Injectable conductive hydrogel restores conduction through ablated myocardium

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Abstract

Abstract Introduction: Therapies for substrate-related arrhythmias include ablation or drugs targeted at altering conductive properties or disruption of slow zones in heterogeneous myocardium. Conductive compounds such as carbon nanotubes may provide a novel personalizable therapy for arrhythmia treatment by allowing tissue homogenization. Methods: A nanocellulose-carbon nanotube conductive hydrogel was developed to have conduction properties similar to normal myocardium. Ex vivo perfused canine hearts were studied. Electroanatomic activation mapping of the epicardial surface was performed at baseline, after radiofrequency ablation, and after uniform needle injections of the conductive hydrogel through the injured tissue. Gross histology was used to assess distribution of conductive hydrogel in the tissue. Results: The conductive hydrogel viscosity was optimized to decrease with increasing shear rate to allow expression through a syringe. The DC conductivity under aqueous conduction was 4.3·10-1 S/cm. In 4 canine hearts, when compared to the homogeneous baseline conduction, isochronal maps demonstrated sequential myocardial activation with a shift in direction of activation to surround the edges of the ablated region. After injection of conductive hydrogel, isochrones demonstrated conduction through the ablated tissue with activation similar to baseline in all 4 hearts. Gross specimen examination demonstrated retention of the hydrogel within the tissue. Conclusions: This proof-of-concept study demonstrates that conductive hydrogel can be injected into acutely disrupted myocardium to restore conduction. Future experiments should focus on evaluating long-term retention and biocompatibility of the hydrogel through in vivo experimentation.

Condensed Abstract

A novel conductive hydrogel was developed as a method to treat substrate-based arrhythmias by homogenizing conduction through disrupted myocardium. In *ex vivo* canine hearts, the hydrogel was injected evenly through ablated regions of myocardium. Electroanatomic activation mapping of the epicardial surface demonstrated a shift in conduction to surround edges following ablation of the region. Following injection of the conductive hydrogel, conduction improved through the ablated region approximating baseline in all 4 hearts. This proof-of-concept study demonstrates that conductive hydrogel can be injected into acutely disrupted myocardium to restore conduction.

Abbreviations List

CNT = carbon nanotube

DC = direct current

EAM = electroanatomic mapping

NFC = nanofibrillated cellulose

SD = standard deviation

Introduction

Arrhythmias in the structurally abnormal heart may be precipitated by heterogeneous conduction through regions of fibrosis or scar. In cases of myocardial infarction, the mechanism for arrhythmogenesis is usually due to the presence of viable myocardium interspersed amidst scarred tissue that leads to reentry or enhanced automaticity. Currently, methods to prevent arrhythmia focus on impacting these slow, viable zones through use of either antiarrhythmic medications that alter activation characteristics or ablation to destroy (ie, homogenize) the tissue region. However, both approaches carry significant limitations either due to medication side effects, including the potential for proarrhythmia, or creation of new scar. Furthermore, existing technology is limited by an inability to completely eliminate all arrhythmia circuits (eg, due to circuits deep in the mid-myocardial region).

Regenerative approaches for enhancing conduction through regions of myocardial scar, preventing the fibrotic remodeling that is thought to contribute to arrhythmogenesis, or reversing the damage done from an infarct or other injurious events reflect new approaches to treatment of patients with myocardial disease(1-4). Use of stem cells or biologically compatible, synthetic compounds have been studied extensively, although with greater focus on effects on myocardial contractility and ventricular function rather than arrhythmogenicity. Certain compounds, such as carbon nanotubes, have been studied in a variety of biological approaches, including as a means of potentially enhancing myocardial health(5-11). One promising aspect of carbon nanotubes is that they are highly conductive. We and others have previously shown that applying 3D printed patches composed of carbon nanotubes intermixed with biocompatible compounds could restore conduction across the surface of a previously injured region(12,13). We sought to evaluate whether an injectable, conductive carbon nanotube-based hydrogel would impact conduction properties through a region of iatrogenically disrupted (ablated) myocardium.

