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Improved conduction and increased cell retention in healed MI using mesenchymal stem cells suspended in alginate hydrogel

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Abstract

Introduction—Mesenchymal stem cells (MSCs) have been associated with reduced arrhythmias; however, the mechanism of this action is unknown. In addition, limited retention and survival of MSCs can significantly reduce efficacy. We hypothesized that MSCs can improve impulse conduction and that alginate hydrogel will enhance retention of MSCs in a model of healed myocardial infarction (MI).

Methods and results—Four weeks after temporary occlusion of the left anterior descending artery (LAD), pigs ($n=13$) underwent a sternotomy to access the infarct and then were divided into

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Conflict of interest None.

two studies. In study 1, designed to investigate impulse conduction, animals were administered, by border zone injection, 9–15 million MSCs ($n=7$) or phosphate-buffered saline (PBS) (control MI, $n=5$). Electrogram width measured in the border zone 2 weeks after injections was significantly decreased with MSCs (-30 ± 8 ms, $p<0.008$) but not in shams (4 ± 10 ms, $p=NS$). Optical mapping from border zone tissue demonstrated that conduction velocity was higher in regions with MSCs (0.49 ± 0.03 m/s) compared to regions without MSCs (0.39 ± 0.03 m/s, $p<0.03$). In study 2, designed to investigate MSC retention, animals were administered an equal number of MSCs suspended in either alginate (2 or 1 % w/v) or PBS ($n=6$ /group) by border zone injection. Greater MSC retention and survival were observed with 2 % alginate compared to PBS or 1 % alginate. Confocal immunofluorescence demonstrated that MSCs survive and are associated with expression of connexin-43 (Cx43) for either PBS (control), 1 %, or 2 % alginate.

Conclusions—For the first time, we are able to directly associate MSCs with improved impulse conduction and increased retention and survival using an alginate scaffold in a clinically relevant model of healed MI.

Keywords

Mesenchymal stem cells; Electrograms; Cardiac regeneration; Conduction velocity; Optical mapping; Connexin 43; Biomaterials; Alginate; Impulse conduction; Impulse propagation; Myocardial infarction; Myocardial scar; Ventricular tachycardia; Slow conduction; Large animal model; Electrophysiology; Sudden death; Stem cell engraftment

1 Introduction

Sudden cardiac death (SCD) due to healed myocardial infarction (MI) is the most common cause of mortality from heart disease. Current therapies for SCD are largely inadequate, making it a major unresolved clinical problem. Cell therapy has emerged as a promising approach for the treatment of MI [1, 2] and has been associated with restoration of electrical activity and reduced arrhythmia vulnerability [3–5]. For example, mesenchymal stem cells (MSCs) are the subject of ongoing clinical trials [6], and results to date demonstrate that MSCs are safe and improve function [7–9]. Studies from our laboratory suggest that MSCs may enhance impulse propagation in tissue damaged by acute ischemia [10] and cryoinjury [11] by a passive, electrotonic mechanism. Others have shown an association between MSCs and reduced arrhythmia propensity in animals [12] and patients [7] during acute MI, suggesting amelioration of the arrhythmia substrate. However, *in vitro* results suggest that MSCs can slow impulse conduction in neonatal rat ventricular myocyte monolayers [13, 14].

Limited retention and survival of stem cells can significantly reduce efficacy and, thus, represent major barriers for cell therapy in the treatment of MI. Suboptimal engraftment of MSCs may be due to the loss of MSCs during cell injections [15] and the diseased state of the recipient myocardium [16]. Biomaterials have shown significant promise as a way to improve heart function by lessening adverse remodeling associated with MI [17, 18]. In addition, biomaterials such as alginate may provide a supporting matrix for MSCs and may improve the harsh cardiac microenvironment that is seen in healed MI [15, 19]. We hypothesize that MSCs will improve impulse conduction and that alginate will enhance retention and survival of MSCs in a clinically relevant model of healed MI.

2 Materials and methods

2.1 Study groups

This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication no. 85–23, Revised 1996) and was approved by the IACUC of Case Western Reserve University. Animals were divided into two studies. The first was designed to investigate impulse conduction in the infarct border zone ($n=7$) of animals that were administered MSCs compared to animals that received sham phosphate-buffered saline (PBS) injections with no cells (control MI, $n=5$). Sample sizes were not equal because comparisons were made within each group (i.e., before and after treatment). The second study was designed to compare MSC retention in the infarct border zone when administered with alginate or without ($n=6$ per group).

