Human Mesenchymal Stem Cells as a Gene Delivery System to Create Cardiac Pacemakers


Abstract—We tested the ability of human mesenchymal stem cells (hMSCs) to deliver a biological pacemaker to the heart. hMSCs transfected with a cardiac pacemaker gene, mHCN2, by electroporation expressed high levels of Cs⁺-sensitive current (31.1±3.8 pA/pF at −150 mV) activating in the diastolic potential range with reversal potential of −37.5±1.0 mV, confirming the expressed current as Iᵢ-like. The expressed current responded to isoproterenol with an 11-mV positive shift in activation. Acetylcholine had no direct effect, but in the presence of isoproterenol, shifted activation 15 mV negative. Transfected hMSCs influenced beating rate in vitro when plated onto a localized region of a coverslip and overlaid with neonatal rat ventricular myocytes. The coculture beating rate was 93±6 bpm when hMSCs were transfected with control plasmid (expressing only EGFP) and 161±4 bpm when hMSCs were expressing both EGFP+mHCN2 (P<0.05). We next injected 10⁶ hMSCs transfected with either control plasmid or mHCN2 gene construct subepicardially in the canine left ventricular wall in situ. During sinus arrest, all control (EGFP) hearts had spontaneous rhythms (45±1 bpm, 2 of right-sided origin and 2 of left). In the EGFP+mHCN2 group, 5 of 6 animals developed spontaneous rhythms of left-sided origin (rate=61±5 bpm; P<0.05). Moreover, immunostaining of the injected regions demonstrated the presence of hMSCs forming gap junctions with adjacent myocytes. These findings demonstrate that genetically modified hMSCs can express functional HCN2 channels in vitro and in vivo, mimicking overexpression of HCN2 genes in cardiac myocytes, and represent a novel delivery system for pacemaker genes into the heart or other electrical syncytia. (Circ Res. 2004;94:952-959.)

Key Words: gene therapy ■ heart block ■ ion channels ■ pacemakers ■ stem cells

Although electronic pacemakers are currently the mainstay of therapy for heart block and other electrophysiological abnormalities, they are not optimal. Among their shortcomings are limited battery life, the need for permanent catheter implantation into the heart, and lack of response to autonomic neurohumors.¹ For these reasons, several gene therapy approaches have been explored as potential alternatives. These include either overexpression of β₂-adrenergic receptors,² ³ use of a dominant-negative construct to suppress inward rectifier current when expressed together with the wild-type gene Kir2.1,⁴ and implantation of vectors carrying the pacemaker gene, HCN2, into atrium⁵ or bundle branch system.⁶ A problem inherent in some of these approaches²–⁶ is the use of viruses to deliver the necessary genes. Although the vectors have been replication-deficient adenoviruses that have little infectious potential, these incorporate the possibility of only a transient improvement in pacemaker function as well as potential inflammatory responses. The use of retrovirus and other vectors, although not attempted as yet for biological pacemakers, carries a risk of carcinogenicity and infectivity that is unjustified, given the current success of electronic pacemakers. Attempts to use embryonic human stem cells to create pacemakers are still in their infancy and carry the problems of identifying appropriate cell lineages, the possibility of differentiation into lines other than pacemaker cells, and potential for neoplasia (see overview⁷).

With this in mind, we embarked on a project to test proof-of-principle that genetically engineered adult human mesenchymal stem cells (hMSCs) can serve as a platform for carrying the pacemaker gene to the heart. We did this with the understanding that the potential for differentiation into other cell lines exists for hMSCs as for embryonic stem cells, but with the rationale that if the relevant gene is genetically overexpressed then the presence or absence of differentiation...
HCN2 (mHCN2) was measured under voltage-clamp by an Axopatch-1B (Axon Instruments) using whole-cell patch clamp to study membrane currents in control hMSCs and those transfected with mHCN2, the gene encoding the α-subunit of the pacemaker current, I_h. Expressed I_h (ie, \( \frac{\Delta I}{I_{h_{max}}} \)) was measured by an Axopatch-1B (Axon Instruments) amplifier. Patch electrode resistance was 4 to 6 MΩ before sealing. Cells were constantly superfused using a gravitational perfusion system with a complete change of the chamber solutions in about 0.5 minutes. The temperature of the bath as well as of the perfusion solution was kept constant at 35°C and action potentials recorded from near the edge of the coverslip using a perforated patch electrode and an Axopatch 200 amplifier and PClamp 8 software (Axon Instruments). The perforated patch technique was used, and amphotericin B (400 µg/mL, Sigma) was added to the pipette solution.

