Cardiac bone marrow cell injection for chronic ischemic heart disease

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Chapter 2

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Human Adult Bone Marrow Mesenchymal Stem Cells Repair Experimental Conduction Block in Rat Cardiomyocyte Cultures

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Abstract

Introduction: Autologous stem cell therapy is a novel treatment option for patients with heart disease. However, detailed electrophysiological characterization of hMSC is still lacking. We evaluated whether human adult bone marrow-derived mesenchymal stem cells (hMSC) could repair an experimentally induced conduction block in cardiomyocyte cultures.

Methods: Neonatal rat cardiomyocytes were seeded on multi-electrode arrays. After 48 hours, abrasion of a 200-450 μm wide channel caused conduction block. Next, we applied adult hMSC (hMSC group; n=8), human skeletal myoblasts (myoblast group; n=7), rat cardiac fibroblasts (fibroblast group; n=7), or no cells (control group; n=7) in a channel-crossing pattern. Cross-channel electrical conduction was analyzed after 24 and 48 hours. Intracellular action potentials of hMSC and cardiomyocytes were recorded. Immunostaining for connexins and intercellular dye transfer (Calcein) assessed the presence of functional gap junctions.

Results: After creation of conduction block, 2 asynchronously beating fields of cardiomyocytes were present. Application of hMSC restored synchronization between the 2 fields in 5 out of 8 cultures after 24 hours. Conduction velocity across hMSC (0.9±0.4 cm/s) was approximately 11-fold slower than across cardiomyocytes (10.4±5.8 cm/s). No resynchronization occurred in the myoblast, fibroblast or control group. Intracellular action potential recordings indicated that conduction across the channel presumably occurred by electrotonic impulse propagation. Connexin-43 was present along regions of hMSC-to-cardiomyocyte contact, but not along regions of cardiomyocyte-to-myoblast or cardiomyocyte-to-fibroblast contact. Calcein transfer from cardiomyocytes to hMSC was observed within 24 hours after co-culture initiation.

Conclusion: hMSC are able to repair conduction block in cardiomyocyte cultures probably through connexin-mediated coupling.

Introduction

Autologous cell therapy has become a novel therapeutic option for patients with ischemic heart disease.[1] Cardiac cell therapy aims to regenerate myocardial tissue and to improve neovascularization. To this purpose, bone marrow-derived cells, myoblasts, and peripheral blood-derived cells have been used in clinical trials.[2-7] Initial enthusiasm for cardiac myoblast transplantation has somewhat faded because life-threatening arrhythmias have been observed.[6] Grafted myoblasts were found to differentiate into hyperexcitable myotubes with a contractile activity electrically independent of neighbouring cardiomyocytes, because the differentiated myoblasts did not express cardiac connexins (Cx).[8,9] These findings highlight the importance of electrical coupling by any therapeutic cell type to be applied to damaged myocardium.

Several clinical studies have shown the safety and feasibility of autologous bone marrow cell transfer in patients with myocardial infarction, refractory ischemia, or heart failure.[2-5,7] In addition, it was shown that both symptoms and clinical parameters improved after cell therapy.[2-5,7] In these studies, repetitive Holter monitoring showed no proarrhythmogenic effect, and electrophysiological studies demonstrated no increased inducibility of ventricular arrhythmias.[2] However, detailed electrophysiological characterization of adult human mesenchymal stem cells (hMSC) injected in the myocardium is still lacking.

Recently, in vitro immunostaining showed the presence of cardiac connexins along regions of intimate cell-to-cell contact between hMSC and between hMSC and canine cardiomyocytes.[10] The presence of connexins resulted in electrical coupling between adjacent cells, but whether such electrical coupling permits activation of a mass of cardiac tissue over extended distances is not known.

We investigated whether adult hMSC could provide resynchronization of 2 previously asynchronously beating fields of cardiomyocytes. To this purpose, an in vitro model was developed in which a monolayer of beating neonatal rat cardiomyocytes was separated in 2 halves by a channel, thereby creating a line of anatomical conduction block. Next, hMSC were seeded in a channel-crossing pattern and the presence of electrical coupling between the cardiomyocyte fields across the hMSC was assessed using extracellular and intracellular electrophysiological techniques. Immunostaining and intercellular dye transfer were used to investigate the presence and function of cardiac connexins.

Methods

Cardiomyocytes and Cardiac Fibroblasts

Animal experiments were approved by the institutional animal experiments committee and complied with the European Convention of Animal Care. Cultures of cardiomyocytes were prepared as described earlier.[11] Cardiac ventricles of 2-day-old Wistar rats were minced and dissociated using collagenase and DNase. The cells were suspended in Ham's F10

medium (ICN Biomedicals, Irvine, California, USA) with 10% horse serum (HS, Invitrogen, Eugene, Oregon, USA) and 10% fetal bovine serum (FBS, Invitrogen), and pre-plated to allow preferential attachment of non-cardiomyocytes. After 1 hour, the non-adherent cells were collected and plated in micro-electrode arrays (MEA, Multi Channel Systems, Reutlingen, Germany) or in 6-well plates containing uncoated glass coverslips. Culture medium consisted of Dulbecco's Modified Eagle Medium (Invitrogen) with 10% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin (Invitrogen). Cells were grown at 37°C in a humidified CO_2 incubator. Spontaneously beating cardiomyocytes were observed within 48 hours after culture initiation.
Cardiac fibroblasts remained attached to the culture dish after removal of the cardiomyocytes and were grown in DMEM containing 10% FBS and the aforementioned antibiotics. After 24 hours the cardiac fibroblasts were trypsinized, resuspended and seeded into culture dishes with uncoated glass coverslips.