2.2 Creation of MI

Female Yorkshire pigs ($n=13$), weighing 30–40 kg, were sedated with telazol, ketamine, and xylazine (1.5–2.5 mg/kg IM), intubated, and mechanically ventilated. Anesthesia was maintained with 0.5–2 % isoflurane. Access to the right carotid artery was obtained via a cutdown procedure and an arterial sheath was inserted. Selective catheterization of the left anterior descending artery (LAD) distal to the first diagonal branch was performed with a coronary guidewire under fluoroscopic guidance. An arterial balloon (2.5–3.0 mm) was inflated and left in place for 2.5 h followed by deflation of the balloon and reperfusion. All animals were continuously monitored throughout the procedure with 12 lead electrocardiograms, arterial blood pressures, pulse oximetry, and end tidal CO₂.

2.3 Cell therapy procedure

After 4 weeks, all animals underwent a median sternotomy to expose the infarct. Animals were divided into two study groups. In the first study group, for investigating impulse conduction in the infarct border zone, animals were administered 9–15 million 1,1'-diiododecyl-3,3,3',3'-tetramethylindocarbocyanine percholate (DiI)-labeled MSCs (suspended in PBS or alginate), or they received sham injections with no cells (control MI, $n=5$). In the second study group ($n=6$), designed to investigate retention and survival of MSCs, an equal number of DiI-labeled MSCs (two million) from the same lot were suspended in either alginate or PBS (for control) and administered into separate border zone regions within the same animal. In both study groups, all treatments were administered by epicardial injection in a region 1–2 cm in diameter (marked with suture) at the scar border zone near a coronary artery suitable for optical mapping in the left ventricular (LV) wedge preparation. In doing so, only a portion (~5–10 %) of the entire border zone (~20 cm in circumference) was treated. As a result, this procedure may not significantly impact whole heart function; however, the main objective of this study was to investigate impulse conduction and retention at the site of administration. Even though the total number of MSCs administered per animal in the present study appears low compared to that in clinical trials (up to 5 million/kg IV), we directly injected MSCs only at the site where measurements were taken. Thus, the number of cells administered per region of myocardial tissue is arguably similar (1–5 million/cm³).

2.4 Electrophysiological measurements *in vivo*

Electrophysiological recordings *in vivo* were performed immediately before treatment and 2 weeks later. Bipolar epicardial electrograms were obtained using a decapolar catheter (40–500 Hz bandpass filtering) while steady-state pacing (300 or 350 ms PCL) from the proximal bipolar pair and recording at the distal bipolar pair located at the site of injection (Fig. 1). The recording (cell injection) and pacing sites were marked with suture. Two weeks later, a median thoracotomy was performed to reexpose the heart. Epicardial electrograms were then obtained exactly as performed 2 weeks earlier. Electrogram width was measured from the first deflection of the isoelectric interval after pacing to the return to the isoelectric level for three consecutive beats and then averaged. *A priori*, electrogram width >30 ms was considered evidence of slow conduction.

2.5 Programmed electrical stimulation

Programmed electrical stimulation was performed from the epicardium of the LV in each animal just before cell therapy and again 2 weeks after. Up to three premature stimuli were delivered following a steady-state drive train from the same pacing location described above. If ventricular fibrillation (VF) or ventricular tachycardia (VT) were induced, the animal was cardioverted into normal sinus rhythm. This protocol was repeated again for a total of two times.

2.6 Mesenchymal stem cell isolation, expansion, labeling, and alginate suspension

Bone marrow was obtained from the tibia and femur of healthy Yorkshire pigs and passed through a Ficoll-Paque density gradient (GE Healthcare) to eliminate unwanted cell types as previously described [20]. Three to 4 days after plating, hematopoietic and other nonadherent cell types were washed away during medium changes. The remaining cells were expanded in culture until they reached 80–90 % confluence at passage 8 and labeled with DiI.

Sodium alginate 20/40 (generous gift from FMC Biopolymers) was gamma irradiated to a dose of 5 MRad (Phoenix Lab, University of Michigan; molecular weight 37,000 g/mol determined by SEC-MALS) and was covalently modified with the peptide sequence glycine–arginine–glycine–aspartic acid–serine–proline (GRGDSP) containing the RGD cell adhesion sequence as previously described [21]. The 5-MRad 20/40 alginate-RGD was then suspended in Dulbecco's modified Eagle medium-high glucose (DMEM-HG) at 1 or 2 % w/v.

