ and was not significantly different in the PEG hydrogel–injected hearts ($22 \pm 10\%$). Likewise at 13 weeks the infarct volumes were similar with infarcts occupying 23% and 28% of the LV volume for saline and PEG hydrogel injected hearts, respectively.

Animals in the PEG hydrogel injected group had a significantly thicker scar at 4 weeks relative to the saline injected group—3 mm and 1.7 mm, respectively ($P < .01$). Although saline-injected rats showed a 36% decrease in wall/scar thickness, PEG hydrogel injection completely prevented thinning of the scar compared with the sham group at 4 weeks (Fig. 3). At 13 weeks, the scar in the PEG hydrogel group had thinned relative to the wall of the sham group. Though there was still a trend toward an increased scar thickness as compared with the saline, it was no longer significant (30% increase, $P = .069$).

Inflammatory Reaction

As a non-degradable hydrogel was being delivered into heart tissue, the inflammatory reaction was assessed by immunohistochemical detection of macrophages. At 4 weeks, there was a clear and pronounced macrophage response to the hydrogel and this situation persisted out to 3 months (Fig. 4). The macrophages were relatively evenly distributed in the PEG hydrogel–infiltrated regions. By 13 weeks, a low number of putative foreign body giant cells were observed on the borders of the hydrogel strands.

Discussion

In the present study, we show for the first time that the injection of a synthetic non-degradable PEG-based hydrogel into ischemic myocardium is feasible and leads to a retardation of post-infarct LV dilation.

Table 1. Echocardiography at 2, 4, and 13 weeks

Weeks	Parameter	Sham	Sham plus PEG	Infarct plus saline	Infarct plus PEG
2	FS%	49 ± 1.4	47.6 ± 1.3	35 ± 1.3	36.6 ± 2.6
	ESD	3.5 ± 0.1	4.3 ± 0.2[†]	5.6 ± 0.2	5.1 ± 0.3
	EDD	6.9 ± 0.1	8.1 ± 0.2[†]	8.5 ± 0.2	8 ± 0.2*
4	FS%	52.3 ± 0.4	48.3 ± 0.8[†]	34.4 ± 1.6	35.5 ± 2.8
	ESD	3.6	4 ± 0.2[†]	6.1 ± 0.3	5.5 ± 0.3
	EDD	7.5 ± 0.1	7.8 ± 0.2	9.2 ± 0.2	8.5 ± 0.2*
13	FS%	48.4 ± 0.6	34.7 ± 1.2[†]	26.5 ± 1.5	26.1 ± 2.5
	ESD	4.4 ± 0.1	5.8 ± 0.2[†]	7.5 ± 0.3	7.7 ± 0.5
	EDD	8.4 ± 0.1	8.8 ± 0.2	10.2 ± 0.3	10.2 ± 0.3

PEG, polyethylene glycol; FS%, percent of fractional shortening; ESD, end-systolic dimension in mm; EDD, end-diastolic dimension in mm.
*$P \leq .05$ saline vs. PEG-injected infarcted hearts.
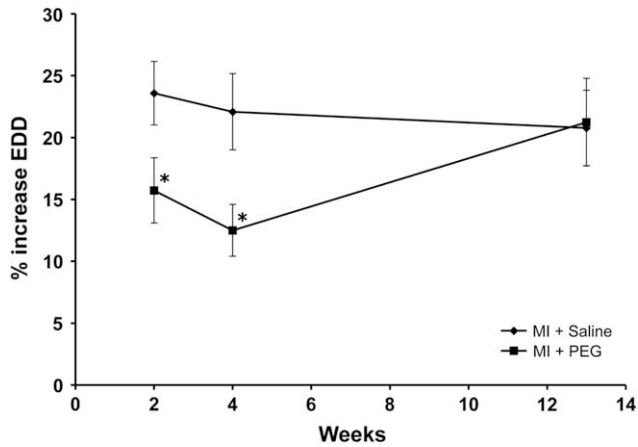[†]$P \leq .05$ sham vs. PEG-injected sham hearts.

Fig. 2. Polyethylene glycol (PEG) hydrogel injections reduce infarct induced diastolic dilation over the medium-term. The change in diastolic dimensions over time is presented as a percentage increase relative to sham diastolic dimensions. There was a significant reduction at 2 and 4 weeks (*$P = .05$ and .02 for 2 and 4 weeks, respectively).

