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PRE-CLINICAL RESEARCH

Intracoronary Injection of In Situ Forming Alginate Hydrogel Reverses Left Ventricular Remodeling After Myocardial Infarction in Swine

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Objectives This study sought to determine whether alginate biomaterial can be delivered effectively into the infarcted myocardium by intracoronary injection to prevent left ventricular (LV) remodeling early after myocardial infarction (MI).

Background Although injectable biomaterials can improve infarct healing and repair, the feasibility and effectiveness of intracoronary injection have not been studied.

Methods We prepared a calcium cross-linked alginate solution that undergoes liquid to gel phase transition after deposition in infarcted myocardium. Anterior MI was induced in swine by transient balloon occlusion of left anterior descending coronary artery. At 4 days after MI, either alginate solution (2 or 4 ml) or saline was injected selectively into the infarct-related coronary artery. An additional group ($n = 19$) was treated with incremental volumes of biomaterial (1, 2, and 4 ml) or 2 ml saline and underwent serial echocardiography studies.

Results Examination of hearts harvested after injection showed that the alginate crossed the infarcted leaky vessels and was deposited as hydrogel in the infarcted tissue. At 60 days, control swine experienced an increase in left ventricular (LV) diastolic area by 44%, LV systolic area by 45%, and LV mass by 35%. In contrast, intracoronary injection of alginate (2 and 4 ml) prevented and even reversed LV enlargement ($p < 0.01$). Post-mortem analysis showed that the biomaterial (2 ml) increased scar thickness by 53% compared with control (2.9 ± 0.1 mm vs. 1.9 ± 0.3 mm; $p < 0.01$) and was replaced by myofibroblasts and collagen.

Conclusions Intracoronary injection of alginate biomaterial is feasible, safe, and effective. Our findings suggest a new percutaneous intervention to improve infarct repair and prevent adverse remodeling after reperfused MI. (J Am Coll Cardiol 2009;54:1014–23) © 2009 by the American College of Cardiology Foundation

Left ventricular (LV) remodeling after myocardial infarction (MI) is often precipitated by early and progressive extracellular matrix (ECM) degradation, infarct expansion, scar thinning, and transition to heart failure (1,2). Current antiremodeling therapies are clearly limited, because many ventricles continue to enlarge (3,4) and morbidity and mortality remain high (5).

Recent experiments in small animals have suggested that direct injection of biomaterials, such as alginate, fibrin, collagen, and self-assembling peptide, into the infarct could act to internally constrain the myocardium from expanding, thereby limiting LV remodeling (6–14). We recently showed that a solution of calcium cross-linked alginate can be injected via a needle into the infarct, where it undergoes phase transition into hydrogel (13,14). The alginate hydrogel implant provides temporary physical support to the damaged cardiac tissue by replacing some of the functions of the damaged ECM while preventing adverse cardiac remodeling and dysfunction after recent and old MI in the rat (13,14). With time, the dissolvable hydrogel gradually

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disappears, and the water-soluble alginate chains are evacuated and excreted by the kidneys (15).

Compared with other biomaterials, a major advantage of the injectable alginate biomaterial solution is nonthrombogenicity (16,17). Therefore, we hypothesized that selective intracoronary injection of alginate solution will result in localized gelation as scaffold in the infarcted tissue, thereby preventing adverse LV remodeling. The present study aimed to test this hypothesis in a large animal model of MI.

Methods

The experiments were performed in compliance with animal welfare regulations of the authors' institutions conforming to the "Position of the American Heart Association on Research Animal Use," adopted by the American Heart Association on November 11, 1984.

Experimental groups. Female domestic swine (50 to 60 kg) were included in two sets of experiments that were designed to determine: 1) the feasibility, safety, and efficacy of intracoronary injection of alginate biomaterial into normal and infarcted (4 days) heart of swine; and 2) the optimal volume of alginate biomaterial for intracoronary injection and the effect on LV remodeling and function.

Injectable alginate solution. The preparation of calcium cross-linked alginate solution (BL-1040, BiolineRx, Jerusalem, Israel) has been previously described (13). In brief, 2 stock solutions were prepared: the first consisted of 2% weight/volume sodium alginate (VLVG, NovaMatrix, FMC Biopolymers, Drammen, Norway) solution, whereas the second was composed of 0.6% weight/volume calcium D-gluconate solution. In both cases the materials were dissolved by stirring at room temperature. Each solution was filtered separately through a sterile 0.2-μm membrane into a sterile container under a laminar flow cabinet. Equal volumes from each stock solution were combined by homogenization (for several minutes). Biotin-labeled alginate biomaterial was prepared for detection of the injectable implant into the infarcted heart. The biotin-labeled alginate was cross-linked by calcium ions, as described previously (13).

Swine model of MI. An anteroseptal ST-segment elevation MI was produced in female domestic swine weighing 50 to 60 kg by 60 (first set of experiments for feasibility and safety) or 90 min (second set of experiments for volume determination and remodeling) balloon occlusion in the mid-left anterior descending (LAD) coronary artery (18). The animals were pre-medicated with aspirin 500 mg, clopidogrel 300 mg, lopressor 95 mg, and amiodarone 300 mg intravenously. Aspirin was continued throughout the entire study period and clopidogrel until the day of injection. Continuous blood pressure, oximetry, and electrocardiographic (ECG) changes were monitored throughout the procedure. A 12-lead ECG was performed before and after MI and before and after alginate biomaterial delivery. The ST-segment changes in a 12-lead ECG and duration of PR, QRS, QT, and corrected QT intervals were measured and analyzed in lead 2.