The DiI-labeled MSCs were then suspended in PBS or 5 MRad 20/40 RGD-modified alginate (1 % or 2 % w/v) at a concentration of 2 million MSCs/ml of PBS or alginate and mixed thoroughly. Just prior to administration, 40 µl of CaSO₄ (8.4 g CaSO₄ in 40 µl sterile water) was then added to each milliliter of the MSC/alginate suspension to crosslink the alginate and immediately transferred to sterile syringes for injection.

2.7 Confocal immunofluorescence (IF) and histology

Immunostaining was performed according to previously published protocols [22]. In all experiments, tissue samples were obtained from the transmural surface immediately

opposite to that imaged in the optical mapping studies and prepared for confocal microscopy (three to four samples per wedge). Slides were incubated with the primary antibody rabbit connexin-43 (Cx43) polyclonal IgG (Sigma, C-6219) at 4 °C. The slides were then washed with PBS and then incubated for 2 h with corresponding secondary antibody donkey anti-rabbit IgG DyLight 488 (Jackson IR) diluted in PBS with blocking serum. After washing with PBS, TO-PRO-3 Iodide (Invitrogen, Carlsbad, CA) was added and washed. An upright spectral scanning confocal microscope (Model TCS-SP2; Leica Microsystems, Heidelberg, Germany) equipped with 633 diode (for TO-PRO-3 iodide) and 488 argon laser (Dylight 488) was used for confocal analysis. Images were collected by sequential excitation and analyzed using Leica Confocal software. To assess fibrosis in the infarct border zone, standard Masson trichrome staining was performed in transmural slices, and the area of blue staining was normalized to total tissue area (blue + red).

2.8 Optical mapping

Two weeks after cell therapy, hearts were explanted and immediately placed in ice-cold cardioplegia. Transmural sections of the border zone were dissected and perfused through a coronary vessel with oxygenated tyrode solution in a heat-controlled, tyrode-filled optical recording chamber. DiI fluorescence was detected using optical mapping techniques as described previously [23]. This allowed the precise overlay of MSC location with action potential activity. MSC retention was quantified by thresholding DiI fluorescence one standard deviation above the mean pixel value for the entire mapping field using image analysis software (ImageJ 1.42i).

Di-4-ANEPPS was used for voltage staining after MSCs were detected. The optical mapping system used has been described in detail previously [22, 23]. Briefly, action potentials were optically recorded at a magnification of $\times 1.24$, resulting in a total mapping field of 14 mm $\times$ 14 mm, with 0.9-mm spatial resolution and 0.8-mm² pixel size. Fluorescence was excited with uniform light from multiple light guides using a 250-W QTH lamp (filtered 510 $\pm$ 40 nm) and transmitted to a 16 $\times$ 16-element photodiode array (emission filter >610 nm). A CCD camera that is optically aligned with the photodiode array was used to obtain DiI fluorescence and visible images of the optical mapping field. To eliminate motion artifact, blebbistatin (Sigma) was used. Electrograms, perfusion pressure, and bath temperature were measured continuously during all experiments.

Action potential depolarization and APD were measured using previously described automated algorithms [24]. Activation and repolarization times were assigned for each action potential and confirmed by visual inspection, and local conduction velocity vectors were generated across the array as described previously [10]. These vectors and local APD were projected onto the image of thresholded DiI fluorescence to determine local conduction velocity and APD in areas with and without MSCs.

2.9 Echocardiography

Echocardiographic images (2-D) were acquired in all animals at baseline, 4 weeks after MI creation, and 2 weeks after cell therapy in the parasternal short-axis and long-axis views. Ejection fraction (EF) was determined using the biplane area-length method.

2.10 Statistical analysis

Statistical differences were assessed by ANOVA (with Bonferroni correction), Pearson correlation, Student *T*-test, as appropriate. Results were expressed as mean±standard error of the mean (SEM).

