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Minimally Invasive Delivery of Hydrogel-Encapsulated Amiodarone to the Epicardium Reduces Atrial Fibrillation

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Abstract

Background—Atrial fibrillation (AF) is the most common cardiac arrhythmia. While treatment options for AF exist, many patients cannot be maintained in normal sinus rhythm. Amiodarone is an effective medication for AF but has limited clinical utility due to off-target tissue toxicity.

Methods and Results—Here, we use a pig model of AF to test the efficacy of an amiodarone-containing poly(ethylene glycol)-based hydrogel. The gel is placed directly on the atrial epicardium through the pericardial space in a minimally invasive procedure using a specially designed catheter. Implantation of amiodarone-containing gel significantly reduced the duration of sustained AF at 21 and 28 days; inducibility of AF was reduced 14 and 21 days post-delivery. Off-target organ drug levels in the liver, lungs, thyroid, and fat were significantly reduced in animals treated with epicardial amiodarone gel compared to systemic controls in small animal distribution studies.

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Conclusions—The pericardium is an underutilized therapeutic site and may be a new treatment strategy for AF and other cardiovascular diseases.

Journal Subject Terms
atrial fibrillation; arrhythmia; pericardium; amiodarone; epicardial; hydrogel; device

Introduction

Atrial fibrillation (AF) is a disease of disordered electrical conduction of the cardiac atria and is the most common cardiac arrhythmia\(^1\). It affects 33 million people worldwide and 6 million in the United States, a number expected to double by 2030\(^2\). AF contributes significantly to morbidity, mortality, and healthcare costs with annual healthcare expenditures of $33 billion in 2013\(^3,4\). AF caused 130,000 deaths in 2013\(^2\). AF patients are 2 to 10 times more likely to have embolic strokes originating from the fibrillating atria\(^2\). The pathophysiology of AF is complex and difficult to treat. Heart disease as well as systemic diseases lead to foci of over-excitable atrial cardiomyocytes as well as shortening of atrial cardiomyocyte action potential durations propagation of rapid conduction\(^5\). Together, these changes foster multiple random propagating wavelets, focal electrical discharges, and localized reentrant activity that prevent coordinated atrial contraction\(^6\).

There are several medications to control atrial rhythm in patients with AF, but none have shown a mortality benefit, due in part to the poor success at achieving normal sinus rhythm (NSR)\(^7-9\). Clinical trials have also shown improved quality of life with restoration of NSR when achievable\(^10,11\). Some rhythm control medications increase risk of arrhythmia, heart failure, or death and are contraindicated in many patients with cardiac disease (ironically, the largest population with AF)\(^1\). Due to the low success rates and toxicity, only 16% of patients with AF are treated with rhythm control strategy\(^12\). Invasive procedures such as AF ablation are available for patients with symptoms refractory to medical treatment but the outcomes are poor for more advanced disease stages (persistent AF)\(^13\).

Amiodarone is a lipophilic medication approved in the United States for the treatment of ventricular arrhythmias and used off-label for the treatment of AF\(^14\). Amiodarone prolongs atrial refractoriness and action potential duration, reducing early excitation in both normal and diseased hearts\(^15\). In a recent Cochrane analysis, amiodarone had the lowest number needed to treat (NNT), 3, to prevent recurrence of AF and significantly reduced the recurrence compared to dronedarone and sotalol\(^8\). Amiodarone is an effective medication but its use is associated with significant off-target tissue toxicity. Off-target tissue levels are estimated to be 10 to 500 times higher than cardiac levels in patients taking chronic amiodarone\(^16,17\). Risk of pulmonary fibrosis, thyroid toxicity, and liver dysfunction range from 18–75% of patients taking chronic amiodarone\(^16,18\). Thus, amiodarone is recommended only as an agent of last resort by American, European, and Canadian guidelines\(^19\).

Recently, we described a catheter-based technique using a novel device to deliver hydrogels to the cardiac epicardium under fluoroscopic guidance\(^20\). This delivery technique creates a temporary compartment on the heart’s surface for hydrogel delivery and in situ...
polymerization allowing precise localization and gelation. This technique could be used to deliver hydrogels with various therapeutic materials and mechanisms of action. Hydrogels are under investigation for various cardiac applications but few specialized delivery few methods have been designed\textsuperscript{21–25}. Intracoronary administration of hydrogels could lead to thrombosis or myocardial infarction. Intramyocardial injection by left ventricular catheter such as Noga Myostar injection catheter could also cause embolic events. Intra-myocardial injection at the time of cardiac surgery would limit eligible patients.

Here, we explore the efficacy of an amiodarone-containing hydrogel delivered to the epicardium of the cardiac atria in a large animal model of AF. Amiodarone delivered in this way could have multiple advantages over systemic dosing, the most important of which are localized drug delivery to the affected cardiac muscle and lessened off-target organ toxicity. This technology represents a new strategy for the treatment of AF, utilizing an existing effective medication that was formally limited by off-target organ toxicity. More broadly, this strategy could facilitate delivery of new cardiac therapeutics to the heart including stem cells, microRNAs, viral based therapies, exosomes and protein in a precise and effective manner.