Methods

The protocol was approved by the Institutional Animal Care and Use Committee at Mayo Clinic in Rochester, MN.

Conductive hydrogel preparation and evaluation

Single-walled carbon nanotubes (CNTs; 76.9 mg, P3, 3-6-wt% R-COOH; Carbon Solutions Inc., Riverside, CA, USA) with a bundle diameter of ˜4–5 nm and a bundle length of ˜1 μm were purified with nitric acid and added to an aqueous solution of a nonionic, noncytotoxic surfactant Pluronic F127 BioReagent (10 mL, 0.1-wt%; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). After heating for 8 hours at 70°C, the mixture was sonicated for 16 hours creating a 0.77-wt% dispersion of CNTs. Nanofibrillated cellulose (NFC; 2 g[?]2 ml, 3-wt%, carboxymethylated nanocellulose, charge density ˜515 μeq/g; Innventia AB, Stockholm, Sweden) was Electron Beam sterilized and then combined with 2 ml of the dispersion using a SpeedMixer^{TM} (FlackTek Inc., Landrum, SC, USA) at 2000 rpm twice for 2 minutes each. The result was a homogeneous hydrogel with dry matter of 20-wt% and NFC:CNT dry weight ratio of 4:1. Our method for assessing conductivity of the hydrogel has been described previously(12).

Rheology

The rheological properties of the conductive hydrogel were analyzed using the Discovery HR-2 rheometer (TA Instruments, UK) with a Peltier plate. The measurements were performed at 25 °C, and the samples were allowed to reach equilibrium temperature for 60 s prior to each measurement. An aluminum plate-plate (20 mm, gap = 500 μm) was used and the shear viscosity was evaluated by increasing the shear rate from 0.1 to 1000 s^{-1} at 25 °C.

Ex vivo heart preparation

A total of 4 mongrel dogs (25-40 kg in weight) were placed under deep sedation with isoflurane inhalational anesthesia. A left lateral thoracotomy allowed visualization of the heart and great vessels. Therapeutic heparinization was confirmed (activated clotting time > 300 s) after which the aorta was cannulated to allow cardioplegia solution to be administered in a retrograde fashion. After asystole was achieved, cardiectomy was performed and the heart was immediately cooled to 4°C. A large animal Langendorff perfusion apparatus (Radnoti, Covina, CA, USA) was used to perfuse the heart*ex vivo* in a warmed basin (*Figure 1*)(14). The hearts were perfused with a modified Krebs-Henseleit solution which was oxygenated with 95% O2 and buffered to 5% CO2(15). The perfusate solution consisted of the following: NaCl (118 mmol/L), $NaHCO_3$ (25 mmol/L), mannitol (16 mmol/L), d-glucose (11 mmol/L), KCl (4.7 mmol/L), sodium pyruvate (2.3 mmol/L), $CaCl_2$ (2.0 mmol/L), KH_2PO_4 (1.18 mmol/L), $MgSO_4$ (1.17 mmol/L), and human insulin (10 U/L; Novolin; Novo Nordisk, Plainsboro, NJ). The perfusate temperature was maintained at 37degC+-1degC.

Ablation and hydrogel injection in beating heart and approach to cardiac mapping

Upon establishing the *ex vivo* suspension and stable function of the heart, patches were attached around the basin to permit *ex vivo* electroanatomical mapping (EAM; CARTO3 system, Biosense Webster, Diamond Bar, CA, USA). A 3.5 mm tip irrigated ablation catheter (ThermoCool Navistar; Biosense Webster, Diamond Bar, CA, USA) was used to perform both mapping and focal ablation. First, during normal sinus rhythm, point-by-point mapping of the epicardial surface of the lateral left ventricle was performed. Each point was adjudicated to establish an appropriate timing reference. After this, a point with homogeneous conduction and voltage was selected within the mapped region as a target for ablation at 30 Watts for 1 minute with normal saline irrigation at 30 ml/min. We then remapped the region including within the scar region and around in regions of normal tissue. The hydrogel was then injected using an 18-gauge needle by sequentially injecting circumferentially around the area of ablated myocardium by pushing the needle into the tissue and slowly injecting the hydrogel as it was withdrawn with each injection. Injections were then performed in perpendicular lines spaced evenly 1 mm apart through the center of the lesion in a grid pattern. After this, remapping was done of the region with again careful attention to annotation of individual points. The time between injection and remapping was within minutes. All mapping was done during sinus rhythm.

