A degradable, bioactive, gelatinized alginate hydrogel to improve stem cell/growth factor delivery and facilitate healing after myocardial infarction

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Abstract

Despite remarkable effectiveness of reperfusion and drug therapies to reduce morbidity and mortality following myocardial infarction (MI), many patients have debilitating symptoms and impaired left ventricular (LV) function highlighting the need for improved post-MI therapies. A promising concept currently under investigation is intramyocardial injection of high-water content, polymeric biomaterial gels (e.g., hydrogels) to modulate myocardial scar formation and LV adverse remodeling. We propose a degradable, bioactive hydrogel that forms a unique microstructure of continuous, parallel capillary-like channels (Capgel). We hypothesize that the innovative architecture and composition of Capgel can serve as a platform for endogenous cell recruitment and drug/cell delivery, therefore facilitating myocardial repair after MI.

Introduction

Over the past few decades, advances in coronary reperfusion and drug therapies have been effective in limiting extension and expansion of ischemic injury, resulting in improved myocardial infarction (MI) survival rates. Among patients surviving acute MI, left ventricular (LV) remodeling attempts to compensate for tissue loss and maintain pump

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The authors declare that they have no conflict of interest.

function. However, over time this may further impact global LV function leading to progressive heart failure (HF) [1]. Up to a third of MI patients develop HF at 5 years post-MI [2], and HF is the most frequent discharge diagnosis, with about 670,000 new HF patients identified yearly [3]. These patients have limited treatment options [4]; moreover, current post-MI treatments, though effective, have reached a therapeutic plateau. A goal of treatment is to delay progression of adverse remodeling that may exacerbate the systolic and diastolic dysfunction. Lifestyle changes and pharmacological interventions for risk factor control (e.g., hypertension, diabetes, hyperlipidemia) are recommended at all stages of HF. Angiotensin converting enzyme inhibitors (ACEIs) and β-blockers are the primary approach in patients at high risk for HF with or without structural heart disease [5,6].

Despite extensive research using therapeutic cells, growth factors, and other materials, there is currently no definitive therapy to regenerate myocardium; modulate scar tissue formation, structure, and composition; or prevent post-MI adverse remodeling.

A relatively new approach uses a combination of therapeutic cells with bioresorbable polymeric biomaterial hydrogels in an effort to improve their residence time in the targeted myocardial region [7]. A potential breakthrough post-MI therapy is intramyocardial injection of a novel, degradable, bioactive material that has a unique capillary-like microstructure of uniform channels (termed Capgel, Fig. 1) [8,9]. We hypothesize that intramyocardial injection of Capgel will modulate scar tissue formation and stimulate repair of ischemia-injured/infarcted myocardial tissue to help preserve cardiac contractile function.

Role of stem cells

Cell-based therapies delivered following MI are designed to improve long-term outcomes and represent a current focus of multiple clinical trials [10–12]. Circulating stem cells migrate into damaged tissues and, together with resident stem cells, contribute to tissue repair and regeneration or fibrosis with loss of pump function [13]. Each organ's extracellular matrix is uniquely structured to maintain a milieu intérieur in which cell adhesion, differentiation, growth, and survival are supported and become pivotal for tissue regeneration and organ function [14]. Although the heart has been thought to be a post-mitotic organ, much evidence supports a certain degree of tissue plasticity and cellular dynamism [15]. However, cardiomyocytes have a very limited intrinsic capacity to regenerate after MI [16].

Once cardiomyocytes are lost and extracellular matrix damaged, there is limited cardiac regeneration [17,18]. The reasons are complex and could be due to a lack of growth factors and sufficient blood flow, as well as presence of inhibitory environmental factors/substances released by necrotic cells in the infarct zone, and/or inhibitory factors or matrix proteins secreted by scar-forming myofibroblast cells [19,20]. These and other reasons (e.g., stem cell lineage selection, timing, delivery modality, differences between animal models and humans) might explain some inconsistencies in results among studies.

Biomaterial-based approaches

Intracardiac injection of biomaterials is a promising approach to modulate post-MI negative remodeling and prevent HF [7]. Considerable data have raised concerns about the very limited and short-lived cell retention after intracardiac stem/progenitor cell delivery [21] and about the unfavorable physical structural remodeling associated with infarct expansion [22]. Even if longer residence time and tissue retention could be achieved, there would be limited engraftment of implanted cells, basically due to loss of extracellular matrix and anoikis-induced apoptosis [19,20]. For these reasons, biomaterials have been combined with cells to improve their residence time and promote cellular engraftment [22]. Injection of

biocompatible materials that structurally and functionally reproduce damaged extracellular matrix may reduce adverse environmental changes caused by ischemic injury and promote recruitment, integration, and growth of endogenous stem/progenitor cells [23,24]. Finding appropriate biomaterials for stem cell/growth factor delivery and/or cardiac remodeling prevention is a challenge. The opportunity to tailor the chemical, physical, and mechanical features of a certain biomaterial is of fundamental importance for therapeutic functionality.