3 Results

3.1 In vivo impulse conduction

In all animals, before and 2 weeks after MSC treatment, *in vivo* impulse conduction slowing at the site of cell injection (marked by suture) was assessed by measuring electrogram (EGM) width from bipolar epicardial recordings made during steady state pacing. Shown in Fig. 2a are three consecutive beats recorded from the same location, before (4 weeks after MI) and 2 weeks after MSC therapy. The EGM recording before cell therapy demonstrates a wide (124–126 ms) and fractionated morphology. Two weeks after cell therapy, EGMs measured from the same location are shorter in width (66–67 ms). On average, MSC treatment was associated with a significant decrease in EGM width at the site of cell injection (-30 ± 8 ms, $p<0.008$), while control animals showed no change (4 ± 10 ms, $p=\text{ns}$). Pacing at a slower cycle length (350 ms, data not shown) also showed a significant decrease in EGM width at the site of cell injection (-26 ± 10 ms, $p<0.05$). These results suggest that epicardial conduction slowing at the site of cell injection is reduced by MSC therapy.

VF/VT was induced in all MSC-treated and control animals during PES before MSC treatment or sham injections (controls). VF/VT could not be reinduced 2 weeks after cell therapy in two of six animals that were treated with MSCs, whereas VF/VT was reinducible in all control animals. This difference, however, did not reach statistical significance. EF measured after MI in MSC-treated animals (0.45 ± 0.02) and controls (0.44 ± 0.02) was equally reduced compared to baseline (0.60 ± 0.03 and 0.58 ± 0.03 , respectively). After 2 weeks, EF in MSC-treated animals (0.44 ± 0.01) or controls (0.45 ± 0.02) was unchanged ($p=\text{ns}$).

3.2 Ex vivo local conduction velocity

After *in vivo* recordings, the heart was removed for optical mapping and tissue sample analysis (seven animals total, one to two samples per animal), to correlate MSC retention with local impulse conduction. The location of LV wedge preparations was chosen based on the site of MSC injection (marked with suture). Just prior to voltage staining with Di-4-ANEPPS, DiI fluorescence was detected using optical mapping techniques as previously described [23]. Shown in Fig. 3a is a representative image of raw DiI fluorescence from the transmural surface of a wedge preparation isolated from an area with MSCs. The dashed line demarcates the location of significant scarring where action potential amplitude was low, either due to the absence of viable tissue or poor dye staining. Shown in panel b is the same image segmented into two regions, MSC+ and MSC-, based on the threshold image of DiI fluorescence.

Optical action potentials were analyzed for activation time during steady state pacing (panel C, symbol) from which isochrone maps and local conduction velocity vectors were

calculated (panel C). Abnormal impulse conduction is evident by crowding of isochrone lines and small local conduction velocity vectors. However, less crowded isochrone lines with larger conduction velocity vectors are also present, suggesting significant heterogeneity of impulse conduction in the border zone. Panel D shows a superimposed image of local conduction velocity (CV) vectors (from panel C) and the DiI image segmented into MSC+ and MSC- regions (from panel B). In this example, the average local conduction velocity in the MSC+ region (0.49 m/s) was larger than that from the MSC- region (0.31 m/s), suggesting that MSCs reduced slow conduction in the border zone. Summary data over all experiments (Fig. 4) demonstrate that average local conduction velocity was significantly higher in areas with MSCs (0.49 ± 0.03 m/s) compared to areas without MSCs (0.39 ± 0.03 m/s, $p < 0.03$, $n = 12$). In addition, local conduction velocity in MSC+ region was greater than average local conduction velocity in control MI (sham injection) animals (0.32 ± 0.04 m/s, $p < 0.002$, data not shown). Finally, APD measured in MSC+ regions (190 ± 8 ms) was slightly longer than that measured in MSC- regions (166 ± 6 ms, $p < 0.03$).

3.3 Correlation between MSC retention and conduction velocity

To determine if MSCs were mechanistically related to improved impulse conduction in the border zone, the decrease in EGM width was correlated with the area of MSC retention. As shown in Fig. 5, the decrease in EGM width significantly correlated with the area of MSCs ($r = 0.92$, $p < 0.004$), suggesting that MSCs can ameliorate slow conduction in the border zone. When normalized to area of MSC retention, there was no difference in EGM width in the presence of alginate compared to PBS, indicating no deleterious effect of the alginate on impulse conduction.