Abbreviations and Acronyms

- **CT** = computed tomography
- **ECG** = electrocardiogram/electrocardiographic
- **ECM** = extracellular matrix
- **LAD** = left anterior descending
- **LV** = left ventricle/ventricular
- **MI** = myocardial infarction
- **MPG** = myocardial perfusion grade
- **MR** = mitral regurgitation
- **TFG** = Thrombolysis In Myocardial Infarction flow grade
- **TIMI** = Thrombolysis In Myocardial Infarction

Intracoronary injection of the calcium cross-linked alginate solution. In the first pilot experiment, incremental volumes (6, 8, and 10 ml) were injected into the left coronary system by a diagnostic 6-F Judkin's right coronary artery catheter through the left main coronary artery of 5 pigs. The alginate solution was injected manually at pressure and rate similar to contrast media injection during coronary angiography. This pilot experiment showed that intracoronary injection of a volume <10 ml of alginate solution is feasible.

In the next experiment, we tested the feasibility of delivering alginate solution into the infarct by intracoronary injection. One animal died during anesthesia, before MI induction, and thus the final procedure was performed in 8 swine (7 treated with alginate biomaterial and 1 with saline). In this experiment, the administration was similar to intracoronary injection of bone marrow cells (19,20). In brief, 4 days after MI, an over-the-wire angioplasty balloon was inflated at the occlusion site. Calcium cross-linked alginate solution (2 and 4 ml) or saline (2 ml) was infused during 3-min balloon inflation via the guidewire lumen. The balloon was then deflated and the myocardium reperfused for 3 min. The cycle was repeated once to deliver the total volume of alginate solution or saline. After the last infusion, a coronary angiogram was performed to assess perfusion in the target artery.

In the next experiment, either calcium cross-linked alginate solution (2 or 4 ml) or saline (2 ml) was injected into the left coronary artery of normal swine. In this experiment, 3 blood tests for troponin-I levels were obtained before, 12 to 24 h after, and 72 h after injection, after which the animals were killed for post-mortem histology.

In the final safety experiment, 2 ml of alginate solution was injected into 3 animals (3 days after MI), and 6 and 8 ml of alginate solution was injected into 2 normal animals. Intracoronary injections were performed using a multifunctional probing catheter (Multifunctional Probing Catheter, Boston Scientific, Galway, Ireland). Three blood tests for troponin-I levels were obtained before, 12 to 24 h after, and 72 h after injection. The computed tomography (CT) angiography was performed using a 64-slice multidetector computed tomography scanner (Brilliance 64, Philips Medical Systems, Cleveland, Ohio) 1 day before and 2 days after injection. The head, chest, abdomen, and pelvis were

scanned for possible perfusion defects suggestive of brain or abdominal parenchymal infarcts or pulmonary emboli.

The second set of experiments was designed to determine the optimal volume and efficacy of intracoronary injection of alginate solution into the infarct. Three to 4 days after MI, either alginate solution (1, 2, and 4 ml) or saline (2 ml control) was injected in a single bolus distal to the site of the LAD coronary artery occlusion by a microcatheter.

Angiographic coronary mapping was performed during the MI procedure, before and 3 min after the injection (day 3), and after 60 days. Angiographic images were recorded and analyzed in a blinded fashion to assess Thrombolysis In Myocardial Infarction (TIMI) flow grade (TFG) (21), and myocardial perfusion grade (MPG) (22).

Echocardiography to assess LV remodeling and systolic and diastolic properties. Echocardiography was performed as part of a pilot study aimed to determine the optimal volume of injectable alginate solution. Echocardiography was performed under anesthesia, before MI, 3 to 4 days after MI, and then at 30 and 60 days after MI, using a phased-array transducer with an ultrasound system (GE [Buckinghamshire, United Kingdom] Logiq book XP, equipped with S-RS [2–3.6 MHz broadband] phased array cardiac transducer). Images were recorded and end-diastolic and end-systolic frames were selected from standard apical and parasternal views. To evaluate diastolic properties, pulsed-wave Doppler interrogation of mitral inflow was performed in a 4-chamber view. Transmitral flow velocities were recorded by positioning the sample volume at the level of the tip of the mitral valve leaflets. The peak of the transmitral flow velocity pattern, the E-wave (early diastolic filling), and the A-wave (late diastolic filling) were measured. Each variable was quantified by averaging the measurements obtained from 3 consecutive steady-state beats.

Post-mortem morphometric and histological analysis. Histological analysis was performed on hearts from all experiments. After completion of the in vivo studies, the hearts were arrested with potassium chloride, and rapidly excised. A rubber balloon was inserted into the LV and filled with saline to maintain constant intracavity pressure during formalin (10%) fixation. After fixation, the hearts were sectioned into 6 to 8 transverse slices (5 mm each) parallel to the atrioventricular ring. Post-mortem morphometric analysis (scar thickness) was performed on the slice obtained at the level of the papillary muscle. In the CT angiography study, analysis was also performed on representative specimens from the, kidneys, lungs, liver, spleen, and brain.

