Optical mapping of cryoinjured rat myocardium grafted with mesenchymal stem cells

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Costa AR, Panda NC, Yong S, Mayorga ME, Pawlowski GP, Fan K, Penn MS, Laurita KR. Optical mapping of cryoinjured rat myocardium grafted with mesenchymal stem cells. Am J Physiol Heart Circ Physiol 302: H270–H277, 2012. First published October 28, 2011; doi:10.1152/ajpheart.00019.2011.—Mesenchymal stem cells (MSCs) have been shown to improve cardiac electrophysiology when administered in the setting of acute myocardial infarction. However, the electrophysiological phenotype of MSCs in situ is not clear. We hypothesize that MSCs delivered intramyocardially to cryoinjured myocardium can engraft, but will not actively generate, action potentials. Cryoinjury-induced scar was created on the left ventricular epicardial surface of adult rat hearts. Within 30 min, hearts were injected with saline (sham, n = 11) or bone marrow-derived MSCs (2 × 10^6) labeled with 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate (DiI; n = 16). At 3 wk, optical mapping and cell isolation were used to measure optical action potentials and calcium transients, respectively. Histological analysis confirmed subepicardial scar thickness and the presence of DiI-positive cells that express connexin-43. Optical action potential amplitude within the scar at MSC-positive sites (33.8 ± 14.3%) was larger compared with sites devoid of MSCs (35.3 ± 14.2%, P < 0.05) and sites within the scar of shams (35.5 ± 6.9%, P < 0.05). Evidence of simultaneous action potential upstroke, the loss of action potential activity following ablation of adjacent viable myocardium, and rapid calcium transient response in isolated DiI+ cells suggest that the electrophysiological influence of engrafted MSCs is electrotonic. MSCs can engraft when directly injected into a cryoinjury and are associated with evidence of action potential activity. However, our results suggest that this activity is not due to generation of action potentials, but rather passive influence coupled from neighboring viable myocardium.

stem cell; action potential; myocardial infarction

STEM CELL THERAPY IS A PROMISING treatment strategy for patients with ischemic heart disease and has been associated with improved hemodynamic function (11, 16, 27, 29). Despite the high risk of sudden cardiac death associated with ischemic heart disease, much of the electrophysiological focus of cell therapy has been on safety (i.e., proarrhythmia) (11, 16, 27). Safety of cell therapy is clinically very important; however, very few reports have investigated the electrophysiological phenotype of stem cells in situ (18, 23, 26).

In previous studies, our laboratory demonstrated that mesenchymal stem cell (MSC) therapy is associated with enhanced electrical viability (evidence of action potentials) in scarred myocardium when cells are administered at the time of an initial ischemic event (18, 31). However, the mechanism of this enhancement is unclear. MSCs have been associated with cardiac-like properties (1, 13, 22, 30) and gap junction proteins responsible for electrical cell-to-cell coupling (14, 32); however, this finding is not universal, and in some settings MSCs can have a detrimental effect (2). Another gap in knowledge is that, for many patients, stem cell therapy can only be administered after a previous ischemic event, when the infarct has already healed. In this setting, the chemical signals associated with acute ischemia are absent, and it is not clear if stem cells will even engraft. We hypothesize that MSCs delivered intramyocardially to cryoinjured myocardium will engraft but not actively generate action potentials.

MATERIALS AND METHODS

Experimental model. 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 Institutional Animal Care and Use Committee of both Case Western Reserve University and the Cleveland Clinic. Male Lewis rats, weighing 250–300 g, were anesthetized with 3% isoflurane, intubated, and mechanically ventilated with room air at 80 breaths/min with a pressure-cycled rodent ventilator (Harvard Apparatus, model 683, Holliston, MA). The heart was exposed through a median thoracotomy, and a cryoinjury was created on the left ventricular (LV) epicardial anterior wall of all animals by applying for 20 s a round 4-mm-diameter metal probe cooled to −80°C. Two groups of rats were utilized in this study: scar + saline injection (sham, n = 11) and scar + MSC injection (MSC treated, n = 16). In these groups, six and eight animals, respectively, were used for optical mapping studies, and the others for fixed tissue sample analysis and cell isolation.

