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# The beneficial effects of deferred delivery on the efficiency of hydrogel therapy post myocardial infarction

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ABSTRACT

Biomaterials are increasingly being investigated as a means of reducing stress within the ventricular wall of infarcted hearts and thus attenuating pathological remodelling and loss of function. In this context, we have examined the influence of timing of delivery on the efficacy of a polyethylene glycol hydrogel polymerised with an enzymatically degradable peptide sequence. Delivery of the hydrogel immediately after infarct induction resulted in no observable improvements, but a delay of one week in delivery resulted in significant increases in scar thickness and fractional shortening, as well as reduction in end-systolic diameter against saline controls and immediately injected hydrogel at both 2 and 4 weeks post-infarction ($p < 0.05$). Hydrogels injected at one week were degraded significantly slower than those injected immediately and this may have played a role in the differing outcomes. The hydrogel assumed markedly different morphologies at the two time points having either a fibrillar or bulky appearance after injection immediately or one week post-infarction respectively. We argue that the different morphologies result from infarction induced changes in the cardiac structure and influence the degradability of the injectates. The results indicate that timing of delivery is important and that very early time points may not be beneficial.



## 1. Introduction

Approximately 5.7 million Americans are suffering from heart failure [1], a condition that 30%–40% of patients die from within 1 year of diagnosis [2]. Myocardial infarction (MI) is one of the most common causes thereof [3] and though early mortality after MI has declined as a result of significant advances in treatment, this decline has been parallelled by an increase in incidence of heart failure [4]. The gold standard treatment for end stage heart failure remains heart transplantation but the need for donor hearts far exceeds their availability [5]. Thus it is apparent that there is an urgent requirement for alternative therapies.

The heart responds to an MI by undergoing a process termed ventricular remodelling [6]. Initially as a result of the increased compliance of the infarcted tissue the ventricular volume increases, thereby preserving stroke volume via the Starling mechanism in the face of a decreased ejection fraction. However, though this early response probably increases survival, the overall increased stress on the wall can result in further eccentric ventricular enlargement due to hypertrophic lengthening of surviving cardiomyocyes and infarct expansion [7,8]. An ensuing cyclical hypertrophic response by the surviving tissue to the increasing stress can then overwhelm the heart resulting in heart failure. Early reduction in ventricular dilation and wall stress through angiotensin- converting enzyme inhibition is associated with a decreased incidence of heart failure [9,10].

The injection of biomaterials within the ventricular wall is increasingly being explored as a means of counteracting elevated wall stress within the infarcted heart [11]. Though initial studies investigated the ability of these materials to increase retention of stem cells within the ventricular wall, improvements in function and attenuation of remodelling resulting from the biomaterial alone were observed [12,13]. Recent finite element models [14,15] have shown that injection of any non-contractile material within the ventricle wall can reduce the MI induced elevated myofiber stresses through wall thickening and that stiffer materials resulted in a greater stress reduction.

A wide range of hydrogels from purely biological materials such as fibrin [12], collagen [16] and alginate [17] through to the fully synthetic such as polyethylene glycol (PEG) [18] and

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thermoresponsive N-isopropyl acrylamide hydrogels [19] have been shown to result in potentially therapeutic outcomes after delivery. Decreased infarct size [13], increased wall/scar thickness [16–20], reduction of LV remodelling [17–19] and improvement of functional parameters [16,17,19,20] have been demonstrated.

We recently explored the ability of a non-degradable PEG hydrogel to perform as a permanent ventricular support when injected immediately after infarction and demonstrated that the hydrogel reduced left ventricular dilation at 4 weeks post-infarction [18]. However this improvement was lost at 3 months and due to its non-degradability the PEG gel was the focus of an ongoing macrophage based foreign body response. The chemistries developed for these types of hydrogels also allow for the formation of hydrogels crosslinked with peptides that are degraded by cell driven enzymatic cleavage [21,22]. Thus we decided to utilise this type of formulation to extend our investigations into the potential of synthetic engineerable PEG hydrogels as a therapy for MI.