Theoretically, the optimal biomaterial should be easy to manufacture, inexpensive to produce, biocompatible, biodegradable, non-toxic, and minimally immunogenic, and should possess mechanical properties similar to the target tissue [25]. Another critical property is low viscosity to permit the material to be injected. At the same time, in situ rheology of the injected material should exhibit viscoelastic properties to prevent washout from the target zone. Several compounds have been developed for cardiac tissue engineering applications.

Collagen is a group of insoluble proteins, principally found in connective tissue and the extracellular matrix, that confer tensile strength to tissues. Collagen type I is also the major component of post-ischemic myocardial scar. As a protein naturally occurring in animal and human tissues, collagen is non-toxic, minimally immunogenic and biodegradable and has been used as a platform for cell culture and delivery. In a rat hind-limb ischemic model, collagen-based matrices increased the residence time of transplanted stem/progenitor cells [26].

Alginate is a natural anionic polysaccharide present in cell walls of brown seaweed. One of alginate's main uses is in food industry as a thickening and gelling agent. Alginate has also an extensive history of use in the pharmaceutical, dental, and medical industries. Calcium-crosslinked alginate scaffolds have been used as liquid-gel phase transition solutions for intramyocardial/intracoronary injection [27,28] or as monolayers for epicardial patches in both small and large animal models [29]. Their main beneficial effects are based on prevention of post-MI adverse remodeling and promotion of cellular retention when used to deliver cells [27–29].

Fibrin glue is an injectable biopolymer made of two separate components: fibrinogen and thrombin. When mixed with thrombin, fibrinogen is cleaved by thrombin to form fibrin, a fibrous protein involved in blood clotting, and the system changes from a liquid state to a solid state within seconds. Fibrin glue was approved by FDA in 1998 and, since then, has been used mainly and extensively as a surgical haemostatic agent. As a biomaterial for preventing cardiac remodeling after AMI, fibrin glue has been used by itself as an injectable compound [30] or an epicardial patch [31] as well as a delivery vehicle for stem cells, growth factors, proteins, plasmids, and other biologics [32,33].

Polyethylene glycol (PEG) is a non-toxic, non-degradable poly-ether compound with several medical applications, especially in the pharmaceutical industry where it is used as an excipient or a binder to prolong serum drug half-life. PEG is a bio-inert scaffold that prevents protein and cell adhesion, and has been used as an injectable biomaterial to provide passive mechanical support to the infarcted cardiac wall for prevention of post-MI LV dilation and dysfunction [34].

Self-assembling peptides are short peptides, typically between 8 and 16 amino acids in length, to be used as an injectable compound [35]. Self-assembling peptides are soluble at low pH and osmolarity but form nanofibers at physiological pH and osmolarity. The result is a nanostructured fibrous scaffold that allows cell and protein delivery and tissue integration [35] by promoting endogenous and exogenous cell adhesion, retention, and differentiation.

Capgel: hypothesis and preliminary studies

A promising post-MI treatment currently under investigation in clinical trials is the injection of alginate (brown sea algae–derived polysaccharide) gels directly into and around the scarred region of the LV [36–38]. In one approach, a low viscosity calcium alginate solution (IKA-5001, aka BL-1040) is injected into a recently-opened infarct-related coronary artery via a catheter; the solution penetrates into surrounding damaged heart muscle and forms an amorphous, bioresorbable gel [28,36]. Incorporation of this amorphous gel into the damaged myocardium serves as a passive mechanical support for the healing LV and thereby reduces LV wall stress and dilation. These reductions potentially stabilize post-MI cardiac function and architecture in the long term. In another trial, a bio-stable alginate-based gel (Algisyl-LVR) is injected intramyocardially in several spots around the infarcted region of a significantly dilated LV [37]. These injected gel boluses effectively change the shape of the LV, potentially resulting in an improvement of cardiac function.

Of note, all existing alginate-based gel technologies currently in clinical testing for post-MI treatment are passive, unstructured, amorphous gels that only thicken the myocardium and mechanical support or augment the LV wall. The fundamental biology of alginate-based gels in the healing myocardium is understudied, and a recent review also raises the concern that the benefits observed could be temporary and lost once all gel has cleared from the healing myocardium [7].