Adult Human Mesenchymal Stem Cells

Adult hMSC were obtained from the posterior iliac crest of 3 patients with ischemic heart disease scheduled for cell therapy after approval of the institutional medical ethical committee and after written informed consent. Bone marrow cells were separated over a Ficoll density gradient and the mononuclear cells were harvested and washed with Phosphate-Buffered Saline (PBS) containing 0.5% human serum albumin. Cells were pelleted by centrifugation and resuspended in DMEM with 10% FBS and antibiotics. Twenty-four hours after seeding the cells at a density of 10^6 cells/cm^2 in culture flasks, the non-adherent cells were discarded and hMSC were expanded by serial passage, and used from passage 3-6. The hMSC surface antigen profile characterized by a FACSort flow cytometer and CellQuest Software (Becton-Dickinson, Palo Alto, California, USA) matched previously published data.[12] Their ability to differentiate into adipocytes and osteoblasts after appropriate stimulation confirmed that the cultured cells were hMSC. For identification, hMSC were infected with an adenoviral vector encoding enhanced green fluorescent protein (eGFP;hAd5/F50.CMV.eGFP). For immunostaining and dye transfer experiments, hMSC were labelled with red fluorescent protein (DsRed;hAd5/F50.hEF1α.DsRed).[13] The generation, propagation, purification and titration of these Ad vectors were carried out as described elsewhere.[14]

Human Skeletal Myoblasts

Human skeletal myoblasts (a gift of Dr. D. Trono, University of Geneva, Geneva, Switzerland) were prepared as previously described.[15] Myoblasts were cultured in Ham's F10 medium supplemented with 15% fetal calf serum, bovine serum albumin (0.5 mg/ml), fetuin (0.5 mg/ml), epidermal growth factor (10 ng/ml), dexamethasone (0.39 µg/ml), insulin (0.18 mg/ml), creatine (1 mM), pyruvate (100 µg/ml) and uridine (50 µg/ml).

In Vitro Model for Conduction Block

Cardiomyocytes were plated on standard planar MEAs containing 60 electrodes (inter-electrode distance: 200 μm, electrode diameter: 30 μm). The MEAs were pre-coated with Ham's F10 medium containing 10% HS and 10% FBS. The cultures were maintained at 37°C during the measurements. The MEA data acquisition system allowed simultaneous recording of 60 extracellular electrograms (sample rate 5 kHz/channel), which were analysed using MC_Rack software (version 3.2.1.0, Multi Channel Systems).

Local activation time (LAT) was determined by the timing of the maximal negative intrinsic deflection ($-dV/dt_{max}$) of the electrogram recorded at each electrode. The LAT values of all electrodes were used for the generation of color-coded activation maps using 2-D plotting software (S-Plus, version 6.0, Insightfull, Seattle, Washington).

Two days after seeding cardiomyocytes on the MEAs, activation maps were constructed to assess impulse propagation. Next, a 200-450 μm wide channel was abraded in the cell monolayer perpendicular to the activation direction using a 200 μm wide pipette tip. After ensuring the presence of conduction block, we applied either 50,000 eGFP-labelled hMSC (hMSC group; n=8), or 50,000 myoblasts (myoblast group; n=7), or 50,000 cardiac fibroblasts (fibroblast group; n=7), in a channel-crossing pattern. In 7 cultures the channel was not filled with extraneous cells (control group; n=7).

Impulse propagation was assessed at 24 and 48 hours after seeding the cells. In this model, the depolarization wavefront has to traverse 2-5 cells in the channel. The 2 previously asynchronously beating cardiomyocyte fields were considered electrically coupled if the activation map showed conduction of electrical activity through the channel. To rule out the possibility of coincident spontaneous activation in the lower field of cardiomyocytes, graphs were created plotting consecutive LATs of the upper field versus the LATs of the lower fields over a period of 30 seconds. The first LAT on an electrode in the upper field was correlated with the first LAT on an electrode in the lower field, the second LAT on the same electrode in the upper field was correlated with the second LAT on the same electrode below in the lower field, and so on. Therefore, each point on each graph represents one single excitation wave measured in the upper and lower field simultaneously. A culture was considered synchronized only if there was a constant time interval between LATs on either side of the channel leading to a 1:1 correlation between the LATs of both fields.

Intracellular Recordings

Cultures of cardiomyocytes with hMSC seeded in a 200-450 μm wide channel were studied 24-48 hours after hMSC seeding and after ensuring resynchronization. Action potentials of beating cardiomyocytes and hMSC in the channel were recorded by the use of standard patch electrode techniques (glass capillaries filled with [in mM] 10 Na_2ATP, 115 KCl, 1 $MgCl_2$, 5 EGTA, 10 HEPES/KOH (pH 7.4) and a tip resistance of 10 to 30 MΩ).[16]