Cubes of tissues from each slice were fixed with 10% buffered formalin, embedded in paraffin, and sectioned into 5-μm slices. In the first experiment, the slides were immunostained with avidin-peroxidase (Vector Laboratories, Burlingame, California) to detect the biotin-labeled calcium cross-linked alginate. In other experiments, serial sections were stained with hematoxylin and eosin and immunolabeled with antibodies against α-smooth muscle actin isoform (Sigma-Aldrich, St. Louis, Missouri) and von Kossa staining for calcium (Diagnostic BioSystems, Pleasanton, California).

Statistical analysis. Statistical analysis was performed with SPSS version 15.01 (SPSS Inc., Chicago, Illinois). All variables are expressed as mean ± standard error of the mean. Normality was tested with the Kolmogorov-Smirnov test. If normally distributed, differences between baseline and 8 weeks in the same group were assessed with 2-tailed paired Student t tests. Percent of change from baseline echocardiography measurement was calculated for each animal as: ([follow-up parameter (−) baseline parameter]/baseline parameter) × 100. Normality of change was evaluated based on the Shapiro-Wilk test, as well as evaluated graphically by the QQ plots. The difference between means of groups was compared by analysis of variance test. If normality was not found, differences between treatments were evaluated by the nonparametric approach of Kruskal-Wallis. To test the hypothesis that changes in measures of

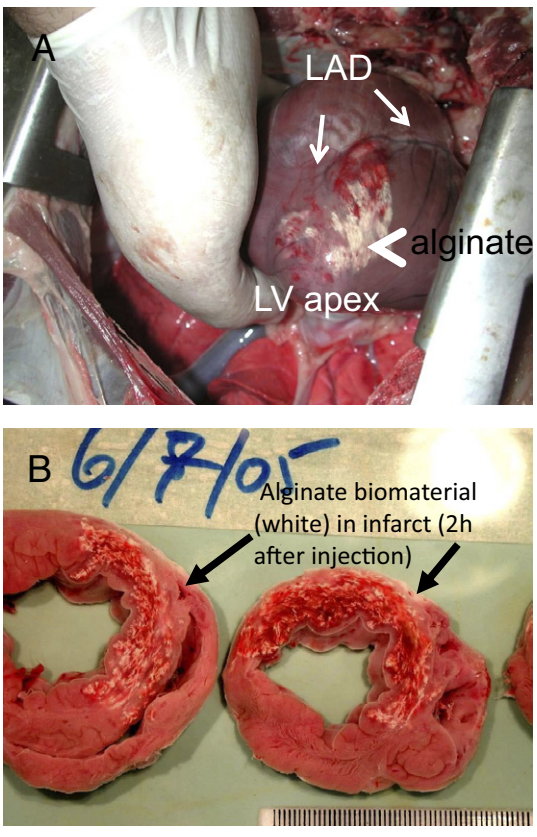

Figure 1 **Macroscopic View of the Infarcted Heart After Intracoronary Injection of Calcium Cross-Linked Alginate Solution**

(A) Two hours after injection, the alginate hydrogel was identified on the infarct surface **(arrows)**. **(B)** Macroscopic view of heart sections revealed hemorrhagic infarct and in situ deposition of the alginate hydrogel **(white areas, arrows)** and consistent distribution of the biomaterial in the granulation tissue. LAD = left anterior descending; LV = left ventricular.

LV variables over time varied among the experimental groups, a general linear model 2-way repeated-measures analysis of variance was used (23). The model included the effects of treatment, time, and treatment-by-time interaction and was followed by a Bonferroni post hoc test. Finally, a linear mix model was applied to analyze the effect of treatment on diastolic properties over time.

Results

Overall, 58 domestic swine were included in 2 sets of experiments. After exclusion of animals who either died during induction of anesthesia or MI (n = 22) or had an inadequate echocardiography image (1), the final analysis included 35 swine. The first set of experiments, designed to determine feasibility, safety, and efficacy, included 16 swine, whereas the second set, designed to determine the optimal volume of injectable alginate implant and its effect on LV remodeling, included 19 swine.

Intracoronary injection formed hydrogel scaffold in the infarcted myocardium. In the experiments designed to determine feasibility and efficacy, transient (60-min) mid-LAD coronary artery occlusion produced significant ST-segment elevation MI as assessed by visual inspection of heart sections: >25% of the LV at the anterior, apical, septal, and right ventricular apex. Four days after MI, we did not observe ischemic ECG changes, conduction blocks, or any type of arrhythmias during and after injection of alginate solution, and there was no mortality after injection.

Two hours after injection, alginate hydrogel could be identified (in a few animals) by visual inspection on the surface of the heart (Fig. 1A). After cross-sectioning, alginate hydrogel was identified as white palpable areas in the infarct of all treated animals (Fig. 1B). In animals treated with biotin-alginate, peroxidase-avidin staining revealed extensive areas of positive brown staining in the infarcted tissue (Figs. 2A and 2C), indicating efficient delivery and deposition of the biomaterial. No positive brown staining was found in control, saline-treated hearts (Fig. 2B). In 1 animal, the biotin-labeled alginate was also embedded at the ECM of the infarct border zone but did not affect viable cardiomyocytes (Fig. 2D). There were no remnants of alginate in either the vascular tree or remote viable myocardium. Furthermore, we detected no intravascular microthrombi by post-mortem histology.

