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Efficacy of intramyocardial injection of Algisyl-LVR for the treatment of ischemic heart failure in swine

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Abstract

Background: Progressive thinning and dilation of the LV due to ischemic heart failure (IHF) increases wall stress and myocardial oxygen consumption. Injectable biopolymers implanted in the myocardial wall have been used to increase wall thickness to reduce chamber volume, decrease wall stress, and improve cardiac function. We sought to evaluate the efficacy of a biopolymer (Algisyl-LVR) to prevent left ventricular (LV) remodeling in a swine model of IHF.

Methods: IHF was induced in 11 swine by occluding the marginal obtuse branches of the left circumflex artery. Eight weeks later, Algisyl-LVR was injected into the LV myocardial free wall in five of the 11 animals. Echocardiographic examinations were done every 2 weeks for 16 weeks.

Results: Within eight weeks of treatment, the ejection fraction increased from $30.5\% \pm 7.7\%$ to $42.4\% \pm 3.5\%$ (treated group) vs. $37.3\% \pm 3.8\%$ to $34.3\% \pm 2.9\%$ (control), $p < 0.01$. Stroke volume increased from 18.5 ± 9.3 mL to 41.3 ± 13.3 mL (treated group) vs. 25.4 ± 2.3 mL to 31.4 ± 5.3 mL (control), $p < 0.05$. Wall thickness in end-diastole of the infarcted region changed from 0.69 ± 0.06 cm to 0.81 ± 0.13 cm (treated group) vs. 0.73 ± 0.09 cm to 0.68 ± 0.11 cm (control), $p < 0.05$. Sphericity index remained almost unchanged after treatment, although differences were found at the end of the study between both groups ($p < 0.001$). Average myofiber stress changed from 16.3 ± 5.8 kPa to 10.2 ± 4.0 kPa (treated group) vs. 15.2 ± 4.8 kPa to 17.9 ± 5.6 kPa (control), $p < 0.05$.

Conclusions: Algisyl-LVR is an effective strategy that serves as a micro-LV assist device to reduce stress and hence prevent or reverse maladaptive cardiac remodeling caused by IHF in swine.

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Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijcard.2017.09.179>.

Keywords

Myocardial infarction; LV remodeling; Myofiber stress; Algisyl; Biopolymers; Stress-related miRNA

1. Introduction

Heart failure (HF) is considered a growing epidemic and hence an important health problem in the US and worldwide because of its high prevalence {5.8 million and 23 million, respectively [1]; which is expected to increase by 25% in the US alone by 2030 [2]}. HF is associated with high morbidity and mortality, has a 5-year mortality rate of 50%, and contributes considerably to the overall cost of health care {\$53.1 billion in the US by 2030 [2]}. Despite progress in managing HF using better medical and device therapies, the incidence continues to increase because the population is aging. For patients with end-stage HF, the only viable option is either a mechanical assist device or a heart transplant, the numbers for which are meager – only 4196 cardiac transplants were performed in 2012 worldwide [3]. Novel therapeutic strategies that improve mortality as well as quality of life for HF patients are therefore a critical priority. Recently, cardiac tissue engineering approaches like injectable biopolymers (e.g., hydrogels) have become a major research focus, and have begun to provide potential ways of treatment for HF, particularly, in post myocardial infarction (MI) HF or ischemic HF.

The complicated pathological changes that follow MI include an initial inflammatory response [4], loss of cardiomyocytes [5–6] and degradation of the LV extracellular matrix by matrix metalloproteases [7–8], progressively leading to LV remodeling [9]. LV remodeling involves wall thinning, infarct expansion at the borderzone [10], scar tissue formation, and eventual LV dilatation followed by ventricular dysfunction [11–12], altering the cellular, structural and mechanical properties of the heart [13–15].

Experiments in large [16–18] and small [19–21] animals, clinical evaluations in humans [22–24], as well as computational models [25–27] have all suggested that injection of biomaterials can limit LV remodeling post MI and improve LV function. Unlike other surgical approaches that modify the size of the dilated ventricle, myocardial injection with biopolymers decreases wall stress and improves regional and global LV function [28].

Reduction of LV wall stress is considered a cornerstone in the treatment of HF. The objective of the present study was to evaluate a therapy for ischemic HF by creating a swine heart ischemic model of HF (at the level of large coronary artery occlusion) and stabilizing the LV remodeling process with injection of biopolymer (Algisyl-LVR) into the LV free wall of a swine failing heart.

2. Methods

2.1. Animal preparation

All animal experiments were performed in accordance with national and local ethical guidelines, including the Guide for the Care and Use of Laboratory Animals, the Public

Health Service Policy on Humane Care and Use of Laboratory Animals, and the Animal Welfare Act, and an approved California Medical Innovations Institute IACUC protocol regarding the use of animals in research.

Eleven male Yorkshire domestic swine, divided in two groups, experimental ($n = 5$) and control ($n = 6$), with body weight of 42.7 ± 1.9 kg were used in this study. The animals were housed at California Medical Innovations Institute – Animal Care Facilities. The pigs had ad libitum access to water and were fed a commercial diet (Teklad 8753). A room temperature of 68–72 °F and humidity of 30% to 70% were maintained. The animals were carefully checked for preexisting diseases and acclimated for a minimum of three days before undergoing the surgical procedures. The pigs were fasted overnight. Sedation was achieved with TKX (Telazol 10 mg/kg, Ketamine 5 mg/kg, Xylazine 5 mg/kg; IM) and surgical anesthesia was maintained with isoflurane 1–2%. Ventilation with 100% O₂ was provided with a respirator and maintained PCO₂ at approximately 35 mm Hg. Electrocardiographic (ECG) leads were attached to the animal's limbs and cardiac electrical signals were monitored on a Physio-Control Lifepak 12 defibrillator. Body temperature was maintained at 36.5 °C–37.2 °C with a heating pad, and pH at 7.4 ± 0.1 .