Immunostaining

Mixed cultures of cardiomyocytes and DsRed-labelled hMSC or skeletal myoblasts or cardiac fibroblasts on glass coverslips were subjected to immunostaining with Cx40-, Cx43- or Cx45-specific antibodies. After 48 hours of co-incubation, the mixed cultures were fixated for 30 minutes in PBS-1% formalin (Merck, Darmstadt, Germany), permeabilized using 0.1% Triton X-100 (BDH Laboratories, Poole, UK) and incubated with goat anti-Cx40 (Santa Cruz Biotechnology, Santa Cruz, California, USA), rabbit anti-Cx43 (Sigma, St Louis, Missouri, USA) or goat anti-Cx45 (Santa Cruz Biotechnology) antibodies at a dilution of 1:100 in PBS and 1% FBS for 24 hours at 4ºC. The cells were then washed with PBS and incubated with secondary Fluorescein Iso Thio Cyanathe (FITC)-conjugated anti-goat antibodies (Sigma; dilution 1:100 in PBS) or FITC-conjugated anti-rabbit antibodies (Sigma; dilution 1:100 in PBS) for 1 hour at 4ºC. Then, the coverslips were incubated in a solution containing Hoechst 33342 (Molecular Probes, Eugene, Oregon, USA; dilution 1:1000 in PBS) for 8 minutes at 4ºC to stain cell nuclei. Finally, the coverslips were mounted onto glass slides using Vectashield mounting medium (Vector Laboratories, Burlingame, California, USA) and sealed with transparent fingernail polish. Stained cells were examined using a fluorescence microscope (Nikon Eclipse, Nikon Europe, Badhoevedorp, The Netherlands) equipped with a digital camera (Nikon DXM1200).

Dye Transfer

Functional gap junction coupling between cardiomyocytes and hMSC was assessed using a fluorescent dye transfer assay.[17] After 2 days of culture on glass coverslips, cardiomyocytes were loaded with 10 µmol/L Calcein-AM (Molecular Probes) for 45 minutes. Calcein-AM, a non-fluorescent cell-permeable compound, is converted by intracellular esterases to Calcein, a 622-Da green fluorochrome retained in the cytoplasm, which can diffuse through functional gap junctions. After incubation of cardiomyocytes with Calcein-AM, culture medium was replaced by HEPES (ICN Biomedicals) buffered salt solution containing 2.5 mmol/L probenecid (Sigma) to prevent cellular release of Calcein. Thereafter, DsRed-labelled hMSC were added to the Calcein-loaded cardiomyocytes. If functional gap junctions between cardiomyocytes and hMSC had been established, Calcein transfer from cardiomyocytes to hMSC should occur through these channels, and hMSC should exhibit both green and red fluorescence. Calcein transfer was assessed 24 hours after co-culture initiation using a computer controlled fluorescence microscope (Zeiss Axiovert 200M, Göttingen, Germany) equipped with imaging software (OpenLab, Improvision, Coventry, UK).

Statistical Analysis

Statistical analysis was performed using SPSS 11.0 for Windows (SPSS Inc, Chicago, Illinois, USA). Data were compared using Student's t-test, analysis of variance, or the two-sided Chi-square test when appropriate. Synchronization between 2 beating cardiomyocyte fields was assessed using linear correlation analysis (Pearson correlation coefficient). A P-value <0.05 was considered statistically significant.

Results

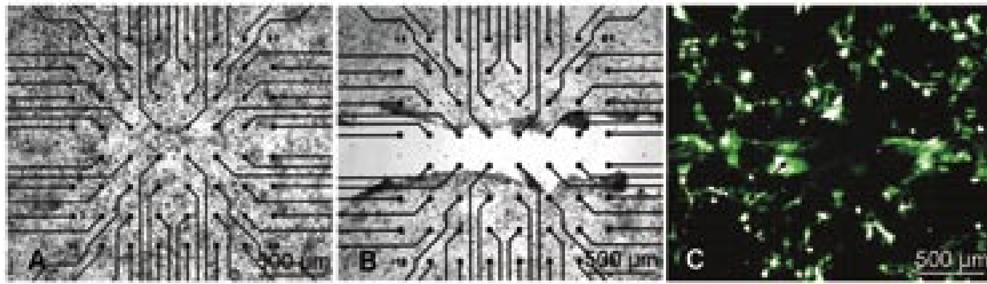
Synchronization of Electrical Activity

A spontaneously beating monolayer of cardiomyocytes had formed in all cultures 2 days after seeding the cells into the MEA (**Figure 1A**). Synchronized beating was present in all cultures at baseline (**Figure 2A**). Application of an anatomical central line of block created 2 asynchronously beating cardiomyocyte fields in all cultures (**Figure 1B**). Average channel width was similar in the 4 groups (**Table 1**). Conduction block was evident by lack of correlation between LATs recorded on electrodes at either side of the channel indicating asynchronous beating of the 2 cardiomyocyte fields (**Figure 2B**). The cross-channel application of 50,000 eGFP-labelled hMSC resulted in restoration of conduction and re-establishment of synchronization between the 2 cardiomyocyte fields in 5 out of 8 cultures after 24 hours (**Figure 1C**). Resynchronization was reflected by the re-establishment of a 1:1 correlation between LATs at either side of the channel, as

Table 1. Characteristics of cultures 24 and 48 hour after application of hMSC, myoblasts, fibroblasts or no extra cells in a channel-crossing pattern.