MSC procedure. Rat bone marrow was isolated by flushing the femurs with 0.6 ml DMEM (GIBCO, Invitrogen, Carlsbad, CA). Clumps of bone marrow were gently minced with an 18-gauge needle and then centrifuged for 5 min at 260 g and washed with two changes of phosphate-buffered saline (PBS) (Invitrogen). The washed cells were then resuspended and plated in DMEM-LG (GIBCO, Invitrogen) with 10% FBS and 1% antibiotic and antymycotic (GIBCO, Invitrogen). The cells were incubated at 37°C, and nonadherent cells were removed by replacing the medium after 3 days. When cultures became 70% confluent, adherent cells were detached following incubation with 0.05% trypsin and 2 mM EDTA (Invitrogen) for 5 min. MSC cultures were then depleted of CD45 + and CD34 + cells simultaneously by negative selection using 10 μl each of primary phycoerythrin-conjugated mouse anti-rat CD45 (BD Biosciences, San Diego, CA) and CD34 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) per 10^6 cells using the EasySep PE selection kit (Stem Cell Technologies). Finally, confluent cells were passaged and plated out at 1:4 to 1:5 dilutions until passage 12 (18). Approximately 2 days before administration, cells were labeled with DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate, Invitrogen). Just before administration, 2 × 10^6 cells were suspended in 200 μl of PBS.
Cells were intramyocardially injected just below (<1 mm) and parallel to the epicardial surface at three to four sites within the cryoinjury of each animal in the MSC-treated group. In the sham group, 200 µl of PBS alone were injected in a similar fashion.

**Optical mapping.** Optical mapping was performed in Langendorff-perfused rat hearts 3 wk after cryoinjury, as described previously (17). Rats were injected with 1,000 units ip heparin and then anesthetized with 250 mg/kg ip sodium pentobarbital. Hearts were excised and immersed in 34°C Tyrode solution containing (mM) 140 NaCl, 4.56 KCl, 0.73 MgCl₂, 10 HEPES, 5.0 dextrose, 1.25 CaCl₂, and 20–2–3 butanedione monoxime (pH 7.4) in an optical recording chamber. To help determine the mechanisms of MSC action, detection of DiI fluorescence was performed before voltage staining (see below) using optical mapping techniques, as described previously (6). This allowed the precise overlay of MSC engraftment with optical action potential activity.

For voltage staining, the heart was placed in 10 ml of ice-cold cardioplegia and 250 µl of 4-(β-[2-(di-n-butylamino)-6-naphthyl]vinyl)pyridium (di-4-ANEPPS, voltage dye) for 30 min. The cardioplegia was bubbled with O₂ 100% to gently agitate the heart and ensure uniform staining of the entire epicardial surface. After completion of staining, the aorta was cannulated and perfused with 33–34°C Tyrode solution (40–60 mmHg). Electrocardiogram, perfusion pressure, and bath temperature were measured continuously during all experiments.

The optical mapping system used has been described in detail previously (17). Briefly, action potentials were optically recorded at a magnification of ×2.1 from 256 sites within an 8.3-mm × 8.3-mm mapping field (0.52-mm interpixel resolution) on the anterior epicardial surface of the LV. Fluorescence was excited with uniform light from multiple light guides using a 250-W QTH lamp (filtered 510 ± 40 nm) and transmitted to a 16 × 16-element photodiode array (emission filter >610 nm), where fluorescence intensity measured at each photodiode was linearly converted to a voltage signal. Voltage signals were low-pass (500 Hz) filtered and had DC offset removal in hardware. Since di-4-ANNEPS emission (>610 nm) does overlap with DiI emission (<605 nm), it is possible that an offset will be added to the optically recorded action potentials. However, since offset is removed in hardware, this will not influence our results. A charge-coupled device camera that is optically aligned with the photodiode array was used to obtain DiI fluorescence and visible images of the optical mapping field relative to anatomical landmarks (e.g., scar and normal tissue). To eliminate motion artifact, 20 mM of 2,3-butanedione monoxime or 5 µM blebbistatin were used.

**Experimental protocol.** All hearts were paced at steady-state cycle lengths of 200, 190, and 150 ms using a unipolar electrode placed on the epicardial surface at 2 × threshold. While not the main objective of this study, arrhythmia vulnerability testing with programmed stimulation was performed followed by Newman-Keuls post hoc analysis. A value <0.05 was considered statistically significant. Data are presented as means ± SD, unless otherwise noted.