We therefore propose for use post-MI an innovative biomaterial hydrogel (Capgel) that is both structured and bioactive [9]. This self-assembled material is composed of low M_w gelatin (hydrolyzed collagen I) and alginate [a negatively charged, linear polysaccharide composed of β-1,4-linked D-mannuronic acid (M) and α-1,4-linked L-guluronic acid (G) monosaccharides]. The hydrogel possesses a regular capillary-like channel morphology (channel diameter ≈ 31 μm; channel density ≈ 100/mm^2) formed by a convection-like process that involves Cu^{2+} ions when the alginate polymer chains contract to the forming gel front [39]. The unidirectional diffusion of Cu^{2+} ions into the viscous solution of alginate tunnels parallel and regular channels, leading to the unique 3-dimensional microstructure of this gelatin/alginate-based hydrogel scaffold. The diameter and density of the gel channels can be tailored to facilitate host-cell ingrowth, vascular formation, and tissue integration. Growth factors and stem-cell recruitment moieties can be potentially loaded in and coupled to the gel. Capgel was developed by our group for use both as a stem-cell tissue scaffold and an injectable stem-cell delivery system.

We previously conducted a preliminary in-vitro study to investigate the morphology and growth of mouse embryonic stem (ES) cells, NIH-3T3 fibroblasts, and mouse multipotent astrocytic stem cells (MASC) [9] on and within Capgel slices. All three cell types were successfully cultured on the gelatinized alginate gel for 2 weeks. ES cells attached and proliferated on Capgel and formed regular cords of healthy cells spreading into the uniform capillary-like channels. The fibroblast-seeded Capgel scaffold maintained viability over 15 days in culture and enabled attachment and proliferation (Fig. 2A).

Because hydrogels and scaffolds made purely of alginate [8] allow for only limited cell adhesion, we incorporated gelatin [9] in an effort to enable adhesion, retention, and growth of endogenous and transplanted cells. Compared to an alginate gel, incorporation of gelatin into the channel-microstructured alginate scaffold [9] significantly enhanced cell adhesion and proliferation in vitro (Fig. 2A).

MASC retained differentiation capacities and spread on Capgel slices, consistently infiltrating and proliferating within capillaries in an oriented manner driven by the 3-dimensional microstructure of the scaffold (Fig. 2B).

We have also recently demonstrated that Capgel can be loaded with therapeutic cells and used as an injectable tissue scaffolding system for use in regenerative medicine/tissue engineering [9]. In this study, neonatal rat pups underwent unilateral common carotid artery ligation to cause a hypoxic-ischemic brain injury. After 24 h, GFP+ MASC-loaded Capgel (≈1 µL) was injected into target area via a 30G needle. One week following the procedure, Capgel injection did not lead to extensive reactive gliosis; viable GFP+ cells expressing astrocyte-like morphologies were found within the gel implant and in the surrounding brain areas.

In light of these findings, we hypothesize that the chemical and structural properties of Capgel will display a positive synergistic effect on (1) stem cell recruitment, retention, and engraftment and (2) dynamics and features of wound healing formation after MI.

This may result in

- angiogenesis, preservation, and formation of viable myocardium at the infarct border zone,

- scar shrinkage and mechanical scaffolding to provide structural support and minimize dilation at the infarct core zone,

there by limiting the loss of pump efficacy (systolic dysfunction) and the maladaptive pathway characterized by a decrease in LV compliance and dilation (diastolic dysfunction) that lead to HF and predispose to sudden cardiac death.

The semi-solid structure and viscoelastic properties of the alginate component of Capgel could provide the cardiac scar tissue with better structural properties and compliance, consequently reducing ventricular wall stress that promotes wall thinning and cardiac dilation. The incorporation of gelatin could enable and enhance endogenous (local and/or circulating) stem cell adhesion, proliferation, and differentiation as well as delivery of therapeutic vectors, drugs, and/or cells.

We further posit that Capgel may serve as a robust platform that provides guidance for 3-dimensional matrix reestablishment, cell orientation, and vascular formation.

Conclusions

Capgel is an innovative bioactive and microstructured hydrogel that enables new modalities for cell/drug delivery treatments of the infarcted heart. Evidence from our studies suggests that this biomaterial is biocompatible and injectable via a fine needle (30G), therefore enabling catheter-based intracardiac delivery. Intramyocardial injection of Capgel may also provide some mechanical support to the infarcted area, resulting in wall thickening, reduced LV wall stress, and limited cavity dilation. The alginate/gelatin matrix in combination with the unique 3-dimensional microstructure of regular capillary-like channels may provide a favorable extracellular environment for cell recruitment, adhesion, and ingrowth; vascular formation, and tissue integration.