	hMSC n=8	myoblasts n=7	fibroblasts n=7	no cells n=7	P-value
Channel width (µm)	350±88	318±59	304±51	371±91	0.34*
24 hours					
Synchronization	5/8	0/7	0/7	0/7	
No synchronization	2/8	7/7	7/7	6/7	
Electrically inactive	1/8	0/7	0/7	1/7	
Detachment of cells	0/8	0/7	0/7	0/7	
Conduction velocity cardiomyocytes (cm/s) [†]	10.4±5.8	9.9±5.8	8.0±7.4	14.1±8.3	0.51*
Conduction velocity hMSC (cm/s)[‡]	0.9±0.4				
48 hours					
Synchronization	5/8	0/7	0/7	0/7	
No synchronization	0/8	7/7	7/7	6/7	
Electrically inactive	0/8	0/7	0/7	0/7	
Detachment of cells	3/8	0/7	0/7	1/7	
Conduction velocity cardiomyocytes (cm/s) [†]	14.4±8.2	11.8±8.1	12.4±5.8	7.6±6.8	0.36*
Conduction velocity hMSC (cm/s)[‡]	0.8±0.4				

*Assessed by analysis of variance; [†]Conduction velocity cardiomyocytes 24 vs. 48 hours: P=0.59 (Student t-test); [†]Conduction velocity hMSC 24 vs. 48 hours: P=0.75 (Student t-test)



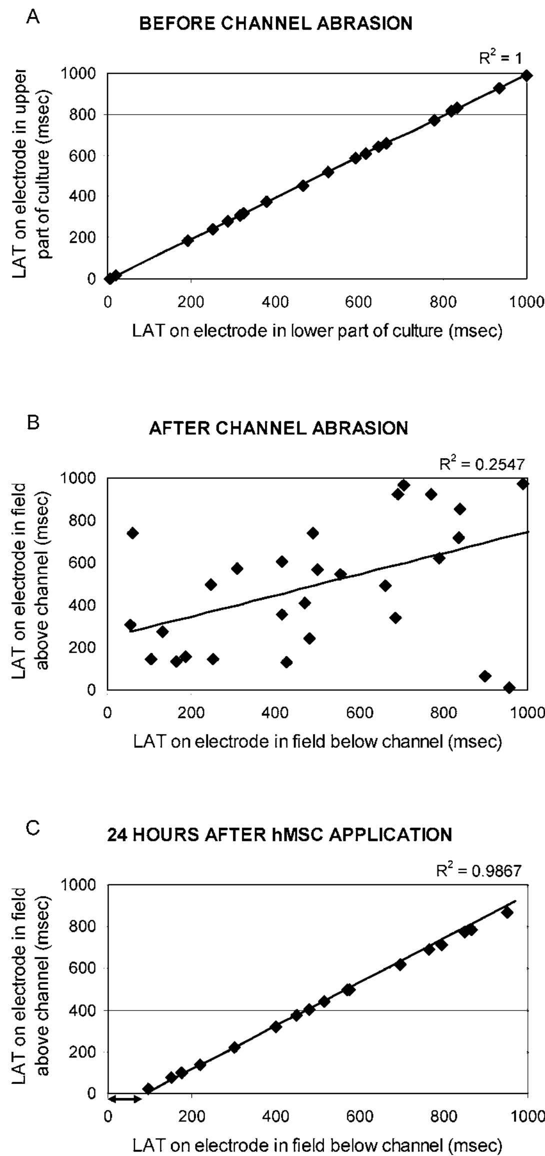


Figure 1
Light microscopy of MEA cultures before (A) and immediately after (B) channel abrasion. Fluorescence microscopy of MEA cultures 24 hour after application of eGFP-labelled hMSC (C).

Figure 2
Before channel abrasion there is a consistent time interval between LATs in the upper and lower field of the culture (all points are on a straight line) and the culture is considered to be activated synchronously with a similar excitation spread in consecutive excitation waves (A). After abrasion, the 2 cardiomyocyte fields beat independently (Correlation between LATs on either side of the channel is lost (B). Twenty-four hours after hMSC application the correlation between LATs is restored, indicating resynchronization. Because of the conduction delay within the channel, the lower field is now activated with an 80 ms delay (C).

demonstrated by a consistent time interval between LATs on either side of the channel (**Figure 2C**). In resynchronized cultures, channel width was 300±71 µm, whereas the 2 cultures that were not resynchronized after 24 hours had a channel width of 450 µm. The remaining eighth cardiomyocyte culture was electrically inactive at 24 hours, precluding any measurement. After 48 hours, synchronization was present in 5 out of 8 cultures, including 1 of the 2 cultures with a channel width of 450 µm. In the remaining 3 hMSC cultures, the cell monolayers had already detached from the MEA culture dish precluding further analysis. In none of the experiments with added myoblasts or fibroblasts, nor in control cultures, synchronization was restored after 24 or 48 hours.