**RESULTS**

**Cryoinjury.** Shown in Fig. 1 is a representative example of the cryolesion scar morphology. Figure 1A is an image of the anterior surface of an MSC-treated heart with the scar in the center (dashed circle). In all hearts, H&E staining confirmed the presence of scarred myocardium (~30–50% of the transmural wall. Figure 1B shows a transmural cross section of the LV wall, demonstrating the scar above normal tissue in a sham heart. Confocal imaging of α-actin (green) demonstrates significantly less striated cardiac myocytes in the scar compared with normal tissue below the scar. Over all experiments (Fig. 1C), the scar thickness in the sham group (549 ± 310 µm) was not significantly different from the MSC-treated group (511 ± 345 µm).
**MSC engraftment.** At the time of optical mapping, all MSC-treated hearts demonstrated significant Dil fluorescence in the scar (Fig. 2). Figure 2A shows a schematic diagram of the whole heart with an image of Dil fluorescence (orange) superimposed on an image of the epicardial extent of the scar (dashed line) that was recorded at the same time using optical mapping techniques. In all hearts, Dil fluorescence was localized to discrete regions that were consistent with the sites of cell injection noted at the time of surgery. Transmural sections (Fig. 2B) of H&E (left) and Dil (right) from two consecutive tissue slices shows Dil+ cells within the scar (dashed line) and close to the epicardial surface. Some Dil+ cells were also observed at deeper layers (arrow). Shown in Fig. 2C are confocal images from a region that did (MSC+) and did not (MSC−) demonstrate significant Dil fluorescence in the optical mapping image. The confocal image from the MSC+ region revealed positive perinuclear Dil fluorescence (red), suggesting MSCs are viable. The confocal image from the MSC− region showed no perinuclear Dil fluorescence. These data suggest that Dil-labeled MSCs engraft and survive when directly injected into the cryoinjury, and that macroscopic engraftment is heterogeneous.

Some cells that were Dil positive in the MSC+ region were also positive for Cx-43 (Fig. 3A). The confocal images, which are all taken from the same scan, show Dil fluorescence (left, red), Cx-43 expression (middle, green), and their colocalization (right, merged). The top images are taken from the border zone, where MSCs are absent (MSC−), and Cx-43 is expressed at intercalated disks (arrows). The bottom images (Fig. 3A) are from the same confocal scan, but in an MSC+ region of the border zone. Cx-43 expression in the scar is evident (arrows); however, the pattern of expression is not the same as that from normal myocytes (MSC−). In one case (asterisk), Dil and Cx-43 are colocalized in the cytoplasm. NaV1.5 expression (Fig. 3B) was significantly higher in the MSC− region of the border zone compared with the MSC+ region.

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**Fig. 1.** A: representative image of heart anterior surface with the scar at center, indicated by the dashed circle. B: representative transmural cross section of the left ventricular (LV) wall of a sham heart demonstrating the scar (700 μm thick) above normal tissue. Confocal images (240 × 240 μm) on the right show significant positive α-actin (green) expression in normal tissue, but not in the scar. Viable nuclei are shown in blue (ToPro). C: scar thickness for sham (n = 11) and mesenchymal stem cell (MSC)-treated (n = 12) groups was not significantly different [P = nonsignificant (NS)]. Values are means ± SE. LA, left atrium.

**Fig. 2.** A: schematic diagram of the whole heart with a representative optical mapping image of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) fluorescence (orange) superimposed on an image of the epicardial extent of the scar (dashed line). The size of the image corresponds to the size of the optical mapping field relative to the whole heart. Dil fluorescence is localized to discrete regions consistent with the sites of cell injection. B: hematoxylin and eosin (H&E) and Dil fluorescence (MSC) across the transmural wall from two consecutive tissue slices showing the presence of MSCs in the scar (dashed line) near the epicardium. Occasionally, MSCs were observed in deeper layers (arrow) below the scar. C, top: confocal image from a region with significant Dil fluorescence on the epicardial surface (MSC+) that shows positive Dil fluorescence (red) from viable cells (ToPro, blue). Bottom: confocal image from a region with no significant epicardial Dil fluorescence (MSC−) shows no DiI fluorescence (red) from viable cells (ToPro, blue). The scale bar represents 15 μm.