**Materials and Methods**

Details of the data, analytic methods, and study materials not provided in this text will be made available to other researchers upon request for purposes of reproducing the results or replicating the procedure.

**Gel synthesis and in vitro analysis**

Poly(ethylene glycol) [PEG] hydrogels based on a 4-arm PEG macromer (20 kDa, Laysan Bio, Arab, AL) with maleimides at each terminus (PEG-4MAL) were cross-linked with dithiothreitol and the bi-cysteine peptide VPM (GCRDVPMSMRGGDRCG). This platform provides structurally defined hydrogels with stoichiometric incorporation of ligands that has been extensively studied for biological applications\textsuperscript{20, 23, 26, 27}. Hydrogel components were adjusted to yield a 5 mL gel of a 4.0% w/v PEG hydrogel containing 50 mg of polysorbate-solubilized amiodarone.

For the release study, amiodarone hydrochloride (Sigma-Aldrich) was loaded into 20 µL hydrogels following solubilization in polysorbate 80. For solubilization, polysorbate 80 solutions (ratios of polysorbate 80:saline 5:95, 10:90, 20:80 and 30:70 v/v) were added to amiodarone to a final concentration of 50 mg/mL. This solution was then heated to 60° C and vortexed vigorously for 1 minute. This amiodarone solution was then added to the hydrogel components and the hydrogel cross-linked with dithiothreitol and VPM (1:1). After gelation, hydrogels were incubated in 500 µL of 10% human serum in PBS. At specified time points, all 500 µL was removed and replaced with fresh 500 µL of 10% human serum in PBS. Supernatants were frozen at −80° C and stored until analysis by high purity liquid chromatography (HPLC) as has been described previously\textsuperscript{28}. Samples were separated on a Kinetex 5µm C18 column (150 × 4.6 mm). The mobile phase, consisting of phosphate buffer (50 mM) with 0.5% formic acid (pH 4.5)-methanol-acetonitrile (45:5:50, v/v/v), was pumped isocratically at a flow rate of 1.0 mL/min. The detection was conducted at 254 nm.
**Pericardial hydrogel delivery in rats**

A rodent model of pericardial drug delivery was used to study amiodarone accumulation to target and off-target tissues. All rodent studies were approved by the Emory University Animal Use and Care Committee and performed in accordance with NIH guidelines. Male Sprague-Dawley rats (200–250 g, Charles River, Wilmington, MA) were anesthetized with isoflurane and intubated. A thoracotomy was performed from the anterior approach, exposing the left lung and heart. The lung was gently retracted with sterile gauze and the pericardium grasped with burr-free atraumatic forceps. A blunted 26 G, 0.5 inch needle with # 5 style blunt tip was attached to a 100 µL syringe (Hamilton Company, Reno, NV) and was used to gently penetrate the tended pericardium. A 100 µL hydrogel containing 1 mg amiodarone (n = 6) was delivered into the pericardial space and gelation confirmed by visualization of gel surrounding the heart confined by the pericardial sac. After 1 month, animals were sacrificed and heart, lung, thyroid, liver, fat and gel remnants collected for tissue amiodarone measurements by HPLC. Additional rats (n=6) were treated with systemic amiodarone via daily intraperitoneal injection (10 mg/kg) for 28 days resulting in a cumulative dose of approximately 100 mg over 1 month depending on animal size and growth rate.