Gross specimen evaluation

Gross examination of the regions of injection was undertaken by first dissecting out the entire transmural region where ablation was performed. We then did two orthogonal incisions through the center of the lesion region to be able to evaluate the myocardium throughout the ablated region. Presence of the conductive hydrogel was based on the gross appearance throughout the region given that the black appearance of the hydrogel is otherwise obvious against the surrounding myocardial tissue. No specific quantification was done of the amount of hydrogel retained within the volume of ablated tissue.

Statistical approach

Mean and SD were calculated based on data from 5 samples for analysis of mechanical hydrogel properties. Statistical significance was determined based on an $\alpha < 0.05$. All statistics were performed in JMP Pro 11 (SAS Institute, Cary, NC, USA).

Results

Characterization of conductive hydrogel

Rheological measurements of the shear viscosity of the conductive hydrogel showed that the hydrogel had good shear thinning properties (*Figure 2A*), i.e. becomes more liquid when a shear force is applied. This allows for ejection from a needle while still keeping its form after it is extruded from the needle head (*Figure 2B*). To evaluate gel formation ability, a solution of 100 mM $CaCl_2$ was added to the extruded hydrogel. The hydrogel exhibited a gelling behavior and after five minutes the hydrogel was able to be lifted using tweezers

(*Figure 2C*). This indicated that the conductive hydrogel can crosslink with the presence of divalent cations to form a stable gel.

Pure NFC as well as NFC/CNT hydrogel were demonstrated to have well-defined dynamic yield stress (G'=G"; 217 and 106 Pa, respectively) with transition from elastic to viscous behavior. DC conductivity under aqueous conduction was $4.3 \cdot 10^{-1}$ S/cm in the homogeneous dispersion of 20 wt% CNT in NFC hydrogel.

Mapping pre-ablation, post-ablation, and post-injection

In 4 *ex vivo* perfused beating canine hearts, a detailed EAM was obtained of the epicardial lateral left ventricle during sinus rhythm at baseline, following ablation, and again following injection of the conductive hydrogel (*Figure 3*). In all 4 animals (100%), we were able to show a disruption of homogeneous baseline conduction by ablation. Following injection of conductive hydrogel, a bridge of improved conduction was demonstrated across the ablated region in all 4 animals (100%).

By placing the mapping catheter on the same site of the muscle in 1 animal, we were able to demonstrate a double potential indicative of far-field and near-field bipolar activation at baseline likely suggestive of endocardial to epicardial activation. Local activation was supported by the steepest negative deflection in the unipolar signal. Far-field and near-field activation appeared to separate with a small isoelectric interval following ablation. Following CNT hydrogel injection, the bipolar signals reapproximated and appeared similar to baseline (*Figure 4*).

Gross specimen evaluation of myocardium post-injection

We performed gross examination of the myocardial specimen following ablation and CNT hydrogel injection. The hydrogel could be seen circumferentially around the ablated lesion at the points where the needle entered the epicardium. Orthogonal transection showed that most of the hydrogel was retained within the ablated tissue. Some hydrogel did extrude from the lesion into areas of nonablated myocardium (*Figure 5*).

Discussion

An injectable carbon nanotube hydrogel was developed by our group with enhanced conductive properties that retain conductivity in aqueous environments. Furthermore, we have demonstrated that injection of such a hydrogel into iatrogenic regions of myocardial injury can enhance conduction through these regions. These data provide support that such a conductive material may impact conduction characteristics of myocardium. They also lay the foundation for future work to enhance understanding of the utility of such an approach and the potential impact on arrhythmogenesis.