**Fig. 3.** A: confocal images of connexin-43 (Cx43) expression from a single scan in the cryolesion border zone. Top: normal ventricular Cx43 expression at the intercalated disk (arrows, green) from the cryolesion border zone, which was negative for Dil fluorescence (MSC−). Viable nuclei are shown in blue (ToPro). Bottom: from the same confocal scan but within an MSC+ region in the border zone, MSCs are shown to expresses Cx43 (arrows). In one case, Dil and Cx43 are colocalized in the cytoplasm (*). B: significantly greater expression of voltage-gated Na+ channel (Nav1.5; green) in the MSC− region compared with the MSC+ region. The scale bar represents 20 μm in A and B.
Previously, our laboratory has shown that optical action potential amplitude can be used to estimate the number of cells exhibiting transmembrane potential activity from normal and scarred myocardium (17). Therefore, to assess the electrophysiological phenotype of MSCs in situ, we directly compared optical action potential amplitude at sites with (MSC+) and without (MSC−) MSC engraftment. Shown in Fig. 4 are optical action potential amplitude (isopotential) maps, where maximum (100%) is equal to the maximum action potential measured in the normal region. At the top is a normal (without scar) Langendorff-perfused rat heart with a depiction of the mapping field (box, Fig. 4A) and a cross section of a heart with scar indicating the depth of Di4-ANEPPS staining (Fig. 4B, red). In this study, di-4-ANEPPS penetrated 200–500 μm into the scar. This suggests that it is unlikely that layers below the scar are influencing optical action potential measured in the scar of MSC-treated hearts or shams. It also suggests that some DiI cells in the scar are not stained with di-4-ANEPPS. Below (Fig. 4C) shows a representative example of optical action potential amplitude distribution in a normal heart. The decrease in amplitude at the periphery is likely due to lens vignetting and reduced excitation light. The action potentials shown to the right indicate a normal morphology across the mapping field.

Shown in Fig. 4D is a representative example from the sham (saline injection) group. There is no evidence of normal action potential activity within the scar (dashed line), except at the scar border zone. In contrast, for an MSC-treated heart (Fig. 4E), the isopotential map demonstrates a region of large optical action potentials with normal morphology from the central portion of the scar (sites a and b). As shown in the image of MSC engraftment (Fig. 4F), regions with MSC+ cells were closely associated with increased optical action potential amplitude.

Over all experiments (Fig. 5), the average optical action potential amplitude within the scar at sites positive for MSCs (>50% DiI fluorescence intensity) was significantly larger than sites negative for MSCs (<50% DiI fluorescence intensity) in the MSC-treated group and also compared with sites within the scar from the sham group. These results were similar for all steady-state cycle lengths tested (200, 190, and 150 ms) and for experiments using blebbistatin (MSC+ 61 ± 2% and MSC− 38 ± 2%; n = 4, P < 0.001). The reason why action potential amplitude was nonzero in sham animals is unknown, but this could be due to a small number of surviving myocytes and residual motion artifact. APD at 80% of resting potential measured in the scar from MSC+ regions (61.2 ± 10.6 ms) was not statistically different from that measured in normal regions remote from the scar (51.4 ± 15.0 ms, P = nonsignificant [NS]) and from normal regions adjacent to MSC+ regions (50.3 ± 7.3 ms, P = NS). Finally, there was no difference in arrhythmia inducibility between sham and treated groups (1 of 6 and 2 of 8, respectively, P = NS), which was relatively low for both groups.

**Mechanisms of action potential activity associated with MSCs.** If optical action potentials observed in the MSC+ region are due to MSCs that generate action potentials, then propagation should be evident. Shown in Fig. 6 are two examples demonstrating the time of dVmax/drmax and absolute AP Amplitude.
amplitude of optical action potentials within the mapping field of hearts treated with MSCs. In both examples, in the MSC+ region (red), action potential dV_m/dt_max is near simultaneous, and amplitude is reduced. This is depicted by the large spacing between isochrone lines in the MSC+ regions and by the simultaneous time of dV_m/dt_max (circles) in the optical action potentials recorded along a line that traverses the scar and MSC+ regions (sites a–f). This suggests that action potential activity observed in the MSC+ region is due to passive electrotonic influence. In contrast, action potential activity outside the scar exhibits propagation. Activation perpendicular to the epicardial surface (from deeper layers) could also explain simultaneous dV_m/dt_max in MSC+ regions; however, pacing is from the epicardial surface just outside the scar (pacing symbol). Some of these action potentials (bottom site f) may be lower in amplitude than average for MSC+ regions (Fig. 5). This could be due to DiI+ cells below the di-4-ANEPPS-stained layer (Fig. 4B).