Together, these findings showed that intracoronary delivery of calcium cross-linked alginate solution is both feasible and safe, and that alginate biomaterial diffuses from the leaky coronary microvasculature and deposits in the infarcted myocardium.

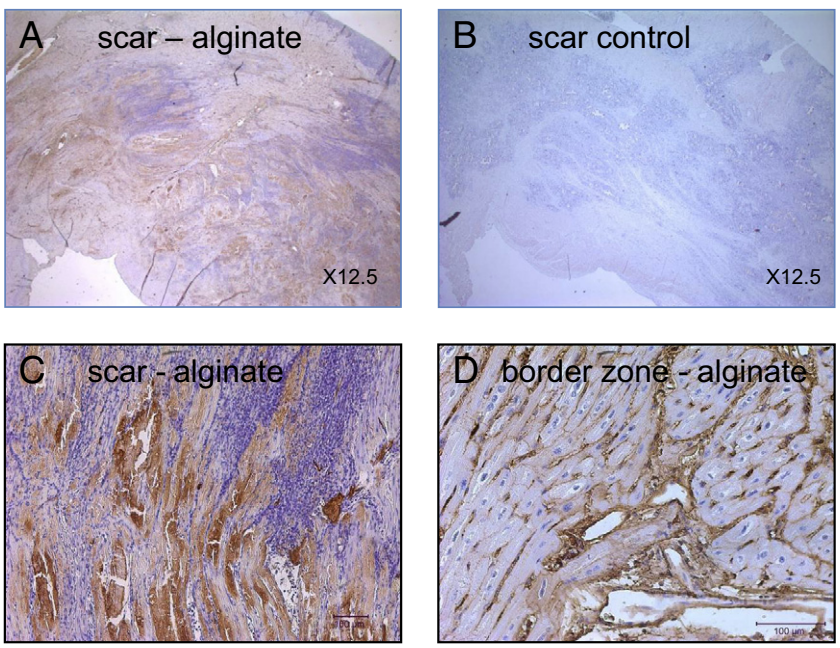

Figure 2 **Microscopic View of Infarcted Myocardium, 2 h After Intracoronary Injection of 2 ml Biotin-Labeled Alginate Solution or Saline**

Upper panels show low-power (×12.5) microscopic view of infarcted myocardium 2 h after intracoronary injection of 2 ml biotin-labeled alginate solution **(A)** or saline **(B)**. **(A)** Positive brown staining confirms extensive and effective delivery of the alginate solution into the infarcted heart. Nuclei of cardiac cells are stained blue by hematoxylin. **(B)** Positive brown staining was absent in saline-treated hearts (×12.5). **(C)** High-power (×100) view of **A**. Note typical inflammation after myocardial infarction. Positive brown staining of avidin-biotin complex indicates effective delivery of the biotin-labeled alginate solution into the infarcted heart. **(D)** In 1 specimen, the alginate biomaterial **(brown staining)** was embedded at the extracellular matrix of the border zone, and did not affect viable cardiomyocytes (×200).

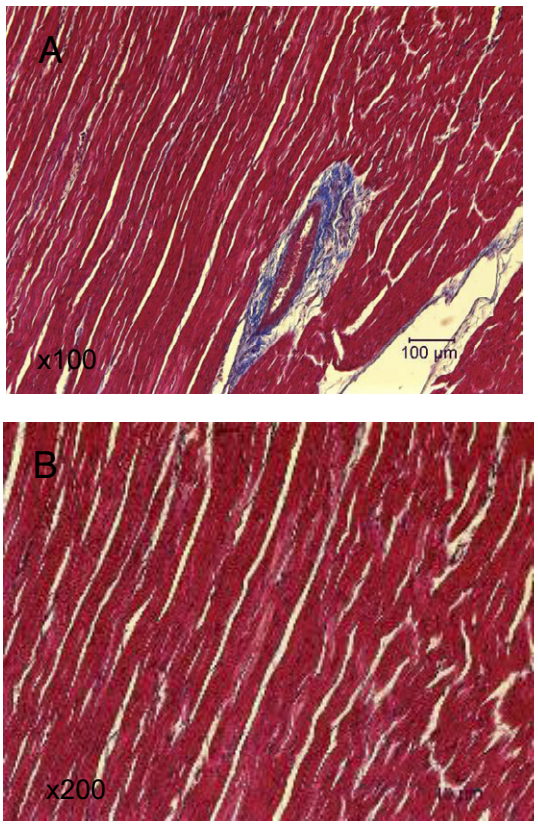

Figure 3 **Masson Trichrome Staining in Naive Pig Hearts, 72 h After Alginate (2 ml) Injection**

Viable myocardium is stained bright red. Fibrosis should be stained bright blue. **(A)** There are no signs of fibrosis in samples from animals treated with intracoronary injection of alginate solution (×100). Only the connective tissue that surrounds the artery (tunica adventitia) is stained blue. **(B)** Higher magnification (×200) confirmed that intracoronary injection of alginate solution does not injure the myocardium.