Future needs of injectable preparations

The conductive hydrogel preparation in our study represented an initial evaluation of a carbon nanotube complex that was made to be expressible through a syringe and needle. However, materials properties need to be considered in the context of the specific use case in which they are applied. In the case of injectable materials, they should liquify upon applying a specific pressure for easy flow out of a container (eg, a syringe) and then immediately solidify upon expression out into whatever material they will be contained in (eg, within myocardial tissue). In the context of our study, while some conductive hydrogel was retained in the myocardium as seen on gross examination, some loss of hydrogel also occurred during injection. We performed the experiments *ex vivo*in a still beating heart with an epicardial injection approach. However, any such injectable material, if delivered endocardially, will have to be optimized to prevent any material loss that could then embolize. Furthermore, the amount injected and retained needs to be predictable and thus further research to evaluate the minimum quantity of material needed to impact conduction will be necessary.

Prior studies have looked at complexing material with alginate or other substances to optimize properties for injection and retention in tissue(16,17). However, most of these studies focused on alginate as a material scaffold that could optimize structural integrity of the tissue or as a delivery tool for stem cells, drugs, or other materials. The impact of complexing other substances with carbon nanotubes and similar conductive

materials will need to be considered in the context of their impact on conductivity of the material itself as well as the impact on the material-myocardial interface in terms of cell-to-material-to-cell interaction. Also, whether commercially available trans-endocardial injection catheters such as the MyoStar catheter (Biosense Webster, Diamond Bar, CA, USA) will be able to accommodate the conductive hydrogel, will need to be further evaluated. Thus, while our work demonstrates proof of concept, these limitations need to be considered prior to *in vivo* studies, particularly if endocardial injections are performed.

Anti-arrhythmic or proarrhythmic?

As with any treatment (eg, ablation or drugs) targeting regions of heterogeneous conduction, therapies meant to alter local conduction should be considered in light of their potential for proarrhythmia. We propose injecting conductive materials into regions of damaged myocardium as a means of "normalizing" conduction. However, it should be noted that we did not account in our study for conduction endocardially or mid-myocardially. Furthermore, while conduction post-nanotube injection seemed similar to baseline, it cannot be assumed to be precisely the same. Use of omnipolar smaller electrodes or optical mapping may allow for enhanced understanding of local conduction direction. While globally it appears that conduction follows the same path as baseline, heterogeneity due to disarray of conduction or conduction abnormalities only revealed with a different wavefront of activation may result in continued arrhythmic potential, as seen in studies of ventricular mapping for substrate characterization.

It is also possible that with chronic retention in tissue, myocardial fibrosis or immune reaction may occur that may alter the tissue-material interface. Furthermore, conductivity may change over time that may result in proarrhythmia in the long-term even if activation appeared to be normalized acutely. Many of these concerns may be clarified in chronic *in vivo* studies. However, animal-based studies may never fully recapitulate human tissue reactions. Furthermore, how integration varies in different types of substrate, whether related to myocardial ischemia and infarction, inflammation-mediated injury, genetic abnormalities, or other causes, needs to be considered.

Comparison to prior work on conductive materials

It is important to consider these findings in light of prior data on the impact of conductive materials on cell-cell interaction in the heart. We have previously reported data on the use of 3D-printed patches to restore conduction across disrupted areas of myocardial activation(12). This has similarly been demonstrated by others. However, scar and arrhythmogenic circuits are often multi-dimensional and not simply reflected along a single surface. Thus, identifying ways to enhance conduction not just along the surface (whether endocardially or epicardially) but transmurally may be critical when developing a solution that may be antiarrhythmic. Each will have theoretical benefits and limitations – a patch may be less invasive, reversible, and easier to apply with a lower risk of complications due to lack of need to inject directly into myocardial tissue. However, injection approaches may more directly address the conduction heterogeneity attributable to 3-dimensional complexity that is likely seen in most cases of scar-related arrhythmia.