2.2. Swine model of heart failure—occlusion of the obtuse marginal branches of the LCX artery

Under sterile conditions, introducer sheaths were percutaneously inserted into the jugular vein to permit administration of fluids and drugs, and the right femoral artery, for pressure monitoring and coronary artery access. Heparin 100 IU/kg was administered IV before instrumentation and was then supplemented as needed to keep an activated clotting time over 200 s. The left circumflex (LCX) artery was accessed using the percutaneous femoral approach. A microcatheter (Cantata 2.9, Cook Medical, Bloomington, IN) was used to access the obtuse marginal branches of the LCX to deliver one to three 2- or 3-mm diameter embolization coils (depending on the anatomical variation in each animal) at two different time points (week 0 and week 2). To decrease the mortality rate due to sudden arterial occlusion, the animals were subjected to four (5, 10, 15, and 20 min. - ischemic preconditioning) balloon occlusions followed by 3-minute reperfusions each time before permanent occlusion with embolization coils. Ventricular arrhythmias were managed with Lidocaine (1–1.5 mg/kg, IV), Amiodarone (30 mg, IV, every 5 min $\times$ 5 times, before ischemic preconditioning, followed by 0.5 mg/min, IV), and magnesium sulfate (3–20 mg/min, IV). After the procedure, all animals received Amiodarone 200 mg, PO, daily, for 4 weeks. The experimental animals recovered for eight weeks before treatment with the polymer. The control animals recovered for sixteen weeks and were not treated with the polymer.

2.3. Left ventricular free wall polymer injection

Eight weeks after MI, the animals underwent the polymer injection procedure with Algisyl-LVR, which consisted of a suspension of insoluble calcium alginate particles and a solution of sodium alginate. Upon mixing, the hydrogel achieved its final material strength of 3–5 kPa within a halftime of 3–4 min.

The chest was opened through a midline sternotomy and an incision was made in the pericardium with the creation of a sling to support the heart. A line was traced circumferentially with a skin marker on the LV free wall, halfway between the base and the apex of the heart, starting on the anterior wall and ending on the posterior wall. A 27-gauge needle was used to make six to seven intramyocardial injections of Algisyl-LVR (0.3 mL each) 1 cm superior and 1 cm inferior, respectively to the circumferential line for a total of 12–14 injections. These injections were made 1 to 1.5 cm apart in a zig-zag pattern between the superior and inferior lines. Intramyocardial polymer injection was associated with ventricular arrhythmias that subsided after the needle was removed. Anti-arrhythmic drugs were not needed. The animals were allowed to recover for eight additional weeks.

2.4. Echocardiography

Transthoracic and transesophageal echocardiograms were obtained in all animals using an iE33 ultrasound system (Philips, Andover, MA) with S5-I and X7–2t transducers, respectively, including one echocardiogram the day of the first intervention (baseline) and one echocardiogram every two weeks until the end of the study. All echocardiographic images were acquired with the animal placed in the supine and left lateral decubitus positions. Images (2D and 3D) were taken in the short axis and long axis views. LVEDV, LVESV, LVSV, LVEF, and LV wall thickness were calculated offline using QLAB 10 (Philips, Andover, MA). Myocardial wall thickness measurements of the infarcted region were obtained on short axis images at the level of the papillary muscles after manually tracing the epicardial and endocardial borders of the LV. The result was the average of the distance from endocardium to epicardium measured at the posterior and lateral walls (infarcted region).

A 3D volumetric analysis was performed offline using commercially available software (TomTec 4D LV-Function, Version 4.6, Build 4.6.3.9, Unterschleißheim, Germany). The long-axis views were aligned to standard apical two-, three-, and four-chamber views to eliminate foreshortening. In each of the apical planes, the positions of the mitral valve and apex were marked. Endocardial borders were manually delineated in all planes in end-diastole (ED). The software then automatically traced the contours dynamically throughout the cardiac cycle. Manual corrections (if necessary) were performed in both ED and end-systolic (ES) frames in order to include the papillary muscles and trabeculae in the chamber volume. The sphericity index (SI) was calculated as the ratio between the greatest short-axis dimension and the greatest long-axis dimension obtained from the endocardial surface in ES. The end-diastolic volume (EDV), end-systolic volume (ESV), and ejection fraction (EF) measurements were derived from the automatically constructed dynamic surface model of the LV. The exported .ucd-files with the coordinates for every frame in the cardiac cycle were converted to .stl-files for LV myofiber stress analysis as described below.

2.5. Left ventricular myofiber stress analysis

Regional myofiber stress was computed using a validated mathematical model, as described elsewhere in detail [26] and shown in a video [27], except for the following differences. First, the in-vivo LV geometries of the mathematical (finite element) models were based on echocardiography (the .stl-files described above) instead of MRI. Second, rather than

making assumptions concerning subject-specific LV pressures [26–27], the ED and ES LV pressures were based on actual measurements. Regional myofiber orientation in the unloaded state of each finite element model was rule-based [26–27]. Briefly, the unloaded finite element model was loaded with the measured ED pressure and diastolic material parameter values were determined, which allowed the ED volume to match that measured using echocardiography. The ED configuration of the model was then used to compute stress in the local myofiber direction in each finite element, and a mean volume-weighted value (to account for different sizes of the finite elements) was determined. Similarly, the unloaded finite element model was loaded with the measured ES pressure and systolic material parameter values were determined, which allowed the ES volume to match that measured using echocardiography. The ES configuration of the model was then used to compute the mean myofiber stress.

2.6. Heart preparation

On the final day of the 16-week period of the study, the heart was arrested in diastole with a saturated solution of potassium chloride, excised and transported to the lab in 0.9% sodium chloride. Transmural biopsy samples were taken from different regions of the LV for histological and PCR analyses, followed by heart fixation with buffered formalin (Carson-Millonig formulation). Additional myocardial samples were taken from the area surrounding the polymer in the ischemic region of the experimental hearts, and from the borderzone area of the control hearts, for cardiomyocytes nuclei counting.