Acknowledgments

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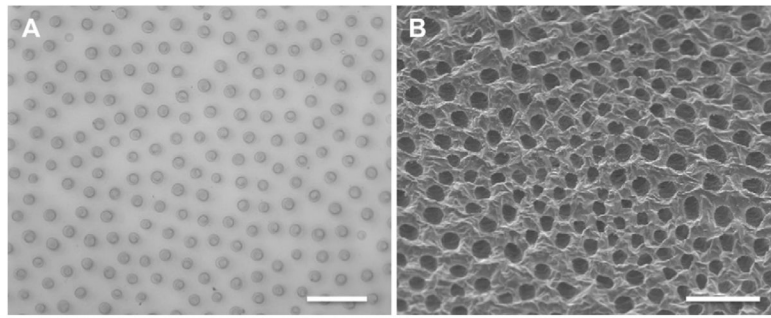
Fig. 1.
Optical microscope (A) and scanning electron microscope (B) images of the morphology of the capillary-like channel microstructure of Capgel in a section perpendicular to channel long axis. Average channel diameter ≈ 31 μm; average channel density ≈ 100/mm^2. Scale bars = 200 μm.
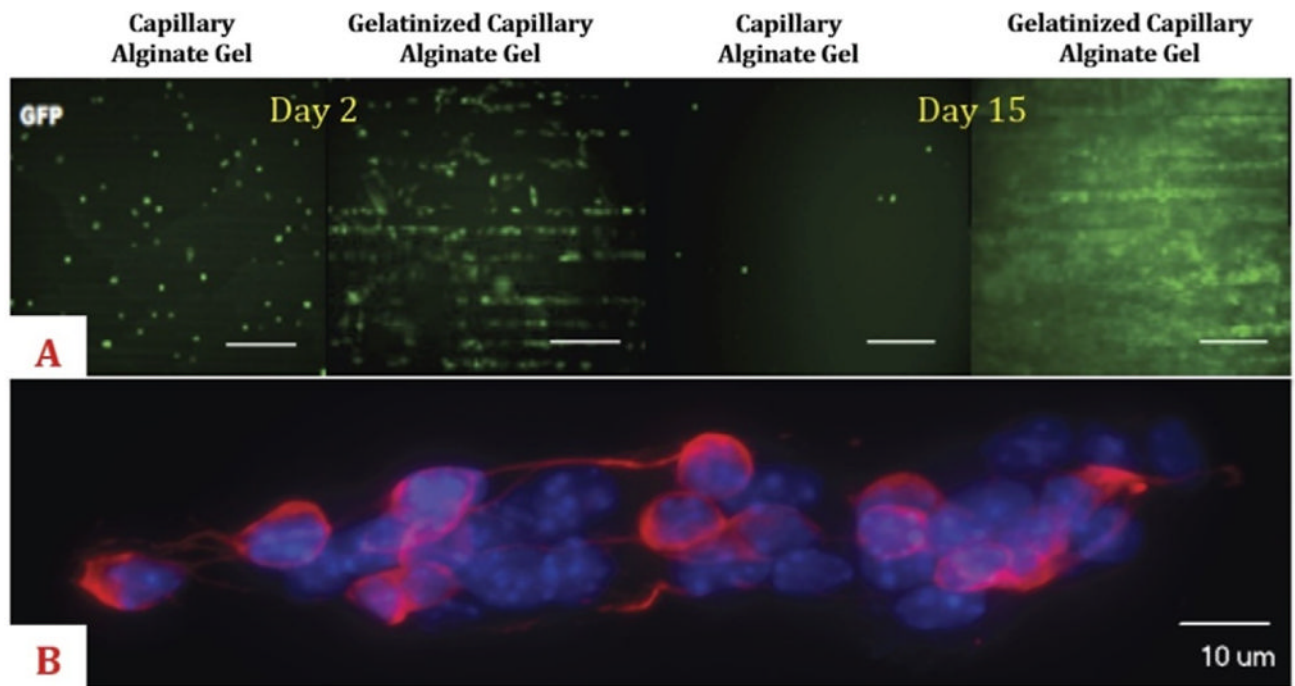
Fig. 2.
(A) Green fluorescent protein (GFP)-labeled fibroblast (NIH-3T3) culture on capillary alginate gel and gelatinized capillary alginate gel (Capgel) slices at 2 and 15 days. Compared to the alginate gel, incorporation of gelatin into the capillary-like channel microstructured alginate gel enhances cell adhesion and proliferation. Scale bars = 300 μM. (B) Spinning disc confocal microscopy images of Multipotent Astrocytic Stem Cells (MASC) oriented along the long axis of a channel of Capgel. Blue = 4′,6-diamidino-2-phenylindole (DAPI); red = β-III tubulin.