Furthermore, prior work has suggested that other materials aside from carbon nanotubes may allow for enhanced conduction between otherwise electrically disconnected myocytes. We did not directly compare the effects of our material with these other materials. However, most work has been on creating a surface over which cells are placed and then evaluating conduction rather than methods that may facilitate enhanced conduction transmurally. Determination of the optimal conductive materials based on chronic maintenance of conduction and biocompatibility will require further study.

Mechanisms of cell-material interaction

One of the key questions when using materials to impact cell to cell interaction is the mechanism that facilitates the cell-material-cell interaction. It is well established that conductive materials can be used to sense electrical activity from a cell and stimulate cells (eg, with pacemakers). However, such ability is often dependent on considerations of source/sink and other factors. The fact is that the electrical impulse propagated by a conductive material from a cell that is activated to a distant cell is not well understood and

will require further study.

Limitations

There are several limitations to our study beyond those noted above. First, evaluation was done on a limited number of hearts. Thus, consistency in results across larger sample sizes needs to be evaluated. Second, these studies reflected acute evaluations of changes in conduction both post-ablation and post-injection. It is possible that lesion maturation may occur over a longer period of time, resulting in dynamic shifts in cardiac activation that may not have been reflected in the acute state. Third, as stated previously, we could not inject a predictable amount of hydrogel each time due to materials properties not permitting appropriate in-tissue retention. Thus, we could not determine a threshold amount of material that would need to be injected to facilitate some change in conduction. In addition, the approach to the testing and mapping (*ex vivo* perfused hearts and epicardial only mapping) may not necessarily extend to in vivo examples, and, thus, before further conclusions can be made, *in vivo* acute and chronic studies will be needed. Finally, we could not evaluate immunogenicity or potential for chronic maintenance of the perceived conduction changes due to the acute and *ex vivo* nature of the study.

Conclusion Our study shows that novel injectable conductive hydrogel can enhance myocardial conduction through regions of tissue injury. Future studies will need to focus on optimizing rheological, electrical conductivity, and in-situ gel formation properties of the conductive hydrogel such that it can be predictably retained within tissue while maintaining conductive properties. In addition, it will be important to improve understanding of the effect on local conduction not just epicardially but transmurally. Finally, establishing both long-term durability of activation normalization and biocompatibility will be key.

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Figure Legends

Figure 1. Langendorff apparatus – The Langendorff apparatus (A) and *ex vivo* perfused heart in warmed basin (B) are shown.

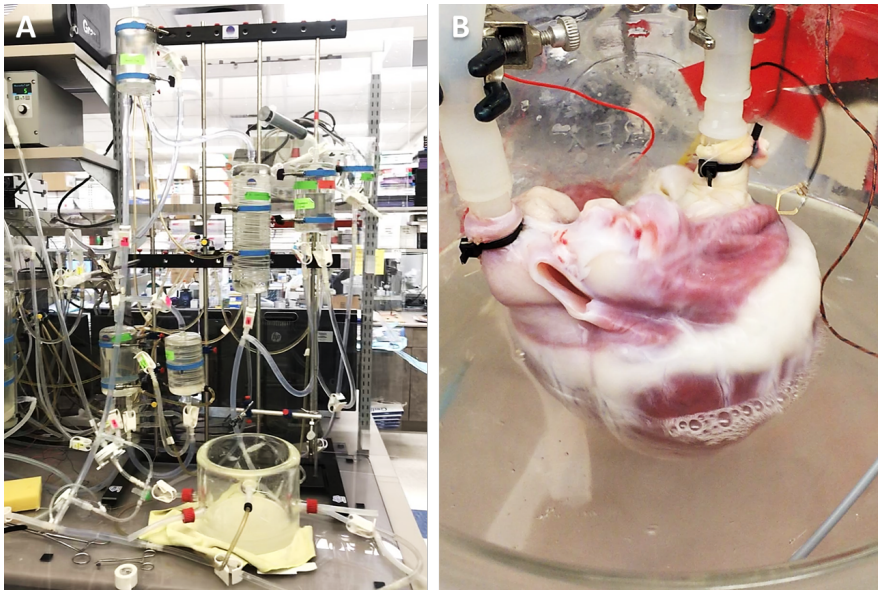
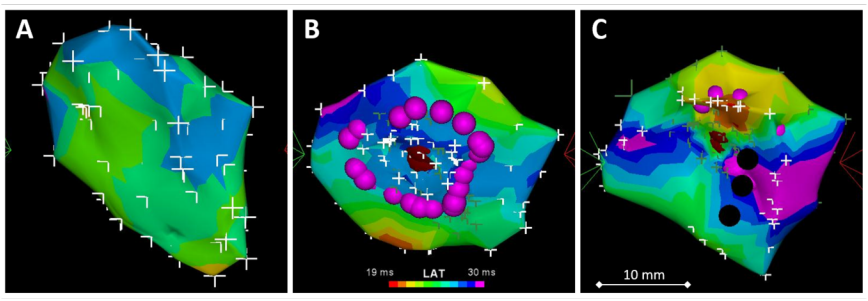