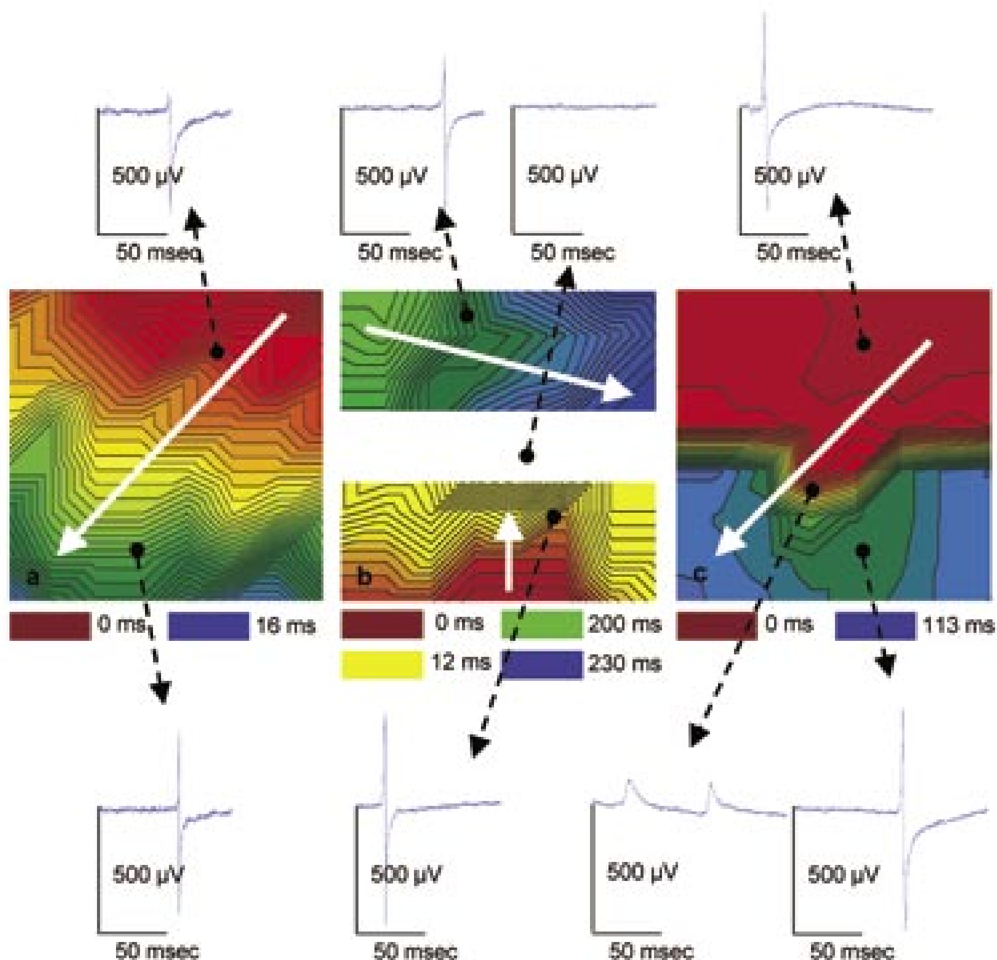


Figure 3
Color-coded activation maps before channel abrasion (A), immediately after channel abrasion (B) and 24 hour after hMSC application (C). See text for explanation.