**Large animal atrial fibrillation model**

The objective of this study was to create a large animal AF model that did not disrupt the pericardial space. All pigs received antibiotics (cephalexin 5 mg/kg IM daily) to prevent hardware infection for the duration of the experiment and colchicine (0.6 mg PO BID) to prevent pericardial inflammation. Male farm pigs of 50–55 kg were used to simulate heart and pericardial size to adult humans. Seven days before gel implantation, animals were sedated and the right neck and internal jugular vein exposed. A chronic resynchronization therapy pacemaker (CRT-P) was implanted (Viva, Medtronic, Minneapolis MN). The CRT-P is designed to clinically deliver 3 leads of output for the purpose of optimizing atrial and ventricular synchrony in heart failure. Unlike the clinical configuration with one lead in the atrial, we positioned all 3 leads (CapSureFix, Medtronic) in the right atria with one in the right atrial appendage, high free wall, and low free wall. Tined, bipolar permanent pacing leads were introduced, advanced under fluoroscopic guidance. Each position was tested for capture threshold less than 2 V and sensing electrogram greater than 2 mV. Lack of diaphragmatic stimulation at maximal output was also confirmed. Leads were connected to the CRT-P and programed to rapidly pace at a rapid intervals in DOO mode (150 beats per minute pacing, with 140 msec atrio-ventricular delay then 80 msec right to left ventricular lead delay) using clinical programmer parameters. Maximal output, 8V, was used. The left neck and jugular vein were also used to implant a single chamber pacemaker (Advisa, Medtronic) with lead terminating in the right ventricular apex and set in VOO mode a rate of 100. Lastly, animals underwent His bundle ablation after receiving bolus of lidocaine (75 mg IV). A Blazer II (Boston Scientific) radiofrequency ablation catheter was advanced from the femoral vein to the AV node and positioned to optimize the His Bundle signal on intracardiac electrogram. Sixty (60) watts of power at 60 C for up to 30 seconds was used for each ablation. Pigs required between 1–4 ablations to achieve heart block. Ventricular arrhythmias generated by ablation were immediately cardioverted with external defibrillation.
Hydrogel implantation was performed 7 days after pacemaker implantation. Animals were placed recumbent on cardiac catheterization suite table (Allura FDA 10: Xper, Philips), and subxiphoid steriley prepped and draped. A micropuncture needle was used to access the pericardial space under fluoroscopic guidance as has been described and used with other pericardial devices\textsuperscript{32}. Our hydrogel delivery device was placed over the atrial epicardium (posterior, inferior) using a posterior directed sheath. The delivery device was advanced outside of the sheath and formed a temporary compartment for hydrogel delivery as has been recently described\textsuperscript{20}. Negative pressure suction held the device in a stable position on the beating epicardium as well as temporarily sealed the hydrogel compartment. Hydrogel components were kept separated in 2 lumens and delivered to the atrial epicardium. The delivery device and suction was held in place for 5 minutes to allow for gelation. The suction was slowly released and device unwound from around the gel without disruption of gel architecture. During gel delivery procedure, animals were invasively monitored for hemodynamic changes with a Swan-Ganz catheter and arterial pressure line.

**Electrophysiologic testing**

At weekly intervals, animals were sedated and electrophysiologic study (EPS) performed using the CRT-P. After cessation of overdrive atrial pacing, atrial rhythm was monitor for 30 minutes via intra-cardiac electrograms and 12 lead EKG. The timing of spontaneous cardioversion into NSR was recorded. If animals did not cardiovert spontaneously, NSR was achieved by synchronized external defibrillation at 180 J to allow for subsequent testing. At least 5 minutes elapsed before further testing to allow for normalization of electrophysiologic conduction. Inducibility of AF was tested by performing atrial pacing at 50 Hz for 10 seconds using the CRT-P right atrial appendage lead. Ten trials were performed for each animal at each time point and number of inductions of AF lasting longer than 1 minute were recorded weekly.

**Tissue processing and amiodarone quantification**

Fresh or frozen tissue was diced into 5 mm\textsuperscript{2} sections and further homogenized using automated TissueLyzer (Qiagen, Germantown, MD). Amiodarone was extracted using acetonitrile, n-hexane, and methanol and analyzed by HPLC as was done for in vitro experiments\textsuperscript{28}. Tissue for histologic evaluation was fixed in formalin, dehydrated using a graded series of ethyl alcohols and xylene, paraffin embedded, and stained with Masson’s Trichrome. Five µm thick sections were imaged using automated slide scanner and representative 10x magnification sections (Nanozoom, Hamamatsu, Bridgewater, NJ). Histologic sections were evaluated by board certified pathologist blinded to the treatment groups. Colormetric segmentation was performed using ImageJ using the threshold function to maximize detection with lower limit of 120 and upper limit set to 190. Measurement of thresholded area was calculated as a percent of the total area of each 10x image. For each animal, three distinct areas of the left atrial were measured and averaged. Blood was collected from peripheral vein for serum markers performed using VetScan chemistry analyzer (Abaxis, Union City, CA) and white blood cell counts using automated hemocytoimeter.
Statistical analysis

Data are expressed as mean ± SEM. Statistical calculations were performed using GraphPad Prism 6 (GraphPad Software Inc) as indicated in the test. Differences between group means were compared using unpaired Student’s t test with correction for repeated measures by the Holm-Sidak method where appropriate. Multiple groups were compared using ANOVA with Tukey’s multiple comparison test.

Results

Amiodarone encapsulation in a synthetic hydrogel achieves extended drug release in vitro

Amiodarone was encapsulated in a poly(ethylene glycol) [PEG]-based hydrogel, a gel platform that has shown significant advantages such as cytocompatibility and controlled polymerization, tunable biophysical and biochemical properties, and minimal inflammation and toxicity. Gel components were also selected due to their use in other clinically approved drugs and devices. The hydrogel was synthesized using a 4-arm PEG-maleimide macromer (Laysan Bio, Arab, AL) cross-linked via the terminal maleimides and dithiol-presenting molecules. Amiodarone was solubilized in different concentrations of polysorbate 80 prior to loading into the gel. Gels were kept under simulated physiologic temperature and motion. Release kinetics showed an initial release bolus of under 20% for amiodarone solubilized with either 5 or 10% polysorbate 80 and 30% and 52% release for amiodarone solubilized with 20 and 30% polysorbate 80, respectively (Fig. 1). Forty-eight hours after encapsulation, gels having amiodarone solubilized in 5% or 10% polysorbate 80 retained more than 40% of the initial dose. All subsequent experiments were conducted using amiodarone (50 mg/ml) solubilized in 10% polysorbate 80 and encapsulated in PEG hydrogel.