2.7. Cardiomyocyte nuclei counting

Blocks of myocardium were embedded in tissue freezing medium in such a way that longitudinal slices (parallel to the longitudinal axis of the myocardial fiber) could be made. Sections of 7 μm -thickness were cut using a clinical cryostat (Leica CM1850), mounted on glass slides, and incubated with Wheat Germ Agglutinin, Alexa Fluor® 488 Conjugate (Invitrogen) for cardiomyocyte boundary determination (green), and Hoechst 33342 (Invitrogen) for cardiomyocyte nuclei staining (blue). Non-repeating fields were surveyed under fluorescence microscopy (Nikon TE-DH100W) for cardiomyocytes in their longitudinal axis, with no tears or sectioning artifacts, and with their nucleus/nuclei in the middle, along the long axis of the myocyte. The number of nuclei in each cardiomyocyte was counted in each field. The frequency distribution of mono-, bi-, and multinucleated cardiomyocytes was determined.

2.8. Reverse transcription and quantitative real time PCR analysis

Total RNA was purified with Trizol Reagent (Invitrogen), according to the manufacturer's instructions. To quantify microRNA (miRNA), reverse transcription and quantitative PCR (qPCR) were performed using the TaqMan® miRNA assay Kit (Applied Biosystems) as previously described [29,30]. Briefly, reverse transcription was performed in a 15 μl reaction mix containing 10 ng of total RNA, 3 μl of miRNA primer mix, 1 mM of dNTP, 50 U of reverse transcriptase, and 3.8 U of RNase inhibitor. Reactions were incubated at 16 °C for 30 min, 42 °C for 30 min, and 85 °C for 5 min. PCR was performed in a 10 μl reaction volume containing 0.5 μl of miRNA primer and TaqMan probe mix, 0.67 μl of RT product (diluted fivefold), and 5 μl of TaqMan Universal PCR Master Mix. The cycling conditions were as

follows: 10 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. U6 small RNA was used as an internal control following the manufacturer's recommendation. For all samples, reverse transcription and qPCR were performed three times, and qPCR was performed in triplicate. Relative gene expression levels between wild type and 3xTg-AD mice were determined using the comparative Ct (2^{-Ct}) method after normalizing to U6.

2.9. Statistical analysis

The statistical analysis was performed using SigmaStat 3.5 (Systat Software, Point Richmond, CA). The data were expressed as means $\pm$ SD. The differences between the various parameters and groups were evaluated using analysis of variance (ANOVA) and Student's t-test. The differences were considered significant at $p < 0.05$.

3. Results

We created a clinically relevant large animal model of IHF, and with medical management and ischemic preconditioning (as described in the Methods section) we reduced an initial mortality rate of $>50\%$ to $<30\%$.

Compared to control, myocardial injection of Algisyl-LVR significantly improved stroke volume, ejection fraction, and wall thickness, and reduced myofiber stress. As shown in Fig. 1A, the treated group showed significant improvement in stroke volume (LVSV) 8 weeks after polymer injection. The LVSV increased from 18.5 ± 9.3 mL at week 8 (day of polymer injection in the experimental group) to 41.3 ± 13.3 mL at the end of the study ($p < 0.05$). LVSV in the control group remained almost the same, 25.4 ± 2.3 mL at week 8 and 31.4 ± 5.3 mL at week 16, $p = 0.18$. The values between both groups were statistically different at week 16 (Fig. 1A, $p < 0.05$, between treated and control groups).

In both the experimental and control groups, LV ejection fraction (LVEF, Fig. 1B) decreased progressively from baseline (day of coronary occlusion) to week 8. Although the LVEF was significantly lower in the treated animals than in the control animals at baseline ($48.0\% \pm 1.9\%$ vs. $51.7\% \pm 2.9\%$, $p = 0.04$), there was no significant difference at week 8 ($30.5\% \pm 7.7\%$ vs. $37.3\% \pm 3.8\%$, $p = 0.13$). In the treated group, LVEF progressively improved after polymer injection, and continued until the end of the study, whereas in the control group, LVEF deteriorated over time ($42.4\% \pm 3.5\%$ vs. $34.3\% \pm 2.9\%$, $p < 0.01$ at 16 weeks after occlusion).

The LV end-systolic volume (LVESV) and the LV end-diastolic volume (LVEDV) in both the experimental and control groups progressively increased over time. LVESV was 32.4 ± 8.4 mL at baseline and 55.6 ± 14.0 mL at 16 weeks in the experimental group versus 29.4 ± 2.9 mL at baseline and 60.6 ± 12.3 mL at 16 weeks in the control group. There was no significant difference between both groups. LVEDV in the experimental group was 62.0 ± 14.3 mL at baseline and 96.9 ± 26.2 mL at the end of the study, whereas in the control group LVEDV was 61.2 ± 8.9 mL and 92.0 ± 16.8 mL, respectively. There was no significant difference in LVEDV between both groups.

Myocardial wall thickness increased after treatment and LV remodeling was prevented by decreased myofiber stress. Average wall thickness of the infarcted region (posterolateral LV wall) in end-diastole in the treated group increased from 0.69 ± 0.06 cm before treatment to 0.81 ± 0.13 cm at the end of the study. Conversely, wall thickness in the control group progressively decreased over time from 0.79 ± 0.02 cm at baseline to 0.68 ± 0.11 cm at the end of the study (Fig. 1C, $p < 0.05$ between treated and control groups at week 16).

The SI was calculated from 2D echocardiograms in end-systole. SI remained approximately the same in the polymer group after treatment, from 0.57 ± 0.05 the day of treatment to 0.56 ± 0.07 at the end of the study, as compared to the control group in which the SI progressively increased from 0.65 ± 0.1 at baseline to 0.69 ± 0.11 (Fig. 1D, $p < 0.001$ between treated and control groups at week 16).