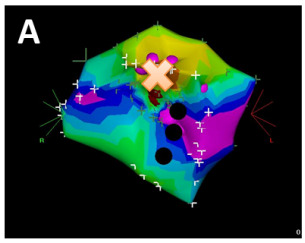
Figure 2. Rheological properties for the conductive hydrogel – Flow curve showing the shear thinning property of the hydrogel (A). Image of the conductive hydrogel after ejection from a needle (B). Conductive hydrogel after 5 minutes of cross-linking in 100 mM $CaCl_2$ solution (C).

Hosted file

`image2.emf` available at https://authorea.com/users/337704/articles/463297-injectable-conductive-hydrogel-restores-conduction-through-ablated-myocardium

Figure 3. Electroanatomical mapping – The electroanatomical propagation map showing activation for the same epicardial area of the lateral left ventricle in a representative animal. At baseline (A), homogenous and rapid activation is demonstrated. There is disruption of conduction as evidenced by clustering of isochrones following ablation (B). Following injection of conductive hydrogel (C), a bridge of improved conduction is restored across the ablated myocardium. Red dots indicate site of ablation, purple dots indicate border zone of abnormal myocardium as determined by voltage (<1.5 mV), and block dots indicate sites of needle entry for hydrogel injection. LAT=local activation time.





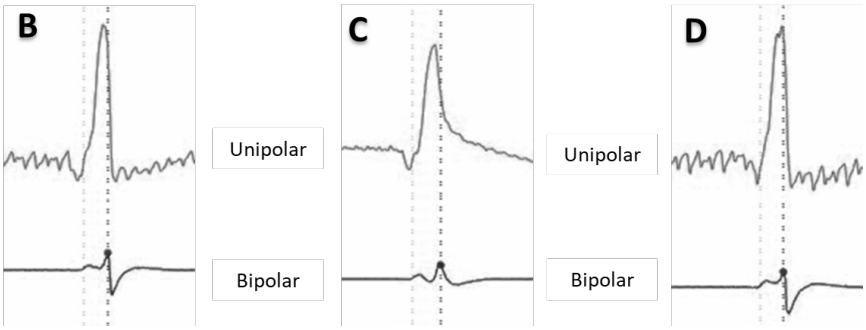


Figure 4. Local activation signals – The Mapping catheter location is shown annotated on the electroanatomical map by the orange X (A). The catheter was placed in the same location at baseline (B), following ablation (C), and following conductive hydrogel injection (D). The steepest negative unipolar deflection corresponds with the sharp near-field bipolar potential suggesting that this point reflects local activation. A far-field potentials closely precedes the near-field activation at baseline. Following ablation there is separation with an isoelectric interval between far-field and near-field potentials. Following conductive hydrogel injection, the bipolar potentials approximate – similar in appearance to the baseline signal.

Figure 5. Hydrogel retention within myocardium – Gross evaluation of the myocardium from the epicardial surface shows the pale ablated tissue and black circumferentially injected conductive hydrogel (A). Orthogonally transected ablation lesions (B & C) demonstrate the hydrogel mostly retained within the ablated

lesion, however, some hydrogel can be seen extruding into regions of unablated myocardium (arrows).