Epicardial delivery of amiodarone-containing gel reduces off target drug accumulation

To test amiodarone tissue distribution, a 100 µL gel containing 1 mg of amiodarone was delivered into the pericardial space of rats via thoracotomy and compared to animals receiving daily systemic (intraperitoneal) amiodarone at a clinically equivalent dose (10 mg/kg/day). After 28 days, animals treated with systemic amiodarone (Fig. 2A, black bars) had higher accumulation of drug in off-target organs compared to epicardial amiodarone-containing gel-treated animals including the fat (36.1±10.7 v. 1.3±0.2 µg/g; P=0.02), lung (7.1±1.4 v. 0.9±0.1 µg/g; P=0.001), thyroid (86.7±20.8 v. 1.9±0.3 µg/g; P=0.002), and liver (2.8±0.9 v. 0.7±0.1 µg/g; P=0.03). Drug levels in the thyroid, lung, and liver were 45 times, 8 times, and 3 times higher, respectively, in systemically dosed animals compared to gel dosed animals.

Cardiac amiodarone levels were low in both groups but higher in systemically dosed rats compared to gel-treated rats at day 7 (5.4±5.1 v. 0.2±0.03 µg/g; P=0.04) and day 28 (2.7±0.4 v. 1.1±0.1 µg/g; P=0.004, Fig. 2B). However, a higher proportion of the total administered dose was present in the heart of gel treated animals after 28 days (0.09±0.001 v. 0.001±0.0003%; P=0.001). Cardiac drug levels in amiodarone gel-treated animals increased from day 7 to 28 suggesting continual drug release over time (0.2±0.03 v. 1.1±0.3 µg/g; P=0.01; Fig. 2B, black bars). Recovered portions of the amiodarone-containing gel
contained drug 7 and 28 days after implant (43.2±33.4 v. 15.0±8.8 µg/g; NS; Fig. 2A),
decreasing as drug was dispersed from the gel.

**Hydrogel was delivered to the atrial epicardium using a minimally invasive procedure**

Using a micro-puncture needle to access the pericardial space percutaneously, a hydrogel delivery device was positioned over the atrial epicardium in all 9 animals (Fig. 3). Gel was delivered to the desired location with procedure times from skin puncture to sheath removal lasting 26.1±10.8 minutes (n=9). Four (4) animals were treated with a 5 mL hydrogel containing amiodarone (50 mg) and 4 with control gel of the same size containing no drug. One additional animal was excluded prior to gel implantation due to infection not related to gel. This amiodarone dose was the maximal quantity that could be incorporated into the gel without significant precipitation. Invasive hemodynamic parameters measured during the implantation procedure did not change acutely, or after 4 weeks (Table 1).

**Atrial overdrive pacing using chronic resynchronization therapy pacemaker (CRT-P) promotes AF**

Overdrive pacing of the cardiac atria in pigs is an established large animal model of AF. Instead of utilizing a neurostimulator generator to achieve cycle lengths of 10 Hz at a single location in the atria, we implanted three leads attached to a CRT-P in the right atria to more closely model the electrical disorganization of AF (Fig. 4). One animal was excluded due to development of pacemaker pocket wound dehiscence and infection 7 days after implantation of CRT-P. Three animals had a pacemaker lead dislodgement detected by CRT-P interrogation and underwent successful lead revision the week before gel placement. One week after atrial overdrive pacing, control animals sustained AF for 0.75±1.5 min after temporary cessation of overdrive pacing (Fig. 5A). After 14 days, AF was sustained for 9.75 ± 13.5 min which is similar in scope to AF achieved by neurostimulator pacing in published studies.

**Epicardial amiodarone-containing gel reduces atrial fibrillation**

Nine pigs underwent implantation of the CRT-P device in the right neck and 3 pacing leads (Medtronic) implanted in the right atrium via the internal jugular vein. In all animals leads were distributed in the right atria in 3 distinct locations: right atrial appendage, upper lateral wall, and lower lateral wall. This configuration achieved sensing and pacing threshold goals but avoided diaphragmatic stimulation. Six of 9 animals required external defibrillation during His ablation from ventricular tachycardia or ventricular fibrillation, and all were successfully cardioverted.