3.4 MSC retention with alginate or without

In the second study group, MSC retention was compared with or without alginate while controlling for MSC dose, animal, and cell lot variability. DiI fluorescence was detected using optical mapping techniques as previously described [23]. Shown in Fig. 6 are representative images of raw DiI fluorescence (bottom) measured from the transmural surface of the wedge preparation. The raw (i.e., unprocessed) images show areas of DiI fluorescence associated with MSCs suspended in 1 or 2 % (w/v) alginate compared to PBS. For each image, the percent area of MSCs was quantified by equally thresholding the intensity of DiI fluorescence. The representative example and summary data show that the retention of MSCs was significantly greater with 2 % alginate compared to PBS or 1 % alginate. Alginate had no direct effect on conduction compared to PBS when normalized to the area of MSC retention (previous section), suggesting that its main benefit is increasing the retention of MSCs.

3.5 Cell survival and Cx43 expression

Immediately after DiI detection, tissue samples adjacent to the wedge preparation imaging surface were prepared for histology and confocal IF analysis to assess fibrosis, MSC survival, and Cx43 expression. Shown in Fig. 7 are images of Masson trichrome staining in border zone tissue samples from a region where MSCs in PBS, 1 % alginate, and 2 % alginate were administered. The percent fibrosis was similar for each group as depicted in the images and summary data (graph, $p = \text{ns}$). Thus, despite significant differences in

engraftment for each group (Fig. 6), there was no difference in fibrosis. This result suggests that when MSCs are administered several weeks after MI, there is little paracrine action that improves myocyte survival.

Shown in Fig. 8 (top) are images of DiI (red) and TOPRO (blue) staining from a region with MSCs in the border zone. With alginate or without (PBS), DiI was most often observed immediately surrounding normal nuclei, suggesting that the majority of MSCs survived 2 weeks after administration. The percent area of surviving DiI+ MSCs normalized to total area of DiI, as defined above, was high and not different ($p=ns$) between PBS ($87\pm5\%$), 1 % alginate ($94\pm4\%$), and 2 % alginate ($96\pm1\%$). The bottom images (Fig. 8) show Cx43 expression (green) and DiI (red) in a region with MSCs in the border zone that includes scar (upper right) and non-scar tissue (lower left). Cx43 expression in both regions is evident. The inset (white box) shows a higher resolution image of Cx43 in the scar near DiI-positive cells (white arrows). The bottom right image shows overlap between DiI and Cx43 (yellow), indicating that DiI-positive cells express Cx43. Note that DiI is a membrane stain, but expression becomes perinuclear over time and, thus, does not depict the entire extent of a DiI-positive cell. Nevertheless, the presence of Cx43 expression suggests structural cell-to-cell coupling.

4 Discussion

In the present study, we address two important barriers for cell therapy in the treatment of MI: the electrophysiological consequences and cell retention and survival. For the first time, we were able to directly associate MSCs with improved impulse conduction in a clinically relevant model of healed MI. In addition, we found that alginate significantly improves the retention of MSCs in the same setting. These findings may lead to more effective therapies for SCD associated with healed MI.

4.1 MSCs improve conduction at the border zone

Our *in vivo* electrophysiology and *ex vivo* optical mapping results suggest that MSCs significantly improve local conduction velocity at the infarct border zone. Moreover, we show a strong correlation between improvements in impulse conduction with the area of MSC retention (Fig. 5). These findings are consistent with our previous studies which suggest MSCs enhance electrical activity when administered following acute MI [10] or cryoinjury [11]. Other laboratories, such as Potapova et al. [25], have shown that human MSCs form gap junctions with adjacent cardiac myocytes *in situ*. Other cell types have also been shown to electrically integrate in the whole heart, such as embryonic stem cell-derived myocytes [3, 26] and donor cardiomyocytes transplanted into a recipient heart [27]. It is important to note that *in vitro* findings suggest that MSCs can slow impulse conduction, which is at odds with our findings [13, 14]. One explanation is that MSCs can slow conduction in well-coupled myocardium but can bridge microscopic block in fibrotic, border zone tissue [28]. It is important to point out that even though we showed a positive correlation between MSC area of engraftment and improved impulse conduction (Fig. 5), there was little impact on fibrosis (Fig. 7). Thus, it is not clear if normal conduction can be restored if the area of engraftment is further increased.

