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An injectable capillary-like microstructured alginate hydrogel improves left ventricular function after myocardial infarction in rats

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ABSTRACT

Background: A new post-myocardial infarction (MI) therapy is injection of high-water-content polymeric biomaterial gels (hydrogels) into damaged myocardium to modulate cardiac negative remodeling and preserve heart function.
Methods: We investigated the therapeutic potential of a novel gelatinized alginate hydrogel with a unique microstructure of uniform capillary-like channels (termed Capgel). Shortly (48 h) after induced anterior MI, Sprague Dawley rats received intramyocardial injection of Capgel directly into the antero-septal wall at the infarct border zone ($n = 12$) or no injection ($n = 10$, controls). Echocardiograms were performed at 48 h (week 0) and 4 weeks (week 4) to evaluate left ventricular function.
Results: Echocardiograms showed 27% improvement of left ventricular systolic function over time with gel injection: fractional shortening increased from 26 ± 3% at week 0 to 33 ± 2% at week 4 ($p = 0.001$). Capgel was present at the injection site after 4 weeks, but was minimal at 8 weeks. The remaining gel was heavily populated by $CD68^+$ macrophages with $CD206^+$ clusters and blood vessels. An *in vitro* experiment was performed to assess Angiotensin-(1–7) released from Capgel. Angiotensin-(1–7) was released from the Capgel in a sustained manner for 90 days.
Conclusions: Use of Capgel, a degradable, bioactive hydrogel composed of gelatinized capillary-alginate gel, appears safe for intramyocardial injection, is associated with improved left ventricular function after MI in rats, and may provide a long-term supply of Angiotensin-(1–7).

1. Introduction

Despite advancement in pharmacological therapies for ischemic heart disease, post-myocardial infarction (MI) management remains challenging. Among novel therapeutic strategies developed to improve tissue salvage and attenuate adverse remodeling after MI, biomaterial has demonstrated great potential for cardiac tissue repair and regeneration [1]. Alginate, an algae-derived polysaccharide, appears feasible due to its biocompatible, non-toxic, and non-immunogenic characteristics and has been approved by Food and Drug Administration for various medical applications [2]. At present, two modalities of injectable alginate-based gels with encouraging preclinical results have reached

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clinical trials. Alginate-based solutions that gel in-situ and administer via intracoronary injection [3,4] are currently under clinical investigation in patients with acute MI [5]; however, 6 months after injection the alginate-based solution did not show superior beneficial effects compared with a saline injection to prevent left ventricular (LV) remodeling [Zeymer et al. PRESERVATION I: bioabsorbable cardiac matrix for the prevention of remodeling of the ventricle after large ST-segment elevation myocardial infarction. Abstract presented at European Society of Cardiology 2015 Congress; 2015 Sept. 1; London, UK]. A different modality of injectable alginate hydrogel, Algisyl-LVR™, in solid form and administered via intramyocardial injection improves heart function and prevents progressive remodeling in dogs with advanced heart failure [6]. Clinical benefits of Algisyl-LVR™ suggesting its ability to modify remodeling were reported among patients with severe heart failure in the AUGMENT-HF trial [7]. Early preclinical and clinical trial results support the promising therapeutic potential of alginate-based approaches for myocardial repair and regeneration.

We have recently developed a novel hydrogel composed of gelatinized alginate with parallel capillary-like channels (hereafter termed Capgel).The present study evaluated the safety and therapeutic potential of Capgel as an injectable system to modify LV function in a rat model of MI. This hydrogel was designed to provide a substrate for endogenous cell homing and growth, as well as a possible reservoir to deliver therapeutic cells or agents. Accordingly, we hypothesized that the unique microstructure of continuous, parallel tubular channels will serve as a 3D matrix to facilitate repair by enhancing cell penetration, tissue formation, and vehicles to deliver therapeutics.

2. Materials and methods

All experiments were performed under the approval of the University of Florida IACUC and in accordance with National Institutes of Health guidelines. All authors have read and agree with the contents of the manuscript.