At weekly intervals, animals underwent EPS. After 2 weeks of overdrive pacing, animals in the control group sustained AF and by 3 weeks, tended to remain in AF for the full 30 minutes after cessation of overdrive pacing. In contrast, animals treated with amiodarone-containing hydrogel spontaneously cardioverted into NSR during the monitoring period and not a single animal maintained AF for the 30 minutes in the treatment group (Fig. 5B). At days 21 (19.5±13.1 v. 1.3±1.5 minutes; N=4; P=0.004) and day 28 (22.5±15.0 v. 5.8±8.3 minutes; N=4, P=0.004) amiodarone containing gel-treated animals had shorter duration of sustained AF (Fig. 5B). While awake, animals have higher heart rates and levels of...
adrenergic stimulation that could influence their propensity for AF. We confirmed that amiodarone-containing hydrogel significantly reduced the duration of sustained AF in awake animals at 19 (23.3±13.5 v. 0.75±1.5 minutes; N=4; p<0.05) and 26 days (25.5±9.0 v. 2.3±2.9 minutes; N=4; p<0.05) after hydrogel delivery. Statistical significance was calculated using Student’s T test corrected for multiple comparisons using the Holm-Sidak method (α=5.000%).

The inducibility of AF was also investigated using 10 second 50 Hz burst stimulation, a maneuver known to trigger AF. Animals treated with amiodarone-containing gel had fewer AF inductions in response to 50 Hz stimulation 14 days (8.0±4.0 v. 2.5±4.4 attempts, P=0.039) and 21 (9.7±0.6 v. 2.5±3.1 attempts, P=0.013) days after gel delivery (Fig. 5C). At 28 days, the positive inductions were lower in amiodarone hydrogel-treated animals, but failed to meet statistical significance (7.5±1.9 v. 3.5±4.1; n=4, NS) due to lack of inducibility in 1 control animal at this time point. Cardiac amiodarone levels in pigs were highest in the left atrium (0.11±0.05 µg/g) and pericardial fat (0.14±0.08 µg/g). Taken together, these results show that a one-time treatment with amiodarone-containing hydrogel is capable of reducing the duration of sustained AF as well as the propensity for induction of AF for at least 1 month in a large animal model of chronic overdrive atrial pacing.

**Amiodarone-containing gel was non-toxic to the heart, liver, and lungs**

Atrial fibrosis has been linked to progression of AF in patients and animal models. Treatments that reduce AF including amiodarone have been reported to reduce atrial fibrosis in animal models. Inflammation, if triggered by gel could also cause atrial fibrosis. Tissue sections from the left atria were fixed and stained with Masson’s Trichrome and fibrosis measured by non-biased colormetric analysis with Image J. There was no difference between control hearts from animals without any cardiac manipulation (normal sinus rhythm) and AF animals. Furthermore, amiodarone content of gel did not increase detectable fibrosis (24.1±6.6 v. 26.2±7.3%; N=4; NS, Fig. 6A) using Tukey’s multiple comparison test.

To rule out the possibility of pulmonary toxicity in animals treated with amiodarone-containing gel, tissue sections from the posterior basal inferior lobe of the lung were collected, and evaluated by 2 board certified pathologist blinded to treatment condition. This location was chosen due to its close anatomic position to the location of gel delivery. In all 4 control gel treated animals and 2 amiodarone containing gel animals, mild focal inflammation was reported including patchy bronchiolitis and mild interstitial pneumonitis-like inflammation. Overall no significant lung inflammatory findings were preset in either group. Serum markers of liver function (albumin, alkaline phosphatase (Alk phos), alanine aminotransferase (ALT) and bilirubin showed no significant change over the course of the experiment (Fig. 6B, Table 2). Creatinine as a marker of kidney function was higher at baseline in the amiodarone-containing gel treated group but remained stable over the course of the experiment (Table 2).

**Discussion**

Here, we describe a new and effective strategy to treat AF by delivering hydrogel-encapsulated amiodarone directly to the heart through the pericardial space. Pigs treated

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with a single dose of amiodarone-containing hydrogel (50 mg amiodarone/5 mL gel) delivered to the atrial epicardium had reduced duration and inducibility of AF for at least 4 weeks compared to empty gel controls (Fig. 5). The gel was successfully delivered in a minimally invasive procedure. Using a rat model to study drug tissue distribution, systemically administrated amiodarone resulted in significant drug accumulation in off-target organs (Fig. 2A) consistent with other studies. In contrast, epicardial gel-treated animals had significantly lower off-target organ drug accumulation, drastically so in the case of the fat, liver and thyroid. Recovered portions of epicardial amiodarone gel still contained drug at 7 and 28 days post-delivery (Fig. 2A). Continued drug availability in the gel is evidenced by increasing cardiac drug levels in this group from days 7 to 28. These data show proof of principle that epicardial hydrogels can 1) deliver therapeutics directly to the heart, 2) achieve continued release over several weeks, and 3) reduce off-target organ accumulation. Thus, epicardial hydrogel delivery may be a beneficial strategy for many cardiac targeted therapeutics.