**Materials and Methods**

Protocols were reviewed and approved by the Columbia University institutional animal care and use committee.

**Human Mesenchymal Stem Cell Maintenance and Transfection**

Human mesenchymal stem cells (Poietics hMSC; mesenchymal stem cells, human bone marrow) were purchased from Clonetics (Walkersville, Md) and cultured in MSC growing medium (Poietics MSCGM; BioWhittaker) at 37°C in a humidified atmosphere of 5% CO2. Cells were used from passages 2 to 4. A full-length mHCN2 cDNA was subcloned into a plR ES2-EGFP vector (BD Biosciences Clontech). Cells were transfected by electroporation using the Amaza Biosystems Nucleofector (Amaza Biosystems) technology. Expression of EGFP after 24 to 48 hours revealed transfection efficiency of 30% to 45%.

**Patch-Clamp Studies of \( I_h \) Expressed in hMSCs**

We used whole-cell patch clamp to study membrane currents in control hMSCs and those transfected with mHCN2, the gene encoding the α-subunit of the pacemaker current, \( I_h \). Expressed \( I_h \) (ie, \( \frac{\Delta I}{I_{h_{max}}} \)) was measured by an Axopatch-1B (Axon Instruments) amplifier. Patch electrode resistance was 4 to 6 MΩ before sealing. Cells were constantly superfused using a gravitational perfusion system with a complete change of the chamber solutions in about 0.5 minutes. The temperature of the bath as well as of the perfusion solution was kept constant at 35±0.5°C. The pipette solution was filled with (in mmol/L) KCl 50, K-aspartate 80, MgCl2 1, Mg-ATP 3, EGTA 10, and HEPES 10 (pH adjusted to 7.2 with KOH). The external solution contained (in mmol/L) NaCl 137.7, KCl 5.4, NaOH 2.3, CaCl2 1.8, MgCl2 1, Glucose 10, HEPES 5, and BaCl2 2 (pH adjusted to 7.4 with NaOH). The membrane capacity was measured by applying a voltage clamp step and current densities are expressed as the value of peak current per capacity.

**Dual Patch-Clamp Studies of Gap Junctions**

Canine cardiac ventricular myocytes were isolated as previously described. Primary cultures of the myocytes were maintained using procedures described for mouse myocytes. They were plated at 0.5 to 1x10^5 cells/cm² in MEM containing 2.5% fetal bovine serum (FBS) and 1% PS onto mouse laminin (10 µg/mL) precoated coverslips. After 1 hour of culture in a 5% CO2 incubator at 37°C, the medium was changed to FBS-free MEM. hMSCs were added and coculture was maintained in DMEM with 5% FBS. Cell Tracker green (Molecular Probes) was used to distinguish hMSCs from HeLa cells in coculture in all experiments.

Glass coverslips with adherent cells were transferred to an experimental chamber perfused at room temperature (≈22°C) with bath solution containing (in mmol/L) NaCl 150, KCl 10, CaCl2 2, HEPES 5 (pH 7.4), and glucose 5. The patch pipettes were filled with solution containing (in mmol/L) KCl 120, NaCl 10, MgATP 3, HEPES 5 (pH 7.2), and EGTA 10 (pCa≈8), filtered through 0.22-µm pores. When filled, the resistance of the pipettes measured 1 to 2 MΩ. Experiments were performed on cell pairs using a double voltage-clamp. This method permitted us to control the membrane potential (V_m) and measure the associated junctional currents (I_J).

**Action Potential Recordings in Coculture**

hMSCs were plated onto fibronectina-coated 9×22-mm coverslips, using a cloning cylinder to restrict the initial plating to an approximate 4-mm diameter circular area. The cells expressed either EGFP alone or EGFP+mHCN2. Four hours later, the cloning cylinder was removed and neonatal rat ventricular myocytes, prepared as described previously, were plated over the entire coverslip. Four to five days later, the coverslips were placed in a superfusion chamber maintained at 35°C and action potentials recorded from near the center of the coverslip using a perforated patch electrode and normal physiological solution containing (in mmol/L) NaCl 140, NaOH 2.3, MgCl2 1, KCl 5.4, CaCl2 1.0, HEPES 5, and glucose 10; pH 7.4. Pipette solution included (in mmol/L) aspartic acid 130, KOH 146, NaCl 10, CaCl2 2, EGTA-KOH 5, Mg-ATP 2, and HEPES-KOH 10; pH 7.2. Recordings were conducted with an Axopatch 200 amplifier and PClamp 8 software (Axon Instruments). The perforated patch technique was used, and amphotericin B (400 µg/mL, Sigma) was added to the pipette solution.