Furthermore we also decided to investigate the impact of timing of delivery post-infarct on remodelling and functional outcomes. There has been substantial variance in the period between infarction induction and biomaterial delivery with delivery from immediately post-infarction, around one week after, and at later time points such as 4 weeks. As the majority of studies were carried out with an immediate delivery or a delay of one week and also it has been suggested that earlier injection may be more favourable [17], we have now carried out a direct comparative study between injecting an enzymatically degradable synthetic PEG hydrogel immediately and 1 week after infarction induction. We assessed the influence of timing of delivery on cardiac function, remodelling and scar thickness.

## 2. Methods

### 2.1. PEG preparation and labelling

Vinyl sulfone derivatized 8-arm PEG of 20 kDa MW (20PEG-8VS) was prepared as described previously [18] 0.3 μL of a 10 mg/ml Alexa Fluor® 660 (Invitrogen Molecular Probes, Eugene, Oregon, USA) solution was mixed with 3 μl of a 15.4 mg dithiothreitol (DTT, Sigma–Aldrich Chemie GmbH, Steinheim, Germany)/10 ml phosphate-buffered saline (PBS, pH 7.5) solution and were allowed to react for 30 min at 37 °C. Gels of 10% m/v were prepared by dissolving 10 mg of 20PEG-8VS in 25 μl PBS and adding 1 μl of the above Alexa/DTT solution. This solution was then mixed with 3.45 mg MMP-1 (GenScript USA Inc., Piscataway, NJ, USA)/75 μl PBS and was injected into the myocardium before the components were able to polymerize.

### 2.2. Induction of MI and injection of PEG hydrogels

Male Wistar rats (180–220 g) were anaesthetized with a mixture of oxygen and 5.0% Isoflurane (Safeline Pharmaceuticals (Pty) Ltd., Johannesburg, South Africa), intubated with a 16G intravenous catheter (B.Braun Melsungen AG, Melsungen, Germany) and placed onto a heated operating board (Braintree Scientific, Inc., Braintree, MA, USA). Throughout surgery the animals were ventilated at 115 breaths/minute while anaesthesia was maintained with a mix of oxygen/2.0% Isoflurane. The heart was exposed via left thoracotomy, performed along the 4th intercostal space. After pericardiotomy, myocardial infarction was induced by permanent ligation of the left anterior descending coronary artery with a 6-0 non-absorbable Polypropylene ligature (Ethicon Inc., Somerville, NJ, USA) 3 mm distal the auricular appendix. Discolouration of the anterior ventricular wall and reduced contractility were hallmarks of a successful occlusion of the artery. Animals were randomized to receive either 100 μl saline or 100 μl PEG-VS/MMP-1 via 2-3 injections into the infarcted area of the myocardium. The rats were further randomized to receive their injection either immediately after infarct induction or in a second procedure after seven days, in which the heart was again accessed via the 4th intercostal space. The chest was stepwise closed and buprenorphine (Temgesic®, Schering-Plough (Pty) Ltd., Woodmead, South Africa) was administered for pain management. The investigator delivering the treatment was blinded until after the infarct was induced. After all *in vivo* measurements, animals were humanely sacrificed on day 28. The hearts were carefully harvested, thoroughly rinsed with saline (Adcock Ingram Critical Care, Johannesburg, South Africa) and fixed in a 4% paraformaldehyde solution (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) for 24 h. All animal experiments were approved by the *Animal Research Ethics Committee* of the *University of Cape Town* and were performed in accordance with the National Institutes of Health (NIH, Bethesda, MD) guidelines.

### 2.3. Echocardiography

Transthoracic echocardiography was performed on days 14 and 28 after coronary artery ligation, using an Acuson Sequoia 512 with a 15L8 Transducer (Siemens AG, Erlangen, Germany). Animals were anaesthetized with a mix of oxygen and 5.0% Isoflurane and placed in left lateral position onto the heated operating board. Anaesthesia was maintained with oxygen/2.0% Isoflurane. Short-axis 2-dimensional and M-mode-derived measurements of the left ventricle were taken at the level of the papillary muscle. The fractional shortening was calculated using the following formula: (EDD-ESD)/EDD*100, (EDD = end-diastolic dimension, ESD = end-systolic dimension). All diameters were measured six times and their mean calculated thereafter. The analyses of the echocardiography images were performed by a blinded investigator.