Achieving rhythm control in persistent AF is challenging due to the confluence of pathophysiologic factors including fibrosis, electrical remodeling, and autonomic nervous system activation. Amiodarone is effective in treating AF, although its use is limited by off-target organ toxicity. The hydrophobicity of amiodarone delays attainment of therapeutic cardiac levels and contributes to buildup of drug in off-target tissues. For outpatient use, patients are often dosed with a 10 gram ‘load’ then treated with 200–800 mg of drug daily. In the present study, a single 50 mg dose of amiodarone delivered directly to the atrial epicardium sustained therapeutic effects over 4 weeks at 5% of the systemic loading dose (10 g), 25% of the daily dose (200 mg) and 0.3% of the cumulative monthly dose (17 g). The reduced dose lessens the chance for off-target organ toxicity.

Prior studies have examined the delivery of cardiovascular agents in the pericardial space and may have some advantages over systemic delivery, especially for newer classes of cardiac therapeutics. The space is in direct contact with the cardiac epicardium. The pericardium is also known to act as a barrier slowing systemic distribution of instilled therapeutics in animal trials. Vascular washout of the therapeutic is eliminated since pericardial fluid is only slowly replenished over several hours. There is no risk of vascular occlusion or embolization since the pericardial space is not within the vasculature.

There are several challenges in utilizing the pericardial space. Few physicians have expertise at accessing a pericardial space lacking effusion – a so called ‘dry’ pericardium. With the advent of epicardial ablations for the treatment of ventricular arrhythmias, as well as the Lariat Device for suturing of the left atrial appendage, invasive cardiologists have gained skill in this technique. In one study, there were no reported complications from pericardial access when a micro-puncture kit was used. Another limitation of the pericardial space is its propensity for inflammation. Our hydrogel has shown minimal inflammation and no local or systemic toxicity in numerous pre-clinical settings. Furthermore, utilizing a one-time intra-pericardial steroid dose in conjunction with the anti-inflammatory medication colchicine, we were able to eliminate clinically significant pericardial inflammation. Further studies will be needed to confirm the safety of this therapy over the long term.
In the current study, we do not know the maximum duration of action of amiodarone delivered in this manner. Gel recovered from rats after 28 days still contained amiodarone and tissue drug levels rose from 7 to 28 days suggesting continual tissue level rise. Longer studies will be needed to understand the duration of drug release and efficacy. For clinical translation, several months of efficacy would be ideal to avoid repeated pericardial procedures. Other formulations such as liposomes or micro particle encapsulation may give prolonged duration of release although will likely deliver a smaller amiodarone quantity per volume of gel. Along with the release profile, it is key to determine the inflammatory profile of any material delivered to the pericardial space.

Several studies have explored the possibility of anti-arrhythmic therapy delivered to the cardiac epicardium. Intra-pericardial injection of sotalol, ibutilide, and esmolol showed acute physiologic effects, but these drugs have not been tested in chronic studies or extended release gel formulations. Infusion of amiodarone acutely into the pericardial space has been shown to increase cardiac amiodarone levels and have electrophysiologic effects. Other groups have developed hydrogels for amiodarone delivery to the heart. These formulations deliver drug over days not months for the post-operative cardiac surgery population and require a spray or direct application. Combination patches with steroid formulation have also been studied in rodents. These preparations require direct delivery to the heart and are not amenable to minimally invasive delivery in patients not undergoing open heart surgery.

A challenge for development of amiodarone-delivering gel is the lack of data on the effective cardiac amiodarone levels needed for suppression of atrial and ventricular arrhythmias. With systemic dosing, plasma drug levels do not correlate well with cardiac drug levels. Autopsy studies of patients receiving acute or chronic amiodarone therapy report cardiac drug levels ranging from 1–40 µg/g. Drug levels achieved in rat and pig studies are lower than that achieved in these clinical settings, although efficacy was still shown in our animal model. This suggests that lower drug levels many be efficacious in some patients. However, this model may lack pathophysiologic factors that could influence the therapeutic threshold and may underestimate cardiac drug levels needed in sicker patient populations. Further studies will be needed to explore efficacious tissue amiodarone levels.

In conclusion, we show the possibility of utilization of the pericardial space for therapeutic hydrogel delivery. Using our minimally invasive delivery device, an amiodarone-containing hydrogel was placed on the atrial epicardium and showed efficacy for at least one month. Complementary small animal studies showed advantageous tissue amiodarone distribution that could result in reduced toxicity from lower off target tissue distribution. Further studies on the duration of action and long term safety are needed prior to translation of this biomaterial strategy into clinical use. However, this technique could be a more efficient mode of delivery for a variety of new therapeutics – such as stem cells, miRNAs, cytokines, gene therapy – that would benefit from more direct cardiac localization.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.
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What is known?

- Atrial fibrillation is the most common cardiac arrhythmia
- Rhythm control may reduce morbidity and hospitalization, but is difficult to attain in many patients
- Amiodarone is an effective rhythm control medication, but has significant systemic toxicity

What the study adds?