**In Vivo Studies in Canine Ventricle**

Stem cells were prepared as above. Under sterile conditions, after sodium thiopental induction (17 mg/kg IV) and inhalational isoflurane (1.5 to 2.5%) anesthesia, 23- to 27-kg mongrel dogs (Team Associates, Dayville, Conn) were subjected to a pericardectomy. We injected 10^5 hMSCs containing HCN2+GFP or GFP alone subepicardially in 0.6 mL of solution into the left ventricular anterior wall, approximately 2 mm deep to the epicardium via a 21-gauge needle. Animals recovered for 4 to 10 days, during which their cardiac rhythms were monitored. They then were anesthetized with isoflurane, as above. Both cervical vagal trunks were isolated, the chest opened, and ECGs monitored. Graded right and left vagal stimulation was performed via standard techniques to suppress sinus rhythm such that escape pacemaker function might occur. Tissues were then removed for histological study.

**Histological Methods**

Unless otherwise indicated, samples of heart tissue were fixed in 10% buffered formalin, embedded in paraffin and sectioned at 4 or 6 micrometers. Some formalin-fixed sections were stained in a routine fashion with hematoxylin and eosin (H&E). Monoclonal mouse antibodies (DakoCytomation) raised against the vimentin and human CD 44 were used. Unstained sections were subjected to a pericardectomy. We injected 10^5 hMSCs containing HCN2+GFP or GFP alone subepicardially in 0.6 mL of solution into the left ventricular anterior wall, approximately 2 mm deep to the epicardium via a 21-gauge needle. Animals recovered for 4 to 10 days, during which their cardiac rhythms were monitored. They then were anesthetized with isoflurane, as above. Both cervical vagal trunks were isolated, the chest opened, and ECGs monitored. Graded right and left vagal stimulation was performed via standard techniques to suppress sinus rhythm such that escape pacemaker function might occur. Tissues were then removed for histological study.

**Statistics**

Results are presented as mean±SEM. Statistical significance was determined by Student’s t test for unpaired data. A value of P<0.05 was considered significant.
Results

Transfection of hMSCs With mHCN2 and Demonstration of Pacemaker Current

Nontransfected hMSCs demonstrated no significant time-dependent currents during hyperpolarizations (Figure 1A). MHCN2-transfected hMSCs expressed a large time-dependent inward current activating on hyperpolarizations up to $-110$ mV and deactivating during the following step to $-20$ mV (Figure 1B). Figure 1C shows the $I_f$ activation curve constructed from tail currents recorded in mHCN2-transfected hMSCs (see inset for sample currents). We fit the data with a Boltzmann two-state model, which yielded a midpoint ($V_{50}$) of $-91.8 \pm 0.9$ mV and a slope factor of $8.8 \pm 0.5$ mV (n=9). $I_f$ was fully activated around $-140$ mV with an activation threshold of $-60$ mV. Inset shows representative tail currents used to construct $I_f$ activation curves. Voltage protocol was to hold at $-30$ mV and hyperpolarize for 1.5 seconds to voltages between $-40$ and $-160$ mV in 10-mV increments followed by a 1.5-second voltage step to $+20$ mV to record the tail currents.

Figure 1. Functional expression of $I_f$ in hMSCs transfected with mHCN2 gene. $I_f$ was expressed in hMSCs transfected with the mHCN2 gene (B) but not in nontransfected stem cells (A). C, Fit by the Boltzmann equation to the normalized tail currents of $I_f$ gives a midpoint of $-91.8 \pm 0.9$ mV and a slope of $8.8 \pm 0.5$ mV (n=9). $I_f$ was fully activated around $-140$ mV with an activation threshold of $-60$ mV. Inset shows representative tail currents used to construct $I_f$ activation curves. Voltage protocol was to hold at $-30$ mV and hyperpolarize for 1.5 seconds to voltages between $-40$ and $-160$ mV in 10-mV increments followed by a 1.5-second voltage step to $+20$ mV to record the tail currents.