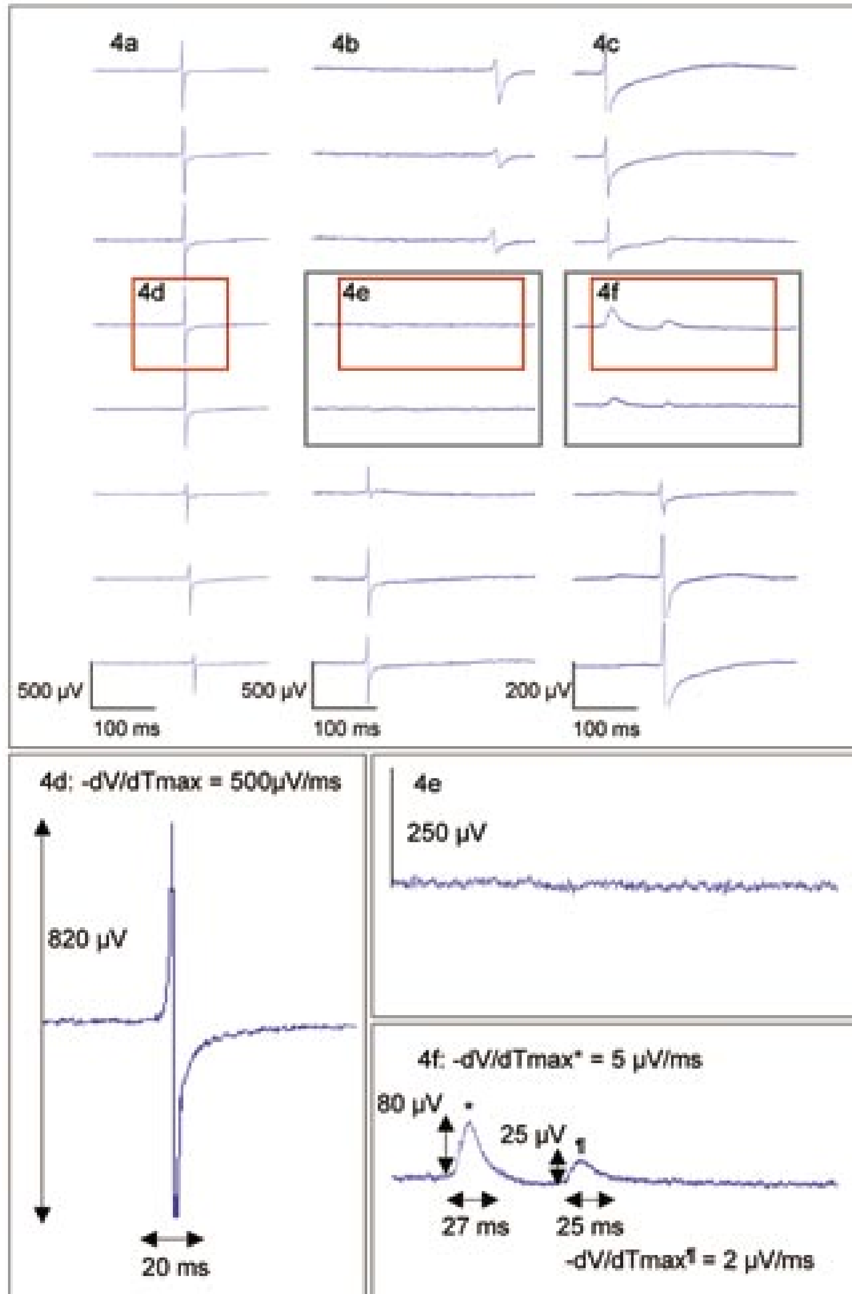


Figure 4
Electrograms recorded at 8 adjacent electrodes in a synchronized culture before channel abrasion (A), immediately after channel abrasion (B) (grey squares indicate electrodes located at the channel level), and 24 hour after hMSC seeding (C). After abrasion no electrical activity is present at the level of the channel (B, E). Resynchronization of the cardiomyocyte fields and the presence of electrical activity in the channel is shown in panel F. Panels D and F give typical recordings of an electrode underneath a cardiomyocyte and a hMSC.

Conduction Velocity Across hMSC-seeded Channel

Activation maps of cardiomyocyte cultures were generated before induction of a conduction block (**Figure 3A**), in the presence of a conduction block (**Figure 3B**), and 24 hours after hMSC seeding (**Figure 3C**). **Figure 3C** indicated the presence of slow conduction through hMSC in the channel. This is confirmed by the rightward shift of the trend line in **Figure 2C** compared to **Figure 2A**: local activation of the electrode in the upper part is 80 ms delayed compared to local activation of the electrode in the lower part.

After 24 hours, conduction velocity across hMSC (0.9±0.4 cm/s) was 11-fold slower than across cardiomyocytes (10.4±5.8 cm/s; P=0.005). After 48 hours, hMSC conduction velocity was 0.8±0.4 cm/s (vs. 0.9±0.4 cm/s at 24 hours; P=NS). Cardiomyocyte conduction velocities were similar both after 24 and 48 hours in the 4 different groups (**Table 1**).

Extracellular Recordings

Before channel abrasion, the whole culture showed synchronized activity (**Figure 3A, 4A**). Immediately after channel abrasion, no electrical activity was recorded by the electrodes located at the side of the channel (**Figures 3B, 4B**), indicating the presence of anatomical conduction block. Furthermore, no electrotonic interaction between the 2 fields of cardiomyocytes was recorded (**Figure 4E**). In the hMSC-seeded channel, $-dV/dt_{max}$ was 5±5 µV/ms, in contrast to a $-dV/dt_{max}$ of 373±243 µV/ms in the cardiomyocyte fields (P<0.001). The amplitude of electrical activity of hMSC (64±19 µV) was significantly lower compared to that of cardiomyocytes (478±156 µV; P<0.001), whereas duration of electrical activity did not differ (cardiomyocytes 35±44 ms vs. hMSC 34±22 ms; P=NS). Typical examples of extracellular recordings of cardiomyocytes and hMSC are shown in **Figure 4D** and **4F**. The configuration of the electrograms of cardiomyocytes was similar in all electrodes, whereas slopes and amplitudes of the hMSC electrograms within the channel were decremental along the path of conduction.

Intracellular Recordings

Intracellular recordings were performed in 4 cultures (**Figure 5**). When comparing resting membrane potential, amplitude and duration of intracellular hMSC and cardiomyocyte recordings, significant differences were found (**Table 2**).

Table 2. Intracellular recordings

	Cardiomyocytes n=5	hMSC n=5	P-value
Resting membrane potential (mV)	-64±9	-23±13	<0.001
Action potential amplitude (mV)	99±17	15±8	<0.001
Action potential duration till 50% repolarization (ms)	192±54	332±47	0.005