Consistent with improved impulse conduction, MSCs have also been shown to reduce arrhythmia burden [12, 5, 4]. Song et al. [5] showed that MSCs reduce the amount of sudden cardiac death in infarcted rats, in addition to decreasing infarct size and fibrosis. The authors do note, however, that more MSC engraftment is required for a more robust increase in electrical coupling and decreased fibrosis. Wang et al. [4] showed that MSCs reduced inducible VT and increased VF threshold during *in vivo* PES. Finally, in a double blind placebo-controlled prospective phase 1 clinical trial of MSCs delivered during acute MI, Hare et al. [7] showed an association between MSCs and decreased premature ventricular contractions (PVCs), a significant decrease in arrhythmic adverse events, and a decrease in overall adverse events at 2 years. It is possible that MSCs may decrease PVCs through cell-to-cell coupling (e.g., passive loading). Although we observed a nonsignificant decrease in arrhythmia inducibility in our study, this result is not surprising since we injected MSCs in a very limited region of the border zone, which has previously been shown to exert, mostly, a local effect [29].

4.2 Mechanism of improved conduction

There are several possible mechanisms that can explain how MSCs improved impulse conduction in the border zone. It is possible that MSCs differentiate into cardiac myocytes [30–33]. However, we have recently shown [11] along with others [34] that MSCs only exhibit signs of electronic influence rather than action potential generation [11]. This and other studies [35, 36] suggest it is unlikely that MSCs differentiate into cardiomyocytes that generate action potentials.

If MSCs do not differentiate into cardiomyocytes but demonstrate passive electrotonic interaction, then they should couple to cells. In the present study, confocal imaging demonstrated Cx43 expression in cells that are Dil positive (MSCs). This finding is consistent with studies from our group [10, 11] and others showing that MSCs have the ability to express Cx43 *in vivo* [37, 38] and *in vitro* [14, 39, 28, 40, 36]. Thus, it is possible that MSCs bridge microscopic barriers in fibrotic tissue, as mentioned above [28]. Finally, it is also possible that MSCs exert their influence on impulse conduction in a paracrine fashion. The role of MSCs as paracrine effectors has been demonstrated in previous studies [41, 13]. It has also been shown that MSCs attenuate the reduction and lateralization of Cx43 in the border zone [4], most likely through a paracrine effect. Our data show no paracrine action on host myocyte survival (Fig. 7), which is consistent with administration of MSCs after acute MI. Collectively, these data suggest that MSCs can improve abnormal impulse conduction in scarred myocardium without differentiating into mature myocytes.

4.3 MSC retention is significantly enhanced with alginate

Up to now, progress in cardiac cell therapy has been hampered by suboptimal engraftment of donor cells [42]. Thus, improving cell engraftment is of major clinical importance and has stimulated further investigation on the use of biomaterials [42]. In the present study, our results clearly show that when MSCs are suspended in alginate, retention is significantly improved. Thus, a significant benefit of alginate, at the least, is improved retention of injected MSCs. Our results are in agreement with prior studies that investigated the use of bioengineered scaffolds as cell delivery vehicles. Le Visage et al. [43] showed an increase in

MSC engraftment using a porous polysaccharide-based scaffold up to 2 months after cell injection in a rat model of acute MI. In addition, Rustad et al. [15] showed that a pullulan-collagen hydrogel enhanced MSC viability, cell survival, and faster healing in a murine model of wound healing. To our knowledge, there have been no studies quantifying MSC engraftment in an alginate scaffold in healed MI. The mechanism of improved retention is not clear. The RDG-modified alginate used in the present study could help the adhesion and spreading of MSCs [44]. Alternatively, Rustad et al. [15] suggest that hydrogels can significantly improve the MSC microenvironment by increasing the secretion of VEGF-A and MCP-1. Hydrogel alone has been shown to improve mechanical function of the infarcted heart; however, its electrophysiological effects are less clear [18]. In the present study, alginate did not appear to have any direct influence on impulse conduction; however, indirectly, it can by increasing cell retention which was strongly correlated with improvement in impulse conduction (Fig. 5).

4.4 Clinical implications

Most cell therapy and tissue engineering strategies have focused on mechanical improvement, hemodynamic benefit, and safety (e.g., proarrhythmia) in the setting of acute MI. In contrast, in the present study, we focus on the electrophysiological benefit of MSCs in a clinically relevant model of healed MI that may be translated to humans. Using MSCs in a targeted fashion to improve conduction at the border zone of healed MI may be more effective than current therapeutic strategies for the treatment of ventricular tachycardia and abnormal substrates associated with healed MI. In addition, considering the larger number of cells required in large hearts (e.g., humans), alginate may also be used to significantly improve MSC engraftment.