### 2.4. Histology

After being fixed for 24 h, the hearts were cut in 4 equal slices and processed through graded alcohol (Illovo Sugar Ltd., Durban, South Africa) and xylene (Saarchem, Gauteng, South Africa) using the Tissue-Tek® Rotary Tissue Processor (Sakura Finetek, Tokyo, Japan). Embedded in paraffin wax (Merck KGaA, Darmstadt, Germany) the sections were cut into 3 μm slices, picked up on glass slides (Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany) and baked on a hot plate at 60 °C (Kunz Instruments AB, Nynäshamn, Sweden) before being dewaxed through xylene, agitated in absolute alcohol and hydrated in running tap water.

#### 2.4.1. Masson's trichrome

This stain was used to identify collagen fibres in the scarred region of the infarcted hearts. A 0.5% Acid Fuchsin solution (Merck KGaA, Darmstadt, Germany) was applied to the slides for 5min, which was followed by rinsing in tap water. In order to remove excess Acid Fuchsin, a 1% Phosphomolybdic acid solution (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) was used to treat the sections for 5min. Subsequently the slides were counterstained for 2min with 2% Light Green SF Yellowish (Sigma Aldrich Chemie GmbH, Steinheim, Germany) before they were dehydrated through alcohol, cleared with xylene and mounted with Entellan® (Merck KGaA, Darmstadt, Germany).

#### 2.4.2. ED1 and neutrophil elastase

ED1 and neutrophil elastase staining were applied to detect macrophages and neutrophils respectively. Slides were antigen retrieved with Proteinase K (Dako Denmark A/S, Glostrup, Denmark), washed with running tap water and Tris-buffered saline (TBS). The primary antibodies were applied as a cocktail of primary antibodies, monoclonal ED1 mouse anti-rat CD68, (AbD Serotec, Oxford, UK), diluted 1/100 in bovine serum albumin (BSA, Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA), and polyclonal rabbit anti-rat Neutrophil Elastase (Abcam, Cambridge UK) diluted 1/200 in BSA. After being washed in TBS for 2 min, slices were treated for 2 h with donkey anti-mouse Cy3 IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA), diluted 1:500 in BSA, and goat anti-rabbit Alexa 488 (Invitrogen Molecular Probes, Eugene, Oregon, USA), diluted 1/50 in BSA.

Finally the slides were washed with TBS and coverslipped with Vectashield® (Vector Laboratories Inc., Burlingame, CA, USA).

#### 2.4.3. Alexa detection

The Cy5-filter on the microscope was used to detect the Alexa Fluor® 660 labelled PEG gel and therefore to determine the distribution of the gel within the myocardium. After being hydrated, the sections were washed in TBS and mounted with Vectashield®, which contains DAPI stain.

#### 2.4.4. Image acquisition

Images were acquired with the Eclipse 90i Microscope (Nikon Corporation, Tokyo, Japan) and captured with the Nikon Digital Camera DXM-1200C (Nikon Corporation, Tokyo, Japan).

**Table 1**
Echocardiographic parameters of saline and PEG hydrogel injected hearts.

| Weeks | Parameter | Saline 0 d | PEG 0 d | Saline 7 d | PEG 7 d |
|---|---|---|---|---|---|
| 2 | ESD (mm) | 4.9 ± 0.1 | 5.2 ± 0.2 | 5.0 ± 0.2 | 4.3 ± 0.3* |
| | EDD (mm) | 7.6 ± 0.1 | 7.8 ± 0.2 | 7.3 ± 0.2 | 7.1 ± 0.3 |
| | FS% | 34.9 ± 1.3 | 33.2 ± 1.7 | 32.0 ± 2.3 | 41.7 ± 0.9 * |
| 4 | ESD (mm) | 5.2 ± 0.2 | 5.6 ± 0.3 | 5.5 ± 0.2 | 4.7 ± 0.2 * |
| | EDD (mm) | 8.1 ± 0.2 | 8.4 ± 0.2 | 8.0 ± 0.2 | 7.7 ± 0.2 |
| | FS% | 35.9 ± 2.4 | 33.3 ± 1.8 | 31.9 ± 2.1 | 41.2 ± 1.4 * |

*$p < 0.05$ vs saline controls and immediately injected hydrogel group.