The myofiber stress was calculated in end-systole and end-diastole, as well as a numerical average (Fig. 2A). The end-systolic myofiber stress in the treated group significantly decreased over time from 28.4 ± 10.7 kPa at week 8 to 17.3 ± 6.8 kPa at the end of the study. The myofiber stress in the control group, on the contrary, increased from 25.5 ± 7.7 kPa to 28.4 ± 6.4 kPa in the same period of time. The values were significantly different between both groups at the end of the study ($p < 0.05$, Fig. 2B). The end-diastolic myofiber stress in the treated group also decreased significantly from 4.2 ± 2.3 kPa at week 8 to 3.2 ± 1.9 kPa at the end of the study, but increased in the control group from 4.8 ± 2.6 kPa to 7.4 ± 4.9 kPa. The difference between treated and control groups were statistically significant at the end of the study ($p < 0.05$, Fig. 2C).

The comparative expressions of three cardiac stress-related microRNAs in the control and polymer groups and non-diseased hearts are shown in Fig. 3. miR-195 ($p < 0.05$), miR-21 ($p < 0.01$), and miR-210 ($p < 0.05$) were significantly upregulated in the control group compared to the expression of non-diseased hearts. On the other hand, compared to the control group, the expressions of miR-195 ($p < 0.05$), miR-21 ($p < 0.05$), and miR-210 ($p < 0.05$) in the polymer-treated group were significantly downregulated (close to the expression of non-diseased hearts). There were no significant differences between the polymer group and the non-diseased hearts.

Algisyl-LVR promoted myocyte division and multi-nucleation. Fig. 4 shows histological sections obtained from one representative heart from the treated group. Fig. 4A shows the encapsulated polymer surrounded by numerous myofibroblasts. Larger magnification of the histological section demonstrates a multitude of multinucleated cardiomyocytes near the capsule that surrounds the polymer (Fig. 4B), as well as new vessel formation. These multinucleated (N3 nuclei) cardiomyocytes (Fig. 4B, C and D) were observed in all treated hearts. Fig. 4C shows a cardiomyocyte with up to 14 nuclei under fluorescence microscopy, whereas Fig. 4D shows the same myocyte with its boundary clearly demarcated.

The frequency distribution of mononucleated, binucleated, and multinucleated cardiomyocytes in the treated and control groups is shown in Fig. 4E and F, respectively. A total of 1113 cardiomyocytes were evaluated in the experimental hearts, and 1109 cardiomyocytes in the control hearts. The percentages of mononucleated and binucleated

myocytes were 21.8% and 22.9%, respectively. The remaining 55.3% of cardiomyocytes were multinucleated cells with 3 to 11 nuclei, as illustrated in Fig. 4A (Mean = 3.2, Median = 3). In the control group, 68.4% of the myocyte population was mononucleated, 19.2% was binucleated, and 12.4% was multinucleated (Fig. 4B, Mean = 1.5, Median = 1).

4. Discussion

The major finding of this study was that injection of Algisyl-LVR in the free wall of the failing heart improves cardiac function and attenuates adverse ventricular remodeling by unloading the stress on myocytes, and maintains the elliptical geometry of the LV; i.e., serves as a micro-LV assist device. When Algisyl-LVR was injected eight weeks after posterolateral MI induction in swine, significant increase in LVEF, LVSV, and wall thickness of the infarcted region were observed. Although we did not find differences in LVESV and LVEDV between polymer and control groups after eight weeks of follow-up, the SI in the treated group remained almost the same, whereas it increased over time in the control group.

Adverse ventricular remodeling after MI is a process that occurs due to an increase in mechanical stress of the injured myocardial wall which leads to infarct expansion and extension of the borderzone [31–32], eccentric ventricular enlargement [15] with gradual increase in ventricular volumes, changes in chamber geometry and ventricular wall curvature [33], and ultimately, progression into HF. The creation of a translational large artery occlusion animal model of HF was important in this study to evaluate the efficacy of Algisyl-LVR in preventing or reversing cardiac remodeling. By preconditioning the targeted branches as described above and occluding the arteries in two separate procedures, we achieved an acceptable mortality rate of <30% and myocardial dysfunction (LVEF <40%) in a relatively short period of time. Furthermore, the development of this chronic swine model of IHF closely mimicked the clinical condition (acute infarction leading to cardiac dysfunction, scar formation, viable myocytes, and fibrosis). This clinically-relevant large animal model of heart failure is a significant achievement that differentiates this study from previous models, such as the canine intracoronary microembolization [34], which lacks clinical relevancy of large coronary artery disease, or the rapid ventricular pacing [35], which recovers after cessation of pacing.

Clinical studies of Algisyl-LVR suggest that treatment with Algisyl can attenuate LV negative remodeling by increasing wall thickness of the infarcted area, decreasing myocardial wall stress, and hence, improving myocardial function. The first in-human results of the safety and feasibility of Algisyl-LVR for the treatment of HF patients were reported in 2015 [23]. Of the 11 patients with advanced HF who received Algisyl-LVR injection, nine had 24 months of follow-up. Seven patients had also undergone a concomitant CABG procedure. There were no adverse events due to Algisyl-LVR injection. LVEF improved from $27.1 \pm 7.3\%$ before the procedure to $34.8 \pm 16.8\%$ 24 months after. LVEDV and LVESV also showed progressive, but not statistically significant, improvement. In 2016, the results of the AUGMENT-HF trial were reported after 12 months follow-up [22]. The trial, which included 78 patients, compared Algisyl-LVR treatment to standard medical therapy (beta-blockers, ACE inhibitors or aldosterone antagonists). Fifty-eight patients completed the study. The major finding was a substantial improvement in peak VO_2

(oxygen consumption) at 12 months, with benefits in exercise capacity, symptoms, and overall improvement in quality of life.