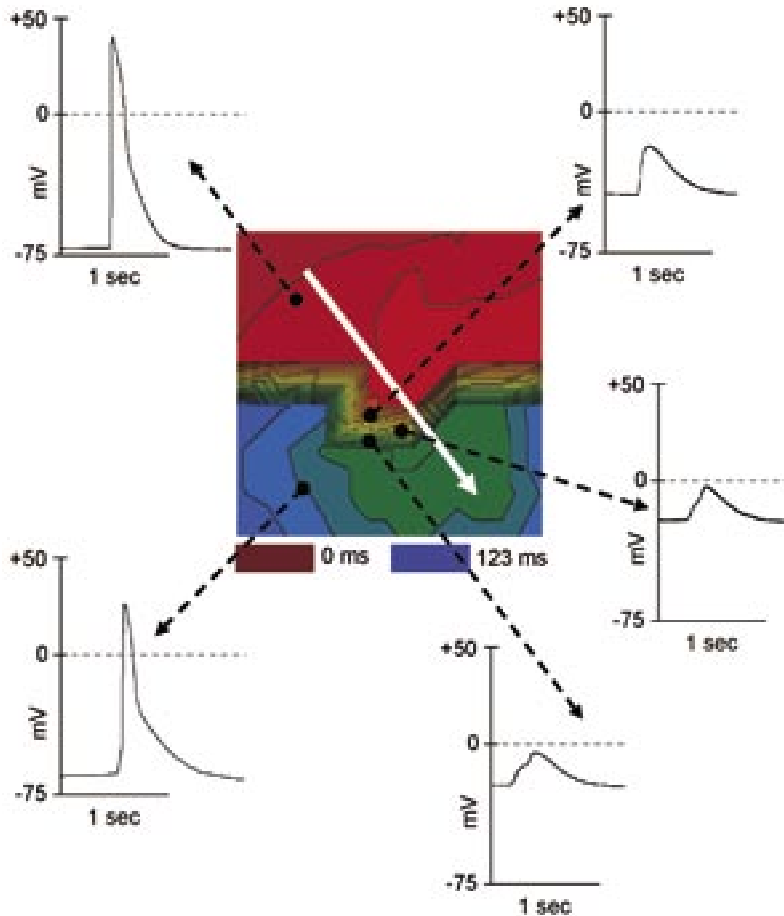


Figure 5
Action potential recordings in a resynchronized culture 24 hour after seeding hMSC in a channel-crossing pattern. Recordings on the left side are membrane potentials of cardiomyocytes, recordings on the right are derived from hMSC within the channel.

Connexin Immunostaining

Immunostaining revealed the presence of Cx43 along regions of intimate cardiomyocyte-to-hMSC and hMSC-to-hMSC contact (**Figure 6A, 6B**). Furthermore, Cx40, Cx43 and Cx45 were observed at the interface of juxtaposed cardiomyocytes (data for Cx43: **Figure 6A**). Beside the presence of Cx43 at the margins of the hMSC, Cx43 was present in the cytoplasm of the hMSC (**Figure 6A, 6B**). hMSC displayed a cytoplasmatic distribution pattern of Cx40 and Cx45, without clustering at contact areas between either hMSC or cardiomyocytes (data for Cx40 and Cx45 are not shown in Figures).

Myoblasts showed a low intensity of punctate cytoplasmatic distribution of Cx43 and Cx45, but no Cx40. These connexins were undetectable at myoblasts-to-myoblasts or myoblasts-to-cardiomyocyte contact areas. No Cx40-, Cx43- or Cx45-positive cardiac fibroblasts were detected in co-cultures with cardiomyocytes.

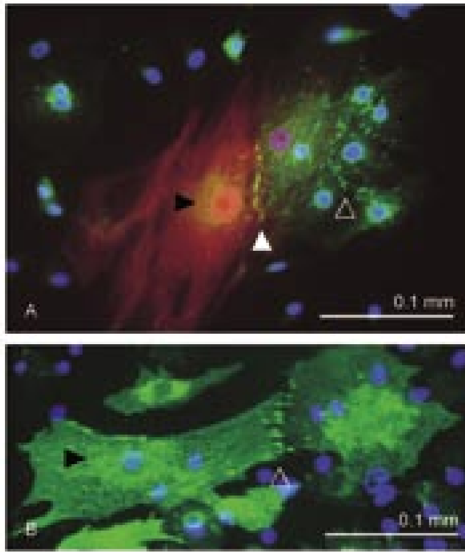


Figure 6
Cx43-positive gap junctions along a region of intimate cell-to-cell contact between a DsRed-labelled hMSC and a cardiomyocyte (A: white arrowhead). Cx43 is also present in the cytoplasm of the DsRed-labelled hMSC (A: black arrowhead, B: black arrowhead), between adjacent cardiomyocytes (A: transparent arrowhead) and between adjacent hMSC (B: transparent arrowhead).

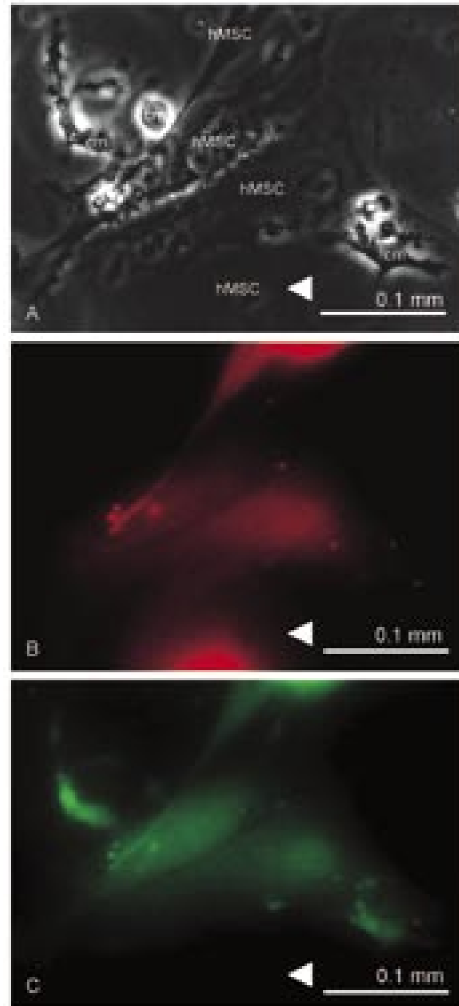


Figure 7
Light microscopy of a mixed culture of DsRed-labelled hMSC and Calcein-loaded cardiomyocytes (cm) (A). Fluorescence in red channel (B), Fluorescence in green channel (C). The hMSC in contact with Calcein-loaded cardiomyocytes display green fluorescence indicative of dye transfer (C), whereas hMSC that have no contact with Calcein-loaded cardiomyocytes do not fluorescence green (A-C: white arrowhead).