We used a non-degradable form of a PEG hydrogel gel to investigate whether a permanent support of the heart is needed to maintain beneficial effects on post-MI remodeling. We also considered that PEG-based hydrogels are mechanically more stable than natural hydrogels such as fibrin, collagen, and alginate, and that PEG ranks among the stiffest hydrogels.[25] Additionally, the PEG gels used in this study exhibit a range of properties that render them suitable for a variety of in situ applications.[31,36] They gel within a few minutes at physiological temperature and pH, do not require the addition of initiators, produce no byproducts, have very low reactivity toward tissue, and are considered to be very biocompatible.[37] The physical properties of the gels may also be varied by choice of molecular mass, functionality, and crosslinker type, and proteinaceous, carbohydrate, and low molecular mass therapeutic agents may be readily coupled and released from the gels, either with or without degradation of the gel itself.

It has been suggested previously that to maximize the attenuation of remodeling, limit deterioration of cardiac function and progression into heart failure when using injected polymers as a stand-alone treatment, stiffer materials should be used.[25,30] The proposed mechanism, an increased mechanical strength of the infarct providing stress relief for the cardiomyocytes, draws support from a recent clinical study in which Sabatine et al showed that high baseline levels of ST2, a marker for biomechanical strain, correlated significantly with higher incidences of cardiovascular death and heart failure after MIs.[38]

Finally, many of the natural biomaterials that have been used so far (fibrin, alginate, matrigel) are known to also enhance angiogenesis, making it challenging to separate the effects mechanical strengthening of the infarcted wall might have from those resulting from improved perfusion.[39,40]

PEG-injected hearts showed reduced adverse remodeling 2 and 4 weeks after MI with a complete prevention of wall thinning and significant 33.3% and 43.3% reductions in end-diastolic diameter increase at 2 and 4 weeks, respectively. PEG-injected hearts also showed a similar trend toward reduced end-systolic dilation. Taken together, the increased scar thickness and decreased heart dimensions should help lessen elevated cardiomyocytes stresses in accordance with Laplace's law.

Indeed, at 1 month, delivery of PEG hydrogel resulted in comparable effects to the angiotensin-converting enzyme inhibitor Lisinopril, namely beneficial remodeling consisting of a significantly decreased EDV and a nonsignificant decrease in ESV, without improving the ejection fraction after ischemic myocardial injury.[41]

However, 3 months after the ischemic event, the remodeling caught up in the PEG-treated group. Luminal dimensions were virtually identical, with only the wall thickness in the PEG group somewhat preserved.

To what degree the inflammatory reaction (foreign body reaction) at 3 months is responsible for the late adverse remodeling is not clear. What speaks against inflammation being the sole reason for the late remodeling in the PEG-treated group is that although long-term implants of non-degradable PEG did damage the non-infarcted heart as confirmed by a substantially decreased fractional shortening at 3 months, the injection of PEG into sham-operated hearts did not lead to increased diastolic dimensions at this time.

A possible explanation for the late remodeling in the treatment group might be a delayed buildup of cardiomyocyte stresses (in the border zone) above a threshold level. (PEG might act as a buffer for stresses initially—permanently but insufficiently reducing stresses. As the heart becomes larger, stresses may well increase slowly, ultimately leading to apoptosis, late adverse remodeling, and ultimately to a common end point in the treatment and control group.)

Engineering slow-degrading hydrogels that break down over a period of several months could be a required compromise to overcome the inflammatory issue while still providing mechanical support for an extended period. The chemistry used in forming the gels employed in this study also allows for the covalent attachment of growth factors and other bioactive moieties.[31] When these gels are polymerized with enzymatically degradable crosslinkers such as peptides, they allow for the slow cell-dependent release of the appended molecules. Therefore this type of gel could not only provide mechanical strength for the critical early stages of the infarct, but also deliver paracrine type signals in a well-defined manner. This type of approach may allow for a more easily achievable therapeutic approach than the delivery of cells with their at-present poorly understood associated complexity.