We also tested the effect of Cs$^+$ to block the expressed current. Cs$^+$ (4 mmol/L) reversibly blocked the inward currents but had little effect on the outward deactivating tail currents, consistent with Cs$^+$ blockade of $I_f$. We constructed the fully activated $I-V$ relationships for the $I_f$-like current in Figure 2D. The plot reinforces the two major observations from the raw data.

Figure 2. Effect of extracellular application of Cs$^+$ and measurement of the reversal potential of $I_f$. $I_f$ was recorded before (A), during (B), and after (C) external addition of 4 mmol/L Cs$^+$. D, Fully activated $I-V$ relationship of $I_f$ in the absence and presence of Cs$^+$. Voltage protocol was to hold at $-30$ mV and hyperpolarize to $-150$ mV for 2 seconds followed by a 1.5-second depolarization to voltages between $-150$ and $-20$ mV to record the tail currents necessary to construct the fully activated current-voltage relation followed by a 0.5-second step to $-10$ mV.

reversal potential is consistent with the mixed selectivity of the $I_f$ channel to [Na$^+$] and [K$^+$]. We also tested the effect of Cs$^+$ to block the expressed current. Cs$^+$ (4 mmol/L) reversibly blocked the inward currents but had little effect on the outward deactivating tail currents, consistent with Cs$^+$ blockade of $I_f$. We constructed the fully activated $I-V$ relationships for the $I_f$-like current in Figure 2D. The plot reinforces the two major observations from the raw data.
by acetylcholine inhibition of adenylyl cyclase. We therefore provided evidence that the mHCN2-transfected hMSCs could influence impulse initiation by cardiac myocytes. Maximum diastolic potential was $-74\pm1$ mV (n=5) in neonatal rat ventricular myocytes cocultured with EGFP expressing hMSCs and $-67\pm2$ mV (n=6) in myocytes cocultured with hMSCs expressing mHCN2 (P<0.05). Spontaneous rate was $93\pm16$ bpm in the former group (n=5) and $161\pm4$ bpm in the latter (n=6, P<0.05). The reduced maximum diastolic potential is consistent with the observed threshold potential of the excitable medium.
pressed current in the mHCN2-transfected hMSCs, and indicates the influence of this depolarizing current on the electrically coupled myocytes. Representative action potentials are shown in Figure 5.

mHCN2-Transfected hMSCs as a Biological Pacemaker in Intact Canine Heart

Given the demonstration of functional coupling of mHCN2-expressing hMSCs to myocytes in vitro, we then injected them into canine heart in situ (see Materials and Methods) to test whether pacemaker function was demonstrable. During sinus arrest, escape pacemaker function can originate in the left or right ventricle, as occurred here, with two of four animals receiving hMSCs expressing EGFP alone developing left and two developing right ventricular escape rhythms. In contrast, five of six animals receiving hMSCs expressing EGFP+mHCN2 developed rhythms originating from and pace-mapped to the left ventricle at a site whose origin approximated that of the hMSC injection. Moreover, the idioventricular rates of these animals was 61±5 versus 45±1 bpm in animals receiving hMSCs expressing EGFP alone (P<0.05). A representative experiment is shown in Figure 6.

Hematoxylin and eosin stain of the site of hMSCs injection revealed normal cardiac myocytes and dense areas of basophilic infiltration adjacent to the needle track (Figure 7A). The hMSCs were easily identified by their size (10 to 20 μm in diameter), large hyperchromatic nuclei, and scanty, deeply basophilic cytoplasm with no matrix. Although the hMSCs had a characteristic appearance with H&E staining, they were more precisely identified by using immunohistochemical stains. The hMSCs stained intensely for vimentin (eg, Figure 7B), a marker of cells of mesenchymal origin. The same regions also were positive for human CD44 (eg, Figure 7C). Interdigitation between hMSCs and myocardium was very clear (eg, Figure 7D).

hMSCs Form Gap Junctions With Cardiac Myocytes In Vitro and In Vivo

To test whether the hMSCs couple electrically with cardiac myocytes, we cocultured hMSCs with adult canine ventricular myocytes. Myocytes were dissociated and plated