- Amiodarone can be delivered directly to the heart encapsulated in hydrogel
- With this method, 50mg of amiodarone reduces atrial fibrillation for one month
- Minimally invasive epicardial delivery of hydrogels is a translatable method to deliver therapeutics to the heart
Amiodarone encapsulation extends drug release. Hydrogels were synthesized with amiodarone solubilized at differing concentrations of polysorbate 80 in saline after which amiodarone release was analyzed over time via HPLC. After 3 days in simulated in vivo conditions (37 C, 1 Hz shaking), hydrogels containing amiodarone solubilized with 5% or 10% polysorbate 80 retained more than 30% of total loaded amiodarone while hydrogels containing amiodarone solubilized with 20% or 30% polysorbate exhibited almost complete amiodarone release. N=2–3, error bars ± SEM.
Figure 2.
Epicardial delivery of amiodarone-containing gel reduces off target drug accumulation. Rats were treated with systemic amiodarone by daily intraperitoneal (IP) injection (10mg/kg/day, n=6) or with a single epicardial amiodarone gel implant (1 mg/50 µl gel, n=6). After 28 days, animals treated with systemic amiodarone had higher drug accumulation in off target organs compared to gel treated controls in fat (36.1±10.7 v. 1.3±0.2 µg/g, P=0.02), lung (7.1±1.4 v. 0.9±0.1 µg/g, P=0.001), thyroid (86.7±20.8 v. 1.9±0.3 µg/g, P=0.002), and liver (2.8±0.9 v. 0.7±0.1 µg/g, P=0.03, A). In contrast, animals treated with amiodarone-containing gel had minimal off target drug accumulation (A, red bars). Significant drug was still present in recovered portions of gel (15.0±8.8 µg/g, A). While overall cardiac drug levels were low in both groups, levels in IP treated animals were higher at 28 days (2.7± 0.4 v. 1.1± 0.1 µg/g; P=0.004). N=6, * indicates p<0.05 comparing indicated groups using Student’s T test; error bars +/- SEM.
Figure 3.
Pericardial gel delivery is minimally invasive and completed using standard fluoroscopy equipment. The pericardial space was accessed by micro-puncture needle from a sub-xiphoid approach using fluoroscopy (A, arrow) with contrast injection and layering along the pericardium (A, arrowheads). Pericardial position was confirmed by wire confinement to cardiac silhouette (B, arrow). The delivery device (C, D arrow) was positioned over the left atrium using fluoroscopy in anterior-posterior (C) and lateral (D) views. Pacemaker leads (*) and Swan-Ganz catheter are visible in some views.
Figure 4.
Atrial overdrive pacing using a 3 lead asynchronous strategy produced atrial fibrillation (AF) in swine. AF was induced by placement of 3 chronic resynchronization pacemaker (CRT-P) leads into the cardiac right atrium (A). Asynchronous and rapid pacing algorithm with intervals ranging from 80 – 170 milliseconds delivered similar frequency of stimulation compared to neurostimulator-induced overdrive pacing but provided longer battery life and allowed for non-invasive electrophysiologic testing (B). Schematic of experimental timeline (C) with AF induction 1 week before gel implant and iterative electrophysiologic studies (EPS).
Figure 5.
Amiodarone containing gel reduces atrial fibrillation (AF) in a large animal model. Pigs were instrumented with chronic resynchronization therapy-pacemaker (CRT-P) in the right neck and all three leads were secured to the right atrium via the right internal jugular vein. Normal sinus rhythm (regular rate, consistent electrical morphology) or AF (irregular rate, variable morphology) was easily distinguishable on intra-cardiac recordings from CRT-P during temporary cessation of pacing (A). The pacing algorithm delivered rapid, sustained, and uncoordinated stimuli to the atria throughout the duration of the experiment mimicking persistent AF. The duration of sustained AF was reduced in animals treated with amiodarone-containing gel at 21 (19.5±13.1 v. 1.3±1.5 minutes, P=0.002) and 28 (22.5±13.0 v. 5.8±7.2 minutes, P=0.004, B). Similarly, the number of inducible AF episodes in response to 50 Hz pacing was lower in the amiodarone gel treated animals at 14 days (8.0±4.0 v. 2.5±4.4 attempts, P=0.039) and 21 days (9.7±0.6 v. 2.5±3.1 attempts, P=0.013, C) after gel delivery. N=4 per group; * p<0.05 using Student’s T test with Holm-Sidak method of correction for repeated measures; error bars +/- SEM.
Figure 6.
Epicardial administration of amiodarone-containing gel had no cardiac or liver toxicity. Left atrial appendage sections were fixed in formalin and stained with Masson’s Trichrome showing varying degrees of fibrosis (A). Amiodarone-containing gel treated animals showed similar percentage fibrosis on colormetric analysis compared to control animals in sinus rhythm as well as AF animals treated with control gel (A). Liver function studies did not vary over time or between groups at weekly time points (B).
Invasive hemodynamics prior to and after epicardial gel delivery. Pigs underwent implantation of amiodarone-containing gel (Amio, n=4) or control gel (Cntrl, n=4) and intra-cardiac pressures monitored by Swan-Ganz catheter and ventricular pigtail catheter. There was no statistical difference between baseline or 4 week pressures over time or between groups in multiple cardiac chambers. Listed P values indicate variation between groups at 4 weeks by Student’s T-test with Holm-Sidak method of correction for repeated measures.