2.1. Preparation of injectable Capgel and characterization of Capgel mechanical properties

Keltone® LVCR sodium alginate was kindly provided by ISP Alginates (now FMC Bioploymers, Philadelphia, PA). All other chemicals used were purchased from Fisher Scientific (Pittsburgh, PA) and Sigma-Aldrich (St. Louis, MO). Capgel was synthesized as previously described [8]. Briefly, a solution of 2% (w/v) alginate and 2.6% (w/v) oligo-gelatin was poured into an alginate-coated glass petri dish; a primary membrane was formed on the top of the gel using a Kimwipe® soaked in 0.5 M $CuSO_4$. The dish was then submerged in a tank of 0.5 M $CuSO_4$ and undisturbed for at least 36 h. The gel was then removed, rinsed extensively in distilled water, sectioned and crosslinked using N-ethyl-N′-(3-dimethylamino propyl) carbodiimide hydrochloride (EDC, Sigma-Aldrich) and N-hydroxysulfosuccinimide (Sulfo-NHS, Pierce) chemistry at a mole ratio of 1:2:2 alginate/EDC/Sulfo-NHS in phosphate-buffered saline at 4 °C for 21 h. The crosslinked scaffolds were then rinsed extensively with sodium citrate followed by normal saline rinsing. Finally, these scaffolds were autoclaved in saline (121 °C, 20 min) and then maintained at 4 °C prior to animal applications. For the in vitro mechanical analysis, the gels were stored at 37 °C in EBM2 basal media for 24 h before testing. Mechanical properties of Capgel and heart slices collected from Sprague Dawley rats were measured using indentation technique as previously described [9].

2.2. Intramyocardial hydrogel injection in healthy rat

A total of 10 normal male Sprague Dawley (~300 g) rats received intramyocardial injection (28G insulin needle) of either 1) saline (n = 5) or 2) Capgel (~20 μL, n = 5). Rats were anesthetized (2–3% isoflurane:oxygen mixture), intubated by mouth, placed on mechanical ventilation connected to a rodent ventilator (Harvard Apparatus model 683, Holliston, MA), and maintained on a 2–3% isoflurane:oxygen mixture. Under electrocardiographic monitoring, the chest of mechanically ventilated animals was then opened by left thoracotomy and the heart was exposed. Injections were performed in the mid-lateral anterior portion of LV wall. The chest was then closed, and the animals were allowed to recover, and then kept alive for an additional 4 weeks.

2.3. Rat myocardial infarction model and hydrogel injection

A total of 39 male Sprague Dawley rats (~300 g) were anesthetized (2–3% isoflurane:oxygen mixture), then intubated by mouth, placed on mechanical ventilation connected to a rodent respirator (Harvard Apparatus model 683, Holliston, MA), and maintained on a 2–3% isoflurane:oxygen mixture. Acute MI was performed via permanent ligation of the left anterior descending (LAD) coronary artery [10]. Briefly, via left thoracotomy the pericardial sac was removed to expose the heart. A small suture anchor was made at the bottom of the cardiac apex to facilitate placement of a ligature around the LAD. After induced anterior MI (~15–20 min), animals assigned to the treatment group received a single injection of Capgel (~20 μL) into the antero-septal wall in the infarct border zone via a 28G needle. The infarct border zone was targeted for Capgel injection with the thought of limiting scar expansion and supporting stunned/hibernating myocardium via neovascularization and cell recruitment stimulated. Animals assigned to the control group were handled in an identical fashion but did not receive any injection because prior studies in this model in our lab showed that a saline control injection using the same volume (20 μL) was not associated with myocardial alterations at 4 weeks. Overall, 39 rats were included in the study. Early mortality (during or shortly after surgery), but before treatment assignment, was 35.9% (n = 14). Two other rats died within 24 h after surgery: one assigned to Capgel and the other assigned to the control group. Another animal was subsequently excluded when no evidence of MI-related LV dysfunction (fractional shortening >45%) was present at week 0 echo. Thus, 22 animals with post-MI LV dysfunction were available for echo evaluation at week 4 and comprise the data presented below: 12 were in the Capgel group and 10 in the control group.

2.4. Echocardiographic assessment

Echocardiographic data (echo) were collected using methodology as previously described [10]. To examine LV diastolic function, mitral inflow velocity was obtained by pulsed-wave Doppler interrogation in an apical view. Image acquisition and data analysis were performed by an experienced technician and analyzed by a cardiologist expert in echocardiography masked to group assignment. **For normal (healthy heart) rats**, all animals were initially examined (pre-surgery) to establish baseline LV function and at 4 weeks post-procedure. **For infarcted rats (MI model)**, echocardiographic data were collected 48 h post-MI (week 0) and again 4 weeks later (week 4). All animals were sacrificed after final echo assessment. Echo-derived measurements from at least three consecutive cardiac cycles were averaged to quantify each variable.

2.5. Histological assessment

After sacrifice, all hearts were rapidly removed, fixed (4% paraformaldehyde), embedded in paraffin and sectioned (5 μm) for staining. Staining included H&E for overall morphology, Trichrome for collagen, and a-smooth muscle actin (mouse anti-SMA, 1:600 Sigma-Aldrich, St. Louis, MO) immunostaining for blood vessels and myofibroblasts. Whole-slide images were captured using Aperio CS ScanScope® (Aperio, Vista, CA). Macrophage (Mac) populations were characterized with immunofluorescent staining using primary antibodies to CD68 (mouse anti-Pan Mac, 1:300, Abcam, Cambridge, MA) [10], CD86