To determine whether action potential activity in the MSC+ region is electrotonic activity from deeper viable layers, we performed optical mapping before and after an endocardial cryoablation procedure. Shown in Fig. 7A at the top (left) is an image of a heart cross section that was TTC stained after the endocardial ablation, to verify the absence of viable tissue below the scar and a viable epicardium. Before ablation, increased optical action potential amplitude and normal action potential morphology (sites a and b) were evident in areas of significant MSC engraftment (top right). After the ablation procedure, optical action potential activity is almost absent compared with the same region before the ablation procedure. Similar results were observed in all hearts ablated, suggesting that the mechanism of action potential activity in MSC+

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bathed in warm oxygenated Tyrode solution, a technique our laboratory has used previously to measure normal action potential activity from the epicardial surface without influence from deeper layers (8). Therefore, MSCs likely survived the ablation procedure.

Finally, cells were isolated from four hearts in the MSC-treated group (Fig. 8). All cells with a normal ventricular morphology (rod shaped, striations) were Dil−. Upon field simulation at 1 Hz, these Dil− cells contracted and exhibited normal calcium transients (Dil−, top). Dil+ cells (MSCs) were mostly rounded and exhibited no striations. The majority of Dil+ cells (20 out of 23) exhibited no contraction or calcium transients upon field stimulation. However, a small percentage of Dil+ cells (3 of 23) reproducibly exhibited calcium transients in response to field stimulation without evidence of contraction. However, these calcium transients were relatively slow and were unable to capture at 1 Hz (Dil+, bottom). These results suggest that Dil+ cells are not mature cardiac myocytes that can respond to normal rates and contract. Moreover, the slow calcium response is consistent with the lack of NaV1.5 that was observed in tissue (Fig. 3). These data provide additional evidence that Dil+ cells in situ do not generate action potential activity, but rather contribute in a passive (i.e., electrotonic) manner.

DISCUSSION

In the present study, we investigated the in situ cellular electrophysiology of MSCs engrafted in nonischemic scarred myocardium. We found that MSCs can engraft when directly injected into the cryoinjury and are associated with evidence of action potential activity. However, these cells do not generate action potentials. The action potentials we measured could be due to a passive (i.e., electrotonic) effect from viable myocardium adjacent to and below the scar.

**MSC survival and engraftment.** Dil fluorescence imaging using optical mapping techniques and in tissue samples using confocal immunoﬂuorescence suggest that MSCs engraft and survive when directly injected into the cryoinjury. However, the macroscopic engraftment is heterogeneous and limited to sites of injection at the epicardium (Fig. 2). The heterogeneous engraftment pattern is in agreement with previous findings, when MSCs were delivered by a transendocardial route (20) and skeletal myoblasts were directly injected epicardially (6). Therefore, despite the absence of acute ischemia, which is associated with chemical signals that could retain MSCs (9, 31), our results suggest that MSCs still engraft and survive.

**Cellular electrophysiological MSCs in situ.** Most cell therapy studies have focused on scar reduction, hemodynamic benefit, and safety (proarrhythmia) (1, 11). In contrast, very few studies have investigated the electrophysiology of stem cells in situ. In the present study, we found that MSC+ regions were associated with a larger optical action potential amplitude compared with areas devoid of MSCs. These results are consistent with our laboratory’s previous studies in which MSCs were delivered intravenously during acute ischemia and were associated with enhanced action potential activity (18, 31). Unlike these past studies, in the present study, we were able to directly correlate regions of MSC engraftment (DiI fluorescence) with optical action potential amplitude. Other laboratories have shown evidence of functional electrical integration of stem cells in the whole heart (33)(26). Roell et al. (23) showed that embryonic cardiomyocytes were associated with improved electrical viability and decreased ventricular tachycardia inducibility after cryoinjury. However, in the present study, we focused on MSCs, which have been recently shown to improve patient outcome (10).