No harm with intracoronary injection of alginate biomaterial to normal heart. The next experiment was designed to determine the effect of intracoronary injection of alginate biomaterial in normal heart. Domestic swine (n = 3) were treated with intracoronary injection of alginate solution (2 or 4 ml) or normal saline (2 ml) delivered into the left main coronary artery. During and after injection, no ischemic ECG changes, conduction blocks, or other types of arrhythmias were observed. Serial troponin-I measurements before, 12 to 24 h after, and 72 h after biomaterial injection) were within normal range. Three days after injection, post-mortem histological examination of swine hearts and Masson trichrome staining of representative slides showed normal myocardium without any evidence of biomaterial deposition, necrosis, inflammation, or fibrosis at the territory of injection (Fig. 3). No intravascular thrombi were detected.

Intracoronary injection of alginate biomaterial does not produce remote infarcts. Three MI (day 3) and 2 normal swine received intracoronary injection of 2 ml of alginate solution. Two normal swine received 6 and 8 ml of alginate without elevated levels of troponin-I, 12 to 24 and 72 h after injection. The CT angiography in both MI and normal swine excluded distal embolization of the biomaterial and remote infarcts in the brain, lungs, kidneys, spleen, gut, and liver. These findings were confirmed by post-mortem histology with no evidence of intravascular thrombi or damage to the heart, lungs, kidneys, liver, spleen, lymph nodes, and bone marrow.

Intracoronary injection of alginate biomaterial prevents LV remodeling after MI. The second set of experiments (90-min coronary artery occlusion) was designed to determine the optimal volume of alginate implant that improves LV remodeling after MI. The changes in echocardiography variables from baseline (day 3 after MI before injection) to 30 and 60 days after MI are presented in Table 1 and Figure 4. At 60 days, control swine experienced a significant increase in LV diastolic area (44 ± 8%; p = 0.01), LV systolic area (45 ± 24%; p = 0.18), and LV mass (35 ± 11%; p = 0.02) from baseline. In contrast, intracoronary injection of alginate biomaterial prevented and even reversed LV enlargement. For example, after 60 days, intracoronary injection of 2 ml of biomaterial reversed LV diastolic and systolic dilation (p < 0.01 compared with control). Furthermore, 2 ml of alginate injection attenuated the increase in LV mass, compared with control (−2 ± 8% vs. 35 ± 11%; p = 0.02). Similarly, injection of 4 ml alginate (n = 4) reversed enlargement of LV (p < 0.01 compared with control).

Table 1 **Effect of Various Volumes of Injectable Alginate Biomaterial on Changes (Δ) in LV Parameters From Baseline to 30 and 60 Days After MI**

	Control (n = 4)		1 ml Alginate Biomaterial (n = 4)		2 ml Alginate Biomaterial (n = 7)		4 ml Alginate Biomaterial (n = 4)		p Value (ANOVA)	
	Δ30–3 Days	Δ60–3 Days	Δ30–3 Days	Δ60–3 Days	Δ30–3 Days	Δ60–3 Days	Δ30–3 Days	Δ60–3 Days	Δ30–3 Days	Δ60–3 Days
LV diastolic area	19 ± 6	44 ± 8	14 ± 10	20 ± 17	1 ± 4	−1 ± 4	−1 ± 2	−6 ± 4	0.11	0.006
LV systolic area	25 ± 16	45 ± 24	19 ± 23	1.6 ± 8	−1 ± 4	−13 ± 4	2.4 ± 1	−9 ± 10	0.35	0.02
LV mass	−8 ± 14	35 ± 11	29 ± 16	73 ± 36	−7 ± 7	−2 ± 8	−16 ± 14	11 ± 18	0.28	0.049
Fractional shortening	−2 ± 13	5 ± 20	6 ± 14	20 ± 17	10 ± 10	31 ± 20	−3 ± 6	15 ± 18	0.81	0.81

Values are %.

ANOVA = analysis of variance; Fractional shortening = [(LV diastolic dimension − LV systolic dimension)/LV diastolic dimension]; LV = left ventricular; MI = myocardial infarction.

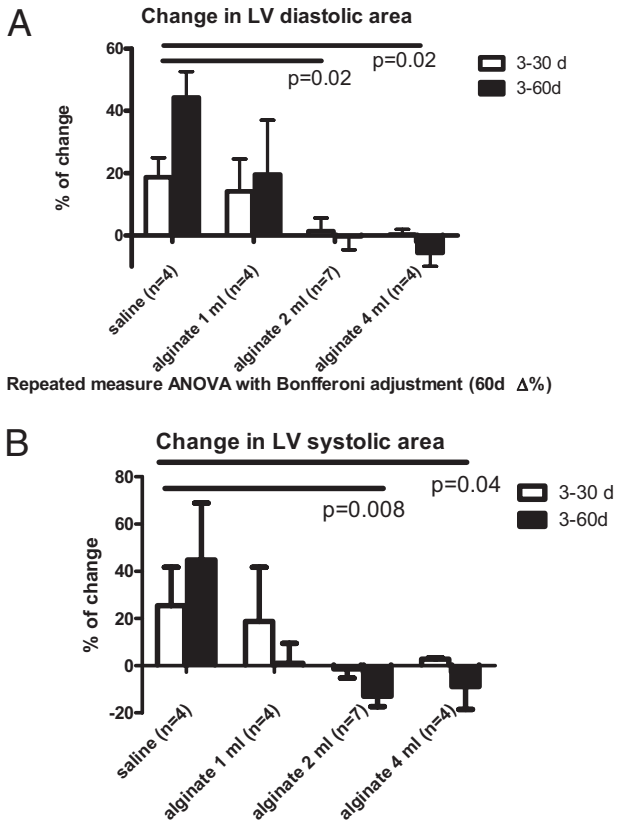

Figure 4 **Effect of Intracoronary Injection of Various Volumes of Alginate on LV Dilation 30 and 60 Days After MI**