Calcein Transfer from Cardiomyocytes to hMSC

Calcein transfer experiments were performed in 10 cultures. Twenty-four hours after start of co-culture of DsRed-labelled hMSC and Calcein-loaded cardiomyocytes, transfer of Calcein to adjacent hMSC was observed (**Figure 7**). Solitary hMSC did not take up the dye, indicating that functional gap junctions are necessary for Calcein transfer from cardiomyocytes to hMSC.

Discussion

The key finding of this study is that 2 previously asynchronously beating fields of cardiomyocytes can be resynchronized by the administration of hMSC in the dividing channel. In other words, by conducting the electrical impulse and subsequent activation of a distal mass of cardiomyocytes, hMSC could repair an experimentally induced conduction block. Impulse transmission across hMSC within 48 hours of co-incubation is characterized by slow conduction, reduced depolarization rates, and low amplitude electrical activity decaying with distance.

Conduction Across hMSC

Successful impulse conduction across an hMSC-seeded channel and resynchronization of previously asynchronously beating fields of cardiomyocytes occurred within 24 hours after administration of hMSC. Gap junctional proteins were found both at the interfaces between hMSC and between cardiomyocytes and hMSC. Calcein dye transfer confirmed the presence of functional gap junctions between cardiomyocytes and hMSC. The slow deflections of extracellular hMSC electrograms occurring synchronously with the rapid deflections in cardiomyocyte electrograms, support the presence of intact electrotonic interaction. Intracellular recordings indicated that conduction across hMSC occurred mainly by passive electrotonic current flow as hMSC action potentials showed a reduced depolarization rate and a reduced amplitude decaying with distance. Outgrowth of cardiomyocytes over seeded hMSC is unlikely to be responsible for the results as cardiomyocytes are found not to migrate within 48 hours and resynchronization did not occur in the control group.

Conduction Velocity

Although impulse conduction across hMSC is relatively slow, it is in line with the conduction velocity across non-excitable Cx43-positive cells reported by Gaudesius et al.[18] Furthermore, cardiomyocyte conduction velocity in our study is in accordance with previously reported data.[19] Although slow conduction could be considered as proarrhythmogenic, until now, clinical studies with hMSC showed no increased inducibility of arrhythmias.[2-5,7]

Conduction through Fibroblasts or Myoblasts

Recently Gaudesius et al. showed that rat cardiac fibroblasts can couple electrically to cardiomyoctes and activate cardiac tissue over distances.[18] They reported a gradual decline of conduction through fibroblasts with increasing channel width. Electrical propagation failed at a width >302 μm, whereas in the present study average channel width was 336±76 μm. Fibroblasts used by Gaudesius et al. stained positive for both Cx43 and Cx45. However, data on electrical coupling between fibroblasts and between

cardiomyocytes and fibroblasts are conflicting. Rook et al. and Laird and Revel also observed electrotonic interaction between cardiomyocytes and fibroblasts.[20,21] However, no Cx43-positive gap junctions between fibroblasts and only a few punctate Cx43-positive spots at cardiomyocyte-fibroblast interfaces were found.[20,21] Other in vitro studies showed that rabbit cardiac fibroblasts stained positive for Cx40 and Cx45, but not for Cx43.[22] Feld et al. reported that cardiac fibroblasts did not cause any significant electrophysiological changes when added to cardiomyocyte cultures.[23] Furthermore, de Maziere et al. showed absence of robust gap junction coupling between cardiomyocytes and cardiac fibroblasts in intact and healthy hearts.[24] Oyamada et al., performing a dye transfer assay in cultures of neonatal rat cardiomyocytes, demonstrated that despite the presence of a few Cx43-positive spots between cardiomyocytes and fibroblasts, no dye transfer to fibroblasts occurred.[25] The results of our study are in line with these observations. No electrical coupling and consequently no resynchronization was observed in any of the fibroblast experiments, which is possibly caused by the absence of Cx43 in these cells. Similarly, cardiomyocytes devoid of Cx43 caused conduction block as reported by Fast et al., whereas hepatocytes transfected with a Cx43 expression vector were able to propagate the electrical impulse in contrast to wild-type hepatocytes without Cx43.[18,19]
Concerning the electrical properties of skeletal myoblasts several studies demonstrated that these cells lack Cx43 and cannot couple electrically to neighbouring cardiomyocytes.[26] Only in myoblasts in very early stages of differentiation the presence of Cx43 was reported. During differentiation Cx43 was downregulated.[27] Similarly, in our study skeletal myoblasts were unable to resynchronize 2 cardiomyocyte fields, which can be explained by the absence of Cx43 at the contact areas.

Study Limitations

In order to perform long-term recordings the attachment of cardiomyocytes to the surface of the MEA should be improved. Although several kinds of coatings of the MEA have been used, the majority of the monolayers detached from the MEA 48 hours following channel abrasion. In addition, electrophysiological properties of hMSC should ideally be tested in co-culture with adult human cardiomyocytes. Unfortunately, cardiomyocytes from healthy humans are hard to obtain and dedifferentiate rapidly during culture.[28] We therefore performed our experiments with neonatal rat cardiomyocytes, which are easily to obtain and exhibit spontaneous electrical activity.

Conclusions

This study showed that 2 previously asynchronously beating fields of cardiomyocytes can be resynchronized by the administration of hMSC. Impulse transmission across hMSC is characterized by slow conduction, reduced depolarization rates, and low amplitude electrical activity decaying with distance.

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