In conclusion, the ability to employ a synthetic hydrogel as a permanent tissue integrated scaffold in an infarcted heart was clearly demonstrated through the use of a label covalently conjugated to the gel, a methodological approach that has not been employed in previous similar studies. However even with a well-dispersed distribution of hydrogel in the infarcted region, therapeutic benefits were only observed
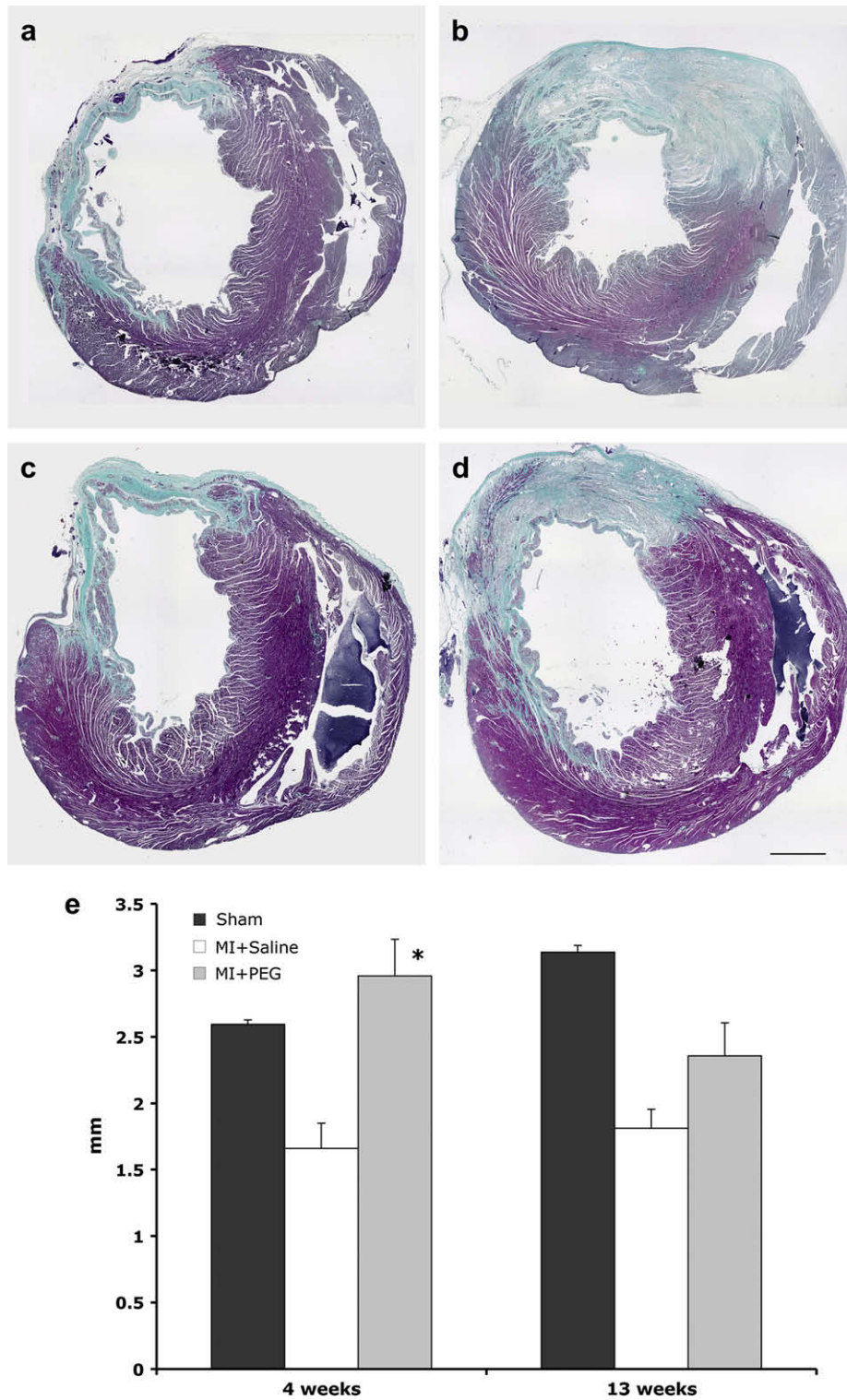
Fig. 3. Polyethylene glycol (PEG) hydrogel injections preserve wall thickness in the medium-term. Micrographs of Masson's trichrome stained infarct scars of at 4 weeks (a, b) and 13 weeks (c, d) for saline (a, c) and PEG hydrogel injected hearts (b, d). Bar represents 2 mm. The wall thickness was quantified by image analysis (e). The wall thinning was prevented at 4 weeks (*$P < .01$).