Comparison of the therapeutic effects of intracoronary delivery of 1, 2, and 4 ml of alginate solution or saline (2 ml) into recent (4-day-old) scar. All volumes of alginate attenuate or prevent left ventriculat (LV) diastolic (**A**) and systolic (**B**) dilation compared with control. Relative change was calculated as ([30 or 60 day measure – 3 day measure]/3 day measure) × 100. Individual values and mean (±SEM). The p values are for treatment effect versus control by repeated-measures analysis of variance (ANOVA) and Bonferroni post hoc test adjusted for multiple comparisons. MI = myocardial infarction.

Animals treated with 1 ml (n = 4) alginate biomaterial experienced a mild favorable effect on LV diastolic and systolic area (p = 0.32 and p = 0.13, compared with control). Thus, the protective effects on LV remodeling were more prominent in animals treated with 2 and 4 ml of alginate biomaterial (Fig. 4).

During a 60-day follow-up, satisfactory transmitral Doppler echocardiography measurements were obtained in animals treated by 2 (n = 7), 1 (n = 2), and 4 ml (n = 2) of alginate or saline (n = 3). During follow-up, control animals maintained a trend for a restrictive LV filling pattern with an increased E/A ratio (Fig. 5). In contrast, this trend was decreased in animals treated with 2 ml of alginate (p = 0.02 for treatment over time interaction) (Fig. 5).

Effect on coronary artery flow and myocardial perfusion. High-quality angiography for TFG and MPG analyses was available from 17 of the 19 MI swine included in the volume determination study. The TFG and MPG, immediately after alginate biomaterial delivery and 60 days later, were assessed in a blinded manner. Intracoronary injection (1 and 2 ml) did not affect coronary flow and myocardial perfusion as assessed by TFG and MPG (Fig. 6). Only 2 swine (1 control and 1 from the 4 ml biomaterial group) had impaired perfusion after intracoronary injection. Thus, injection of 1 and 2 ml of alginate solution does not affect coronary blood flow in either epicardial coronary arteries or in myocardial microcirculation.

Injectable alginate biomaterial increase scar thickness. Postmortem morphometric analysis of the heart sections at the level of the papillary muscles was performed 2 months after biomaterial injection (Fig. 7). No remnants of alginate or infarct calcifications were found by both echocardiography and von Kossa calcium staining. Alginate biomaterial (2 ml) increased scar thickness by 53% compared with control (2.9 ± 0.1 mm vs. 1.9 ± 0.3 mm; $p < 0.01$), and anterior wall thickness by 34% (9.1 ± 0.5 mm vs. 6.8 ± 0.9 mm; p = 0.03). Histological examination showed accumulation of myofibroblasts at the scar tissue without remnants of the biomaterial (Fig. 8A). Examination of control heart sections treated with saline showed positive staining for α-smooth muscle actin predominantly on the vessel walls (Fig. 8B).

Discussion

The present study shows that intracoronary injection of alginate biomaterial is feasible and effective in preventing adverse cardiac remodeling in swine. Our model mimics the scenario of ST-segment elevation MI with coronary reperfusion, and the injection of alginate biomaterial 4 days after MI with 60 days of follow-up is relevant to the healing and repair phase after MI. After intracoronary injection, the alginate solution disseminates through the leaky vessels into the infarct and undergoes phase tran-

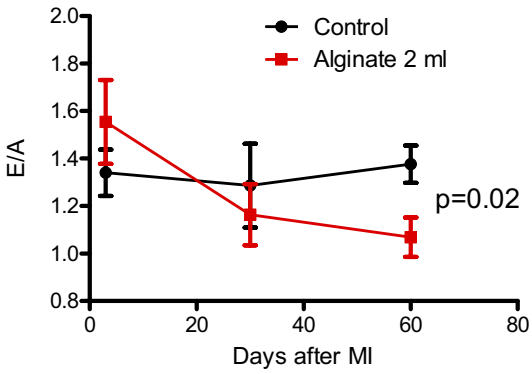

Figure 5 **Diastolic Properties Were Improved in Alginate Biomaterial-Treated Animals by Doppler Echocardiography**

Diastolic properties were evaluated by serial Doppler echocardiography tests. During a 60-day follow-up, control animals maintained a trend for restrictive LV filling pattern presented by increased E/A ratio. In contrast, the E/A ratio decreased in alginate-treated animals (p = 0.02 for treatment over time interaction by a linear mix model). Abbreviations as in Figure 4.