4.5 Limitations

MSCs were not delivered to the entire border zone or in a targeted fashion to prevent VT, so our inability to show a significant reduction in arrhythmia vulnerability may be expected. Additional studies using a targeted approach or more complete coverage are needed. In addition, our epicardial injection method is likely not clinically practical as a primary procedure by itself. However, cells could be delivered by an endocardial injection catheter via a percutaneous approach [7]. Finally, we did not determine the fate of injected MSCs or if Cx43 was expressed at the membrane of host myocytes or administered MSCs in the present study. However, in a previous study using a rat model of chronic injury with rat MSCs, we found that injected MSCs do not generate action potential activity, exhibit striations, or express Nav1.5 [11].

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Fig. 1.

Experimental methods. Schematic diagram of the heart 4 weeks after MI with representative anterior-apical scar. Cells were injected at the border zone of the scar in a region suitable for isolation of the LV wedge preparation (*dashed circle*). Steady state pacing was performed from the proximal end of a catheter placed on the epicardial surface from outside the scar (*pacing symbol*) with $2\times$ threshold current. Bipolar electrograms were recorded before and 2 weeks after cell injections from the distal electrode of the catheter placed at the center of the cell injection site. Pacing and recording (*cell injection*) sites were marked with suture

Fig. 2.

In vivo impulse conduction. **a** Local bipolar electrograms (*EMG*) that were recorded at the infarct border zone during steady state pacing at 300-ms pacing cycle length before and after cell treatment. EGM width was measured from the first deflection from the isoelectric interval after pacing to the return to isoelectric. Summary data for all animals (**b**) show that MSC therapy significantly shortened EGM width compared to before MSC therapy (*bottom left*), whereas sham injections showed no change (*bottom right*)

Fig. 3.

Ex vivo impulse conduction example. **a** Representative image of Dil fluorescence from the transmural surface of a wedge preparation isolated from the area of MSC engraftment. The *dashed line* demarcates the location of significant scarring. **b** The same image segmented into two regions based on the thresholded image of Dil fluorescence, MSC+, and MSC-. **c** Isochrone map and local conduction vectors calculated from analyzing activation time of optical action potentials measured during steady state pacing (*pacing symbol*). **d** Superimposed image of local conduction velocity vectors (**c**) and the Dil image segmented into MSC+ and MSC- regions (**d**). In this example, the average local conduction velocity was higher in the MSC+ region compared to the MSC- region

Fig. 4.

Ex vivo impulse conduction summary. Summary data from all animals show that average local conduction velocity was significantly higher in areas of MSC engraftment (*black*) compared to areas without MSCs (*white*, $p < 0.03$)

Fig. 5.

Impulse conduction and area of engraftment. Decrease in EGM width correlated with the area of MSC engraftment in treated animals. The decrease in EGM width became larger as the area of MSC engraftment increased ($r=0.92$, $p<0.004$), suggesting a mechanistic relationship

Fig. 6.

Area of engraftment with alginate. **a** Image of the heart (*upper left*) with the approximate location of tissue samples chosen for analysis of MSC engraftment. The images on the bottom show raw DiI fluorescence (MSC label) from three areas spanning the transmural wall that were injected with cells suspended in PBS, 1%, or 2 % alginate. Each fluorescence image was thresholded to determine the percentage of MSC engraftment area per imaging field. **b** Summary data from all animals. Engraftment significantly increased only with 2 % alginate (ANOVA with Bonferroni correction, $p < 0.05$)

Fig. 7.

Percent fibrosis. The images show Masson trichrome staining in tissue samples spanning the transmural wall that were injected with cells suspended in PBS, 1 %, or 2 % alginate.

Percent fibrosis (*blue area*) was normalized to total tissue area in each sample ($n=4$ for each group). Despite significant changes in engraftment (Fig. 6), there was no difference in percent fibrosis (ANOVA, $p=ns$)

Fig. 8.

Survival and Cx43 expression. *Top*: Representative confocal IF images from three separate regions where MSCs suspended in 2 % alginate, 1 % alginate, and PBS were injected. With alginate or without, Dil (*red*) was observed surrounding normal nuclei (TOPRO, *blue*), suggesting MSCs survive 2 weeks after administration. The bottom images show Cx43 expression (*green*) and Dil (*red*) in the border zone that includes scar (*upper right*) and non-scar tissue (*lower left*), with a higher resolution image at the inset (*white box*). The *bottom right* shows a high-magnification image of overlap between Dil and Cx43 (*yellow*). The pattern of Cx43 expression suggest cell-to-cell coupling. *Scale bar*= 10 μm