Our study and several others by various investigators [23,26–28,36], demonstrate that myocardial injection of Algisyl-LVR substantially reduced myofiber stress with concomitant positive effects on ventricular geometry. Ventricular wall stress is an important determinant of myocardial function and has been suggested as a diagnostic criterion of HF [37]. Injectable biomaterials reduce wall stress, stabilize ventricular size, and prevent wall thinning [21,38–40]. After Algisyl-LVR is injected into the myocardial wall, it forms a permanent, solid polymer, with the shape of an ellipsoid that undergoes deformation comparable with the surrounding tissue and also acts as a scaffold. The material is inert and causes no inflammation in the vicinity of the implants [18]. The scaffolding effect of the polymer provides physical support of the scar tissue, and with increase in wall thickness, prevents or reverses maladaptive LV remodeling, as demonstrated in the present study. The correct softness-stiffness of the material is important for preservation of LV shape, as recently described by Rodell and colleagues [16]. The material stiffness provided by dual-crosslinking hyaluronic acid hydrogel used in their study allowed the LV wall to maintain a near normal thickness, which reduced myofiber stress. On the contrary, guest-host first crosslinking of the material led to a soft hydrogel that deforms under loading conditions and elongates circumferentially [16]. Ex-vivo MRI analysis of the hearts used in our study (data presented in a follow-up paper) has shown that Algisyl displaces tissue longitudinally and not radially. This probably contributes to the decrease in LV sphericity. Furthermore, the Algisyl implants and the fibrotic tissue that develops around the implants likely anchor the surrounding tissue, which would also attenuate some of the remodeling in the vicinity of the injected material. This speculation remains to be verified.

Recently, several microRNAs (miRNAs or miRs) have emerged as potential new biomarkers of cardiovascular diseases. miRNAs are a class of small, endogenous, noncoding RNAs that regulate gene expression at the post-transcriptional level in both physiological and pathological conditions [41]. Thirteen miRNAs: miR-1, -124-3p, -126, -150, -195, -21, -210, -30a, -342-3p, -423-5p, -499-5p, -622 and -92a, have been found to be differentially regulated in more than one HF cohort, and hence, are of special interest [42]. Of those myocardial stress-associated miRNAs, three were upregulated in the control group that developed HF in the present study, while they were downregulated in the group treated with Algisyl-LVR. miR-21 is upregulated in cardiac fibroblasts in response to pathological stresses that promote cardiac fibrosis in HF [43]. miR-195, which is a member of the miR-15 family, is upregulated in response to myocardial stress and myocardial infarction (death of cardiomyocytes and loss of contractility) [44]. miR210 is upregulated in hypoxic cardiomyocytes [45] and HF, and strongly correlates with the levels of BNP, a marker of HF [41]. These three miRNAs have thus been identified as excellent supplementary biomarkers to evaluate patients with HF. The results of the present study on myocardial stress-associated miRNA are novel.

An interesting and novel histological finding of our study was the substantial amount of multinucleated cardiomyocytes (Fig. 4B, C and D) found in the area surrounding the fibrous capsule containing the Algisyl-LVR. An increased number of myofibroblasts and new vessel

formation were also observed. Several studies [46–48] have shown that injection of alginate can cause an increase in the number of capillaries and arterioles, as well as infiltration of myofibroblasts. Nelson and colleagues [49] have hypothesized that the presence of alginate may create healthier microenvironment through stress shielding or an inflammatory response to the material. Similarly, injectable alginate biomaterials have been reported to support local infarct healing while limiting myocardial remodeling [50]. The presence of multinucleated cardiomyocytes associated with the injection of Algisyl-LVR or any type of biomaterial, however, has not been previously reported in the literature. Multinucleated myocytes of up to four nuclei have been found in young and aging hearts, cardiac hypertrophy, and ischemic cardiomyopathy in humans [51]. In these four conditions, the population of mononucleated, binucleated, trinucleated and tetranucleated myocytes were remarkably similar, where approximately 75% were mononucleated cells, 24% binucleated, and 1% multinucleated. In our study, multinucleated cardiomyocytes of more than four nuclei (up to 14) were found in the hearts treated with the polymer. We hypothesize that the presence of alginate material in the myocardium establishes a stress gradient (smallest near the implant) and hence promotes myocyte division by proliferation of pre-existing myocytes in the direction of the gradient but the cells cannot divide due to the Algisyl constraint (hence, a multinucleated myocyte). This hypothesis remains to be tested in future studies.

4.1. Study limitations

The small sample size of the study due to the initial challenge of creating the model is a limitation. The results of the study, however, remain statistically valid despite the few cases analyzed and compared.

The lack of a sham-operated control group treated with intramyocardial saline injections is a limitation of the present study. Previous studies, however, have compared the effects of Algisyl-LVR [18] and different types of biomaterials [49] to saline injections, and have reported significant beneficial effects such as increased wall thickness, attenuation of LV dilation, improved fractional shortening, improved LVESV and LVEDV, increased LVSV, increased LVEF, and overall improved cardiac function with these hydrogels, as opposed to no effects with saline injections [18,49]. Hence, the comparison of current studies to a saline sham group is not likely to change the conclusions of the study.

4.2. Conclusions and future directions

We have shown that Algisyl-LVR is an effective strategy to prevent or reverse maladaptive cardiac remodeling and myocardial dysfunction in swine IHF. This approach holds promise for treatment of patients once it can be delivered percutaneously via either the epicardium or the endocardium. Furthermore, it will allow therapy to be delivered not only to the myocardial free wall but also the interventricular septum.