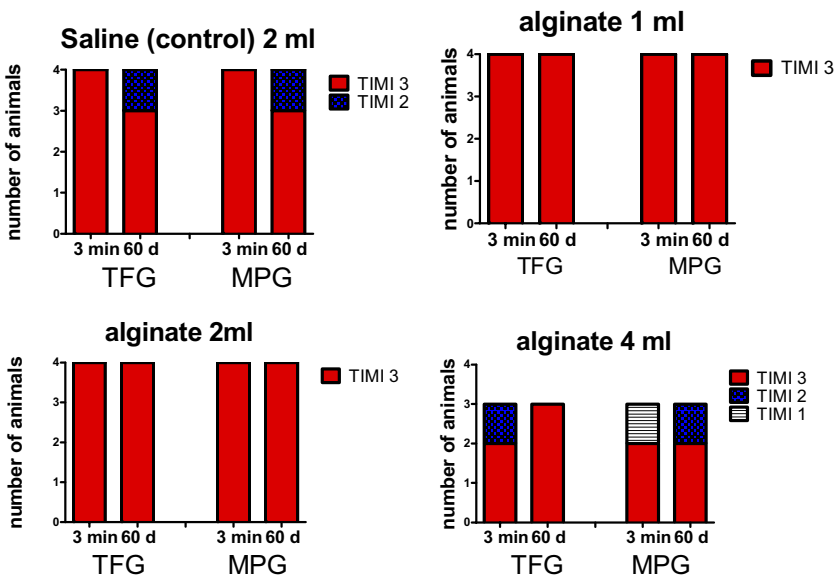

Figure 6 **Alginate Solution (1 and 2 ml) Does Not Affect TFG and MPG 3 Min and 60 Days After Intracoronary Injection**

MPG = myocardial perfusion grade; TFG = Thrombolysis In Myocardial Infarction flow grade; TIMI = Thrombolysis In Myocardial Infarction.

sition into hydrogel. Both replacement of the damaged ECM in the infarcted tissue and the scaffolding effect of the alginate hydrogel provide physical support to the infarcted tissue, enhance healing, and prevent LV dilation. The implant was gradually replaced by myofibroblasts and connective tissue. An increased number of myofibroblasts in the scar is considered a marker of improved healing (24), and is found after biomaterial or

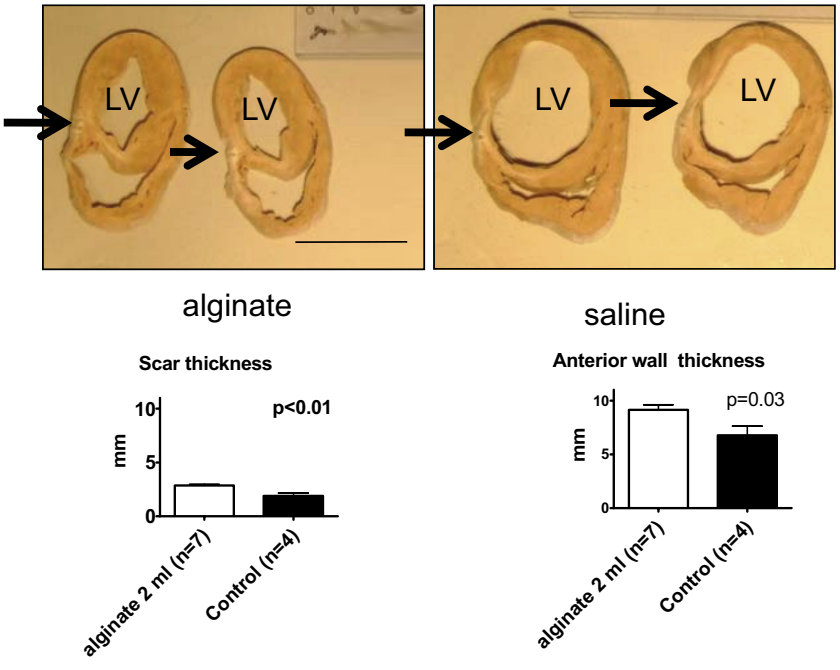

Figure 7 **Alginate Implant Increases Scar Thickness by Post-Mortem Morphometry, 60 Days After MI**

Representative sections of heart treated with alginate **(upper left panel)** or saline **(upper right panel)**. Morphometry shows that injection of alginate solution (2 ml) increases scar thickness **(arrows)** as well as anterior wall thickness **(lower panels)**. Abbreviations as in Figure 4.

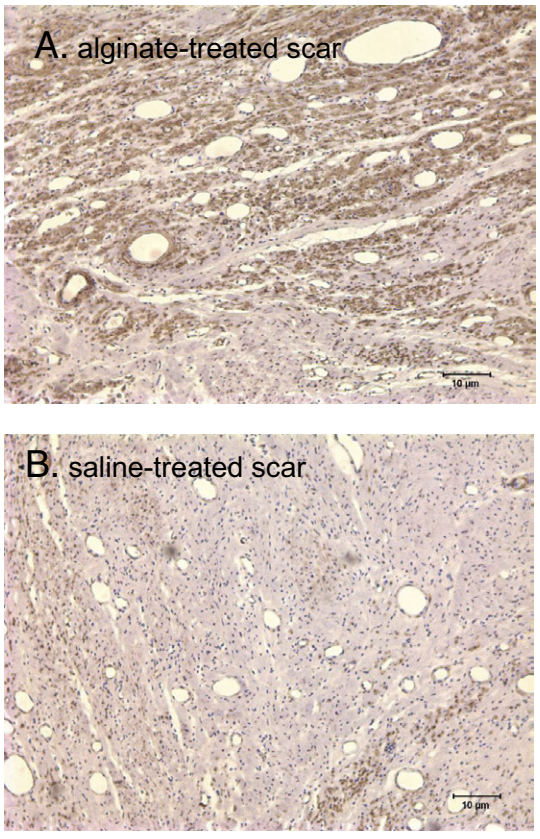

Figure 8 **Representative Micrographs of Infarcted Hearts After Immunostaining for α-SMA, 60 Days After Either Alginate or Saline Injection**

(A) Examination of the scar tissue showed that the alginate-treated scar is populated with numerous myofibroblasts that were stained positive for α-SMA. **(B)** Examination of control sections treated with saline showed positive staining for α-SMA predominantly on the vessel walls. SMA = smooth muscle actin.

cell therapy (13,25–27). Thus, injectable alginate biomaterial supports local infarct healing while limiting more generalized myocardial remodeling.