**Electrophysiological mechanisms of MSCs in situ.** Several reports suggest that MSCs differentiate into cardiac myocytes (1, 13, 25, 30). Rota et al. (25) showed that bone marrow cells can acquire a cardiomyogenic and vascular fate after 30 days. Dai et al. (4) showed that muscle-specific and endothelial markers are present in DiI+ MSCs at 6 mo, but not as early as 2 wk. However, even after 6 wk, these cells did not take on an adult cardiac phenotype. MSCs co-cultured with embryonic cardiomyocytes show the presence of cardiac makers (e.g., troponin and connexin), but no evidence of action potential generation (24). The action potential activity we measured over an aggregate of DiI+ MSCs exhibited signs of electric influence from deep layers rather than action potential generation (Fig. 6, reduced amplitude and simultaneous dV/dtmax). Once deeper viable myocardial layers were eliminated by ablation, optical action potential amplitude within the scar was essentially eliminated, except at the epicardial border zone of the scar (Fig. 7). Moreover, isolated Dil+ cells (MSCs) did not exhibit the morphological characteristics of ventricular myocytes (rod shape, striations), nor did they demonstrate rapid calcium transients during field stimulation (Fig. 8). These findings are supported by previous studies that have demonstrated electrotonic interactions between MSCs and cardiomyocytes (32), and from studies that have shown optically recorded action potentials from cells that cannot generate action potentials (7).

In the present study, confocal imaging demonstrated Cx-43 expression in Dil+ cells (MSCs). This finding is consistent with others showing that MSCs have the ability to express Cx-43 and Cx-45 in vivo (10, 14) and in vitro (2, 19, 21, 32). Moreover, it has been shown that MSCs are able to functionally couple with other cells (21, 32). Studies from our group (5, 6) and others (12, 15, 23) have shown the importance of connexin expression in engrafted cells. This may explain the electrotonic influence we observed. However, the pattern of Cx-43 cellular expression (and NaV1.5) we measured is unlike that seen in contracting host myocytes, suggesting that these cells after 3 wk are not mature, contracting, cardiac myocytes.
**Implications.** A potentially important clinical implication of our work is that MSCs might be able to engraft, survive, and passively enhance electrical viability when directly injected into chronic myocardial scar. In addition, as in clinical trials using bone marrow cells (16, 27, 29) or purified MSCs (3, 11), we did not report an increase in arrhythmias. It is even possible that the passive electrical nature of MSCs may suppress premature ventricular contraction activity in patients (11). However, it is unlikely that MSCs in this setting are able to generate action potential activity. Finally, it is also important to consider the loading effect MSC may have on conduction and APD (15). We were unable to determine whether conduction was altered in normal tissue next to MSC+ regions; however, APD was not significantly different.

**Limitations.** The cryoinjury model is not good for, and was not chosen to test, arrhythmia inducibility. Cryoinjury was used because arteries remain patent compared with left anterior descending coronary artery occlusion, and the location and extent of cryoinjury injury, as well as the location of cell injection relative to cryoinjury, could be reproduced across all animals. In addition, cryoinjury is not an accurate model of chronically healed myocardial infarction. However, chronic myocardial infarction in rats leads to very thin walls (thickness <1 mm), which makes it nearly impossible to inject cells into the scar.

It is possible that optical action potentials recorded from the scar include fluorescence from active tissue below the scar. However, we show that, when hearts are superfused with di-4-ANEPPS, the staining does not exceed 500 μM of depth from the epicardium (17). Moreover, 95% of di-4-ANEPPS fluorescence is measured from a depth of <0.5 mm, even when staining is uniform across tissue depth (8). Since the optical action potentials measured in the MSC+ region were, at the least, 50% of the amplitude measured in normal tissue, it is very unlikely this was from deeper layers. Even if deeper layers were influencing action potential amplitude measured in the scar, this should be equal across all groups. But MSC+ regions had significantly higher action potential amplitude compared with regions that had no MSCs (Fig. 5). Alternatively, confocal imaging from the epicardial surface in the whole heart could be used to avoid fluorescence from deeper tissue layers (26).

We cannot exclude paracrine effects in this study. MSCs release cytokines and growth factors that stimulate endogenous repair mechanisms (9, 28) associated with increased angiogenesis, downregulation of proapoptotic proteins, and recruitment of cardiac stem cells. That said, it is important to note that the action potential amplitude in the MSC− areas was the same as that in sham-treated animals, suggesting that the presence of the MSCs may be required for the electrical changes we observed.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