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Abbreviations:

HF	heart failure
MI	myocardial infarction
LV	left ventricle
LVEF	left ventricular ejection fraction
LVSF	left ventricular stroke volume
SI	sphericity index
ES	end-systole
ED	end-diastole
LVESV	left ventricular end-systolic volume
LVEDV	left ventricular end-diastolic volume

References

- [1]. Roger VL, Epidemiology of heart failure, *Circ. Res* 113 (6) (2013) 646–659. [PubMed: 23989710]
- [2]. Heidenreich PA, Albert NM, Allen LA, Bluemke DA, Butler J, Fonarow GC, Ikonidis JS, Khavjou O, Konstam MA, Maddox TM, Nichol G, Pham M, Piña IL, Trogon JG, American Heart Association Advocacy Coordinating Committee.; Council on Arteriosclerosis, Thrombosis and Vascular Biology.; Council on Cardiovascular Radiology and Intervention.; Council on Clinical Cardiology.; Council on Epidemiology and Prevention.; Stroke Council, Forecasting the impact of heart failure in the United States: a policy statement from the American Heart Association, *Circ. Heart Fail* 6 (3) (2013) 606–619. [PubMed: 23616602]
- [3]. Lund LH, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dipchand AI, Dobbels F, Goldfarb SB, Levvey BJ, Meiser B, Yusen RD, Stehlik J, International Society of Heart and Lung Transplantation, The registry of the International Society for Heart and Lung Transplantation: thirty-first official adult heart transplant report—2014; focus theme: retransplantation, *J Heart Lung Transplant* 33 (10) (2014) 996–1008. [PubMed: 25242124]
- [4]. Deten A, Volz HC, Briest W, Zimmer HG, Cardiac cytokine expression is upregulated in the acute phase after myocardial infarction. Experimental studies in rats, *Cardiovasc. Res* 55 (2) (2002) 329–340. [PubMed: 12123772]
- [5]. Weisman HF, Bush DE, Mannisi JA, Weisfeldt ML, Healy B, Cellular mechanisms of myocardial infarct expansion, *Circulation* 78 (1) (1988) 186–201. [PubMed: 2968197]
- [6]. Narula J, Haider N, Virmani R, DiSalvo TG, Kolodgie FD, Hajjar RJ, Schmidt U, Semigran MJ, Dec GW, Khaw BA, Apoptosis in myocytes in end-stage heart failure, *N. Engl. J. Med* 335 (16) (1996) 1182–1189. [PubMed: 8815940]
- [7]. Thomas CV, Coker ML, Zellner JL, Handy JR, Crumbley AJ, 3rd, Spinale FG, Increased matrix metalloproteinase activity and selective upregulation in LV myocardium from patients with end-stage dilated cardiomyopathy, *Circulation* 97 (17) (1998) 1708–1715. [PubMed: 9591765]
- [8]. Tyagi SC, Campbell SE, Reddy HK, Tjahja E, Voelker DJ, Matrix metalloproteinase activity expression in infarcted, noninfarcted and dilated cardiomyopathic human hearts, *Mol. Cell. Biochem* 155 (1) (1996) 13–21. [PubMed: 8717434]
- [9]. Sutton MG, Sharpe N, Left ventricular remodeling after myocardial infarction: pathophysiology and therapy, *Circulation* 101 (25) (2000) 2981–2988. [PubMed: 10869273]

- [10]. Hutchins GM, Bulkley BH, Infarct expansion versus extension: two different complications of acute myocardial infarction, *Am. J. Cardiol* 41 (7) (1978) 1127–1132. [PubMed: 665522]
- [11]. Jeremy RW, Hackworthy RA, Bautovich G, Hutton BF, Harris PJ, Infarct artery perfusion and changes in left ventricular volume in the month after acute myocardial infarction, *J. Am. Coll. Cardiol* 9 (5) (1987) 989–995. [PubMed: 3571760]
- [12]. Teo SK, Vos FJ, Tan RS, Zhong L, Su Y, Regional ejection fraction and regional area strain for left ventricular function assessment in male patients after first-time myocardial infarction, *J. R. Soc. Interface* 12 (106) (2015) 10.1098/rsif.2015.0006.
- [13]. Pfeffer MA, Braunwald E, Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications, *Circulation* 81 (4) (1990) 1161–1172. [PubMed: 2138525]
- [14]. Gupta KB, Ratcliffe MB, Fallert MA, Edmunds LH, Jr., Bogen DK, Changes in passive mechanical stiffness of myocardial tissue with aneurysm formation, *Circulation* 89 (5) (1994) 2315–2326. [PubMed: 8181158]
- [15]. Holmes JW, Borg TK, Covell JW, Structure and mechanics of healing myocardial infarcts, *Annu. Rev. Biomed. Eng* 7 (2005) 223–253. [PubMed: 16004571]
- [16]. Rodell CB, Lee ME, Wang H, Takebayashi S, Takayama T, Kawamura T, Arkles JS, Dusaj NN, Dorsey SM, Witschey WR, Pilla JJ, Gorman JH, 3rd, Wenk JF, Burdick JA, Gorman RC, Injectable shear-thinning hydrogels for minimally invasive delivery to infarcted myocardium to limit left ventricular remodeling, *Circ. Cardiovasc. Interv* 9 (10) (2016) 10.1161/CIRCINTERVENTIONS.116.004058.
- [17]. Dorsey SM, McGarvey JR, Wang H, Nikou A, Arama L, Koomalsingh KJ, Kondo N, Gorman JH, 3rd, Pilla JJ, Gorman RC, Wenk JF, Burdick JA, MRI evaluation of injectable hyaluronic acid-based hydrogel therapy to limit ventricular remodeling after myocardial infarction, *Biomaterials* 69 (2015) 65–75. [PubMed: 26280951]
- [18]. Sabbah HN, Wang M, Gupta RC, Rastogi S, Ihsar I, Sabbah MS, Kohli S, Helgersson S, Lee RJ, Augmentation of left ventricular wall thickness with alginate hydrogel implants improves left ventricular function and prevents progressive remodeling in dogs with chronic heart failure, *JACC Heart Fail.* 1 (3) (2013) 252–258. [PubMed: 23998003]
- [19]. Henning RJ, Khan A, Jimenez E, Chitosan hydrogels significantly limit left ventricular infarction and remodeling and preserve myocardial contractility, *J. Surg. Res* 201 (2) (2016) 490–497. [PubMed: 27020836]
- [20]. He YY, Wen Y, Zheng XX, Jiang XJ, Intramyocardial delivery of HMGB1 by a novel thermosensitive hydrogel attenuates cardiac remodeling and improves cardiac function after myocardial infarction, *J. Cardiovasc. Pharmacol* 61 (4) (2013) 283–290. [PubMed: 23188125]
- [21]. Dai W, Wold LE, Dow JS, Kloner RA, Thickening of the infarcted wall by collagen injection improves left ventricular function in rats: a novel approach to preserve cardiac function after myocardial infarction, *J. Am. Coll. Cardiol* 46 (4) (2005) 714–719. [PubMed: 16098441]
- [22]. Mann DL, Lee RJ, Coats AJ, Neagoe G, Dragomir D, Pusineri E, Piredda M, Bettari L, Kirwan BA, Dowling R, Volterrani M, Solomon SD, Sabbah HN, Hinson A, Anker SD, One-year follow-up results from AUGMENT-HF: a multicentre randomized controlled clinical trial of the efficacy of left ventricular augmentation with Algisyl in the treatment of heart failure, *Eur. J. Heart Fail* 18 (3) (2016) 314–325. [PubMed: 26555602]
- [23]. Lee RJ, Hinson A, Bauernschmitt R, Matschke K, Fang Q, Mann DL, Dowling R, Schiller N, Sabbah HN, The feasibility and safety of Algisyl-LVR™ as a method of left ventricular augmentation in patients with dilated cardiomyopathy: initial first in man clinical results, *Int. J. Cardiol* 199 (2015) 18–24. [PubMed: 26173169]
- [24]. Anker SD, Coats AJ, Cristian G, Dragomir D, Pusineri E, Piredda M, Bettari L, Dowling R, Volterrani M, Kirwan BA, Filippatos G, Mas JL, Danchin N, Solomon SD, Lee RJ, Ahmann F, Hinson A, Sabbah HN, Mann DL, A prospective comparison of alginate-hydrogel with standard medical therapy to determine impact on functional capacity and clinical outcomes in patients with advanced heart failure (AUGMENT-HF trial), *Eur. Heart J* 36 (34) (2015) 2297–2309. [PubMed: 26082085]
- [25]. Lee LC, Wall ST, Genet M, Hinson A, Guccione JM, Bioinjection treatment: effects of post-injection residual stress on left ventricular wall stress, *J. Biomech* 47 (12) (2014) 3115–3119. [PubMed: 25065728]