Injectable biomaterials for heart repair. Progressive thinning and enlargement of the infarct zone (infarct expansion) are events that occur during the first days after MI (2). Injectable biomaterials can reduce wall stress by increasing scar thickness and stabilizing chamber size (6,8,9,12,28). By thickening the scar, wall stress is reduced (Laplace law) and the degree of outward motion of the infarct that occurs during systole (dyskinesis) is also reduced. This effect is analogous to an external device that constrains the myocardium from expanding (29). Injectable biomaterial could also repair or prevent mechanical complications after MI. A recent preliminary study showed that injection of polyvinyl alcohol polymer into inferior MI in 6 sheep resulted in decreased severity of acute mitral regurgitation (MR) compared with baseline (30). Using injectable alginate solution in a dog model of inferior MI (n = 30) showed that the severity of ischemic MR can be reduced during a 6-month follow-up (31). This new approach offers a percutaneous alternative for relieving ischemic MR by correcting papillary muscle position and relieving the tethering that causes ischemic MR. Finally, injectable biomaterial can also create an improved environment for myocardial repair (6,10), as well as a platform for controlled delivery of therapeutic genes and proteins (32–35).

Intracoronary administration of alginate biomaterial solution. Ideally, injectable biomaterial for myocardial repair should possess the following characteristics: 1) gels at the infarct site only; 2) rapid, controllable gelation without detrimental effects on myocardial function and remote organs; 3) sufficient tissue-bulking properties to support damaged myocardium; 4) bio-erodible; 5) nonimmunogenic; and 6) nonthrombogenic and can be injected selectively into the infarct-related artery.

Alginate, a polysaccharide found in brown seaweed, meets many of these criteria. It has been used extensively in the food, pharmaceutical, and medical device industries. It is biocompatible, and its cross-linked hydrogel form is similar in structure to that of ECM (17). The injectable biomaterial is delivered into the infarcted tissue through damaged, permeable vessels in the infarct zone. In contrast, when alginate solution was delivered into normal heart, with intact coronary vessels, it did not penetrate into the myocardium or into remote organs with intact vessels. The remnants of the biomaterial that did not deposit in the heart dissolved in the bloodstream and were excreted by the kidneys (15). To partially mimic the clinical scenario, animals were treated with aspirin throughout the study and with clopidogrel up to the day of alginate injection. No intravascular thrombi were detected by post-mortem histology in infarcted or in normal hearts. This may be explained by the nonthrombogenic properties of alginate (16), and by the fact that physiologic concentrations of calcium in the blood are insufficient for intravascular conversion of alginate solution into gel. In the present study, we show that intracoronary injection of 2 ml of alginate solution is more effective than 1 ml, showing similar beneficial effects to that of 4 ml in preventing LV remodeling.

Study limitations. First, the functional experiments were designed to determine the optimal injectable volume of alginate implant. Thus, our functional findings should be considered preliminary and interpreted with caution. Furthermore, because of the relatively small number of animals in each group, differences between some variables did not achieve statistical significance. However, our findings were recently confirmed by another group using a dog model of MI (31). Second, the absence of cardiac magnetic resonance imaging data is a weakness. Cardiac magnetic resonance imaging is rapidly becoming the standard reference modality for assessment of LV dimensions, global LV function, and myocardial mass in pre-clinical research. Third, the effect of injectable alginate biomaterial on diastolic dysfunction is uncertain and needs to be substantiated by hemody-

namic measurements of LV end-diastolic pressure. In the present study, Doppler echocardiography measurements were used for estimating diastolic properties and suggest a possible benefit from the injection of biomaterial into a recent infarct. This finding is in accord with the improved LV remodeling and systolic function in biomaterial-treated animals, as well as our previous experiments in rats (13). However, a bias may have been created because Doppler measurements were not available in all swine because of technical difficulties.

Finally, our pig model mimics ST-segment elevation MI with early reperfusion, a scenario that is generally associated with improved infarct healing and LV remodeling (36). It would be practical to test the efficacy of our approach in a large animal model of permanent coronary occlusion with extensive infarct. Nevertheless, based on our work in a rat model of extensive MI (13), we predict that this approach would be effective in a large infarct.

Conclusions

Our experiments provide a novel catheter-based strategy of injectable, bioresorbable alginate implant to improve cardiac remodeling after MI. Biomaterial injection has several advantages over current approaches to treatment of LV remodeling after MI. The injectable implant increases scar thickness and provides physical support for improved healing and repair. The ability to deliver biomaterial into the infarct by intracoronary injection can revolutionize patient treatment after MI and could prevent mechanical complications, heart failure, and death.

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