<table>
<thead>
<tr>
<th>Hemodynamics (mmHg)</th>
<th>Baseline</th>
<th>4 weeks</th>
<th>Δ baseline</th>
<th>p</th>
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<tbody>
<tr>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Right atrium</td>
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<tr>
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<td>0.4</td>
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<tr>
<td>Amio</td>
<td>5.5±1.7</td>
<td>7.3±4.0</td>
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<td></td>
</tr>
<tr>
<td>Right ventricle, diastole</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cntrl</td>
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<td>4.8±1.7</td>
<td>0.0</td>
<td>0.54</td>
</tr>
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<tr>
<td>Right ventricle, systole</td>
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<tr>
<td>Cntrl</td>
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<td>20.5±4.5</td>
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<tr>
<td>Amio</td>
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<tr>
<td>Pulmonary capillary wedge</td>
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<tr>
<td>Cntrl</td>
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<td>Amio</td>
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<td></td>
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<tr>
<td>Left ventricle, diastole</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cntrl</td>
<td>7.3±4.5</td>
<td>5.8±1.0</td>
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</tr>
<tr>
<td>Amio</td>
<td>6.8±1.7</td>
<td>6.5±2.1</td>
<td>−0.3</td>
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<tr>
<td>Left ventricle, systole</td>
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<tr>
<td>Cntrl</td>
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<td>Amio</td>
<td>65.6±4.7</td>
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</table>
Table 2

Serum markers of liver and kidney function studies and white blood cell (WBC) counts. Pigs were treated with control gel containing no amiodarone (Cntrl, n=4) or amiodarone containing gel (Amio, n=4). Prior to gel implantation procedure and 4 weeks later, serum albumin, alkaline phosphatase (Alk phos), alanine aminotransferase (ALT) and bilirubin were measured as markers of liver function and did not differ between groups. Serum creatinine was measured as a marker of kidney function and was higher in amiodarone-containing gel treated animals compared to control (2.0±0.3 v. 1.1±0.1 mg/dL, P=0.03) although levels were elevated at baseline before gel delivery and remained stable in the amiodarone group. WBC count and differential were similar between groups.

<table>
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<tr>
<th>Liver function studies</th>
<th>Baseline</th>
<th>4 weeks</th>
<th>Δ baseline</th>
<th>p</th>
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</thead>
<tbody>
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<td>Albumin (g/dL)</td>
<td>Cntrl</td>
<td>3.4±0.7</td>
<td>3.8±0.2</td>
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<tr>
<td></td>
<td>Amio</td>
<td>3.5±0.3</td>
<td>3.2±0.3</td>
<td>−0.3</td>
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<tr>
<td>Alkphos (U/L)</td>
<td>Cntrl</td>
<td>77.0±32.9</td>
<td>92.5±58.0</td>
<td>15.5</td>
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<tr>
<td></td>
<td>Amio</td>
<td>122.8±48.0</td>
<td>136.0±41.0</td>
<td>13.3</td>
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<tr>
<td>Alt (U/L)</td>
<td>Cntrl</td>
<td>49.3±19.3</td>
<td>49.5±8.3</td>
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<tr>
<td></td>
<td>Amio</td>
<td>55.0±10.0</td>
<td>55.0±15.3</td>
<td>0.0</td>
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<tr>
<td>Total bilirubin (mg/dL)</td>
<td>Cntrl</td>
<td>0.3±0.1</td>
<td>0.3±0.1</td>
<td>0.0</td>
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<tr>
<td></td>
<td>Amio</td>
<td>0.3±0.1</td>
<td>0.2±0.0</td>
<td>−0.1</td>
</tr>
<tr>
<td>Other laboratory tests</td>
<td>Baseline</td>
<td>4 weeks</td>
<td>Δ baseline</td>
<td>p</td>
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<tr>
<td>Creatinine (mg/dL)</td>
<td>Cntrl</td>
<td>1.1±0.1</td>
<td>1.8±0.4</td>
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<tr>
<td></td>
<td>Amio</td>
<td>2.0±0.3</td>
<td>1.9±0.2</td>
<td>0.3</td>
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<tr>
<td>WBC (×10⁹/L)</td>
<td>Cntrl</td>
<td>14.6±3.9</td>
<td>12.2±3.2</td>
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<tr>
<td></td>
<td>Amio</td>
<td>13.1±4.4</td>
<td>10.4±2.6</td>
<td>−2.7</td>
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</table>