- [26]. Lee LC, Wall ST, Klepach D, Ge L, Zhang Z, Lee RJ, Hinson A, Gorman JH, 3rd, Gorman RC, Guccione JM, Algisyl-LVR™ with coronary artery bypass grafting reduces left ventricular wall stress and improves function in the failing human heart, *Int. J. Cardiol* 168 (3) (2013) 2022–2028. [PubMed: 23394895]
- [27]. Lee LC, Zhihong Z, Hinson A, Guccione JM, Reduction in left ventricular wall stress and improvement in function in failing hearts using Algisyl-LVR, *J. Vis. Exp* 74 (2013) 10.3791/50096.
- [28]. Lee RJ, Hinson A, Helgerson S, Bauernschmitt R, Sabbah HN, Polymer-based restoration of left ventricular mechanics, *Cell Transplant.* 22 (3) (2013) 529–533. [PubMed: 22469060]
- [29]. Fu L, Shi Z, Luo G, Tu W, Wang X, Fang Z, Li X, Multiple microRNAs regulate human FOXP2 gene expression by targeting sequences in its 3′ untranslated region, *Mol. Brain* 7 (2014) 71. [PubMed: 25269856]
- [30]. Shi Z, Luo G, Fu L, Fang Z, Wang X, Li X, miR-9 and miR-140–5p target FoxP2 and are regulated as a function of the social context of singing behavior in zebra finches, *J. Neurosci* 33 (42) (2013) 16510–16521. [PubMed: 24133256]
- [31]. Zouein FA, Zgheib C, Liechty KW, Booz GW, Post-infarct biomaterials, left ventricular remodeling, and heart failure: is good enough? *Congest. Heart Fail* 18 (5) (2012) 284–290. [PubMed: 22612796]
- [32]. Gorman RC, Jackson BM, Burdick JA, Gorman JH, Infarct restraint to limit adverse ventricular remodeling, *J. Cardiovasc. Transl. Res* 4 (1) (2011) 73–81. [PubMed: 21161462]
- [33]. Zhong L, Su Y, Yeo SY, Tan RS, Ghista DN, Kassab G, Left ventricular regional wall curvedness and wall stress in patients with ischemic dilated cardiomyopathy, *Am. J. Physiol. Heart Circ. Physiol* 296 (3) (2009) H573–84. [PubMed: 19122166]
- [34]. Sabbah HN, Stein PD, Kono T, Gheorghiadu M, Levine TB, Jafri S, Hawkins ET, Goldstein S, A canine model of chronic heart failure produced by multiple sequential coronary microembolizations, *Am. J. Phys* 260 (4 Pt 2) (1991) H1379–84.
- [35]. Moe GW, Stopps TP, Howard RJ, Armstrong PW, Early recovery from heart failure: insights into the pathogenesis of experimental chronic pacing-induced heart failure, *J. Lab. Clin. Med* 112 (4) (1988) 426–432. [PubMed: 3171352]
- [36]. Wenk JF, Wall ST, Peterson RC, Helgerson SL, Sabbah HN, Burger M, Stander N, Ratcliffe MB, Guccione JM, A method for automatically optimizing medical devices for treating heart failure: designing polymeric injection patterns, *J. Biomech. Eng* 131 (12) (2009) 121011. [PubMed: 20524734]
- [37]. Alter P, Koczulla AR, Nell C, Figiel JH, Vogelmeier CF, Rominger MB, Wall stress determines systolic and diastolic function – characteristics of heart failure, *Int. J. Cardiol* 202 (2016) 685–693. [PubMed: 26454537]
- [38]. Wall ST, Walker JC, Healy KE, Ratcliffe MB, Guccione JM, Theoretical impact of the injection of material into the myocardium: a finite element model simulation, *Circulation* 114 (24) (2006) 2627–2635. [PubMed: 17130342]
- [39]. Rane AA, Christman KL, Biomaterials for the treatment of myocardial infarction: a 5-year update, *J. Am. Coll. Cardiol* 58 (25) (2011) 2615–2629. [PubMed: 22152947]
- [40]. Christman KL, Lee RJ, Biomaterials for the treatment of myocardial infarction, *J. Am. Coll. Cardiol* 48 (5) (2006) 907–913. [PubMed: 16949479]
- [41]. Bronze-da-Rocha E, MicroRNAs expression profiles in cardiovascular diseases, *Biomed. Res. Int* 2014 (2014) 985408. [PubMed: 25013816]
- [42]. Wong LL, Wang J, Liew OW, Richards AM, Chen YT, MicroRNA and heart failure, *Int. J. Mol. Sci* 17 (4) (2016) 502. [PubMed: 27058529]
- [43]. van Rooij E, Olson EN, MicroRNA therapeutics for cardiovascular disease: opportunities and obstacles, *Nat. Rev. Drug Discov* 11 (11) (2012) 860–872. [PubMed: 23080337]
- [44]. van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, Hill JA, Olson EN, Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis, *Proc. Natl. Acad. Sci* 105 (35) (2008) 13027–13032. [PubMed: 18723672]