in the immediate post-infarct healing phase: This in spite of the use of a material such as PEG with its potentially optimal characteristics of a high degree of inertness and stiffness. In reference to the latter, a limitation of this study is the lack of specific data determining the contribution of the PEG to the

mechanical properties of infarcted tissue. This could be addressed in the future by analysis of any alterations in pressure-volume relationships resulting from hydrogel delivery. However, regardless of the outcome of these types of investigations, it seems clear that the successful application
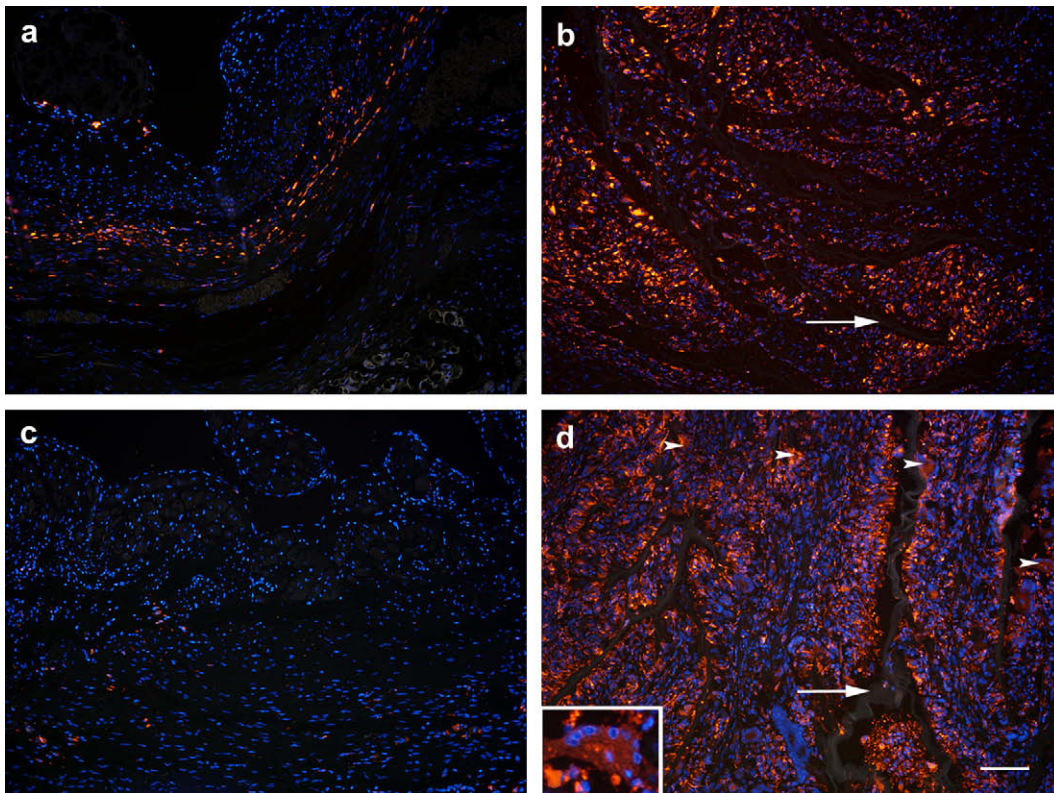
Fig. 4. Inflammatory reaction to polyethylene glycol (PEG) hydrogel. Macrophages were identified by staining for ED-1 (red). Saline injected hearts at 4 and 13 weeks (a, c) and PEG hydrogel injected hearts at 4 and 13 weeks (b, d). Foreign body giant cells were identified by their clustered nuclei (DAPI: blue) at 13 weeks in PEG hydrogel injected hearts (arrowheads). Inset in (d) shows a higher magnification of the cell identified by the rightmost arrowhead clearly demonstrating the characteristic multiple nuclei present within the singular ED1 positive cytoplasm. The PEG hydrogel is visible on the micrograph (arrows) as light gray material. This identification was confirmed by examination of adjacent serial sections for presence of Alexa Fluor 660 label as the label was lost during the immunohistochemical staining procedure (data not shown). Bar represents 100 μm.

of the attractive therapeutic option of an injectable permanent scaffold will most probably require substantial further research toward developing a material with the necessary characteristics and a greater understanding of the degree of stress relief needed to halt pathological remodeling.

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