- [45]. Mutharasan RK, Nagpal V, Ichikawa Y, Ardehali H, microRNA-210 is upregulated in hypoxic cardiomyocytes through Akt- and p53-dependent pathways and exerts cytoprotective effects, *Am. J. Physiol. Heart Circ. Physiol* 301 (4) (2011) H1519–30. [PubMed: 21841015]
- [46]. Landa N, Miller L, Feinberg MS, Holbova R, Shachar M, Freeman I, Cohen S, Leor J, Effect of injectable alginate implant on cardiac remodeling and function after recent and old infarcts in rat, *Circulation* 117 (11) (2008) 1388–1396. [PubMed: 18316487]
- [47]. Mukherjee R, Zavadzkas JA, Saunders SM, McLean JE, Jeffords LB, Beck C, Stroud RE, Leone AM, Koval CN, Rivers WT, Basu S, Sheehy A, Michal G, Spinale FG, Targeted myocardial microinjections of a biocomposite material reduces infarct expansion in pigs, *Ann. Thorac. Surg* 86 (4) (2008) 1268–1276. [PubMed: 18805174]
- [48]. Yu J, Gu Y, Du KT, Mihardja S, Sievers RE, Lee RJ, The effect of injected RGD modified alginate on angiogenesis and left ventricular function in a chronic rat infarct model, *Biomaterials* 30 (5) (2009) 751–756. [PubMed: 19010528]
- [49]. Nelson DM, Ma Z, Fujimoto KL, Hashizume R, Wagner WR, Intra-myocardial biomaterial injection therapy in the treatment of heart failure: materials, outcomes and challenges, *Acta Biomater* 7 (1) (2011) 1–15. [PubMed: 20619368]
- [50]. Leor J, Tuvia S, Guetta V, Manczur F, Castel D, Willenz U, Petneházy O, Landa N, Feinberg MS, Konen E, Goitein O, Tsur-Gang O, Shaul M, Klapper L, Cohen S, Intracoronary injection of in situ forming alginate hydrogel reverses left ventricular remodeling after myocardial infarction in Swine, *J. Am. Coll. Cardiol* 54 (11) (2009) 1014–1023. [PubMed: 19729119]
- [51]. Olivetti G, Cigola E, Maestri R, Corradi D, Lagrasta C, Gambert SR, Anversa P, Aging, cardiac hypertrophy and ischemic cardiomyopathy do not affect the proportion of mononucleated and multinucleated myocytes in the human heart, *J. Mol. Cell. Cardiol* 28 (7) (1996) 1463–1477. [PubMed: 8841934]

Fig. 1.

A) Left ventricular stroke volume over a 16-week period in swine treated with Algisyl-LVR (polymer) and the control group. Animals in the polymer group were treated at week 8. * denotes significance between both groups at week 16. † denotes significance over time in the treated group after injection of Algisyl-LVR at week 8. B) Left ventricular ejection fraction determined from 3D echocardiograms taken over a 16-week period in swine treated with Algisyl-LVR (polymer) and in the control group. Animals in the polymer group were treated at week 8. * denotes significance between both groups at week 16. † denotes significance overtime in the treated group (from 8 to 16 weeks). C) Average wall thickness of the infarcted region (posterolateral wall) in the polymer and control groups. Measurements were obtained from 2D echocardiograms taken at the level of the papillary muscles. Significant changes were observed over time in the polymer group after treatment (†). Differences between both groups were found at week 16 (*). D) The sphericity index (SI) in both the polymer and control groups. SI remained almost the same in the polymer group after treatment but it increased in the control group. The values were statistically different between both groups after week 14 (*).

Fig. 2.

A) Average, B) end-systolic, and C) end-diastolic myofiber stress. In all three cases cardiac myofiber stress was significantly lower in the treated group at the end of the study, as compared to the control group. * denotes significance at 16 weeks between treated and control groups.

Fig. 3.

Comparative expressions of three myocardial stress-responsive microRNAs. miR-195, miR-21 and miR-210 are upregulated in heart failure (control group), while the treated group (polymer) shows similar values to the expressions in normal hearts. * denotes significance between normal hearts and control group. † denotes significance between control and polymer groups.

Fig. 4.

A) Histological section of a representative heart from the treated group showing the polymer (Algisyl-LVR) encapsulated in the myocardial wall. H&E staining, 100 $\times$. B) Multinucleated myocytes (MM) near the borderzone (BZ) with accumulation of myofibroblasts in the infarcted area (treated group). H&E staining, 600 $\times$. C) Nuclei of cardiomyocytes in the treated group. Fluorescence microscopy, Hoechst, 600 $\times$. D) Multinucleated myocyte (MM) with nuclei aligned in the center of the cell, along the long axis. Fluorescence microscopy, WGA (green) and Hoechst (blue), 600 $\times$. Frequency distribution of mononucleated, binucleated, and multinucleated cardiomyocytes in the E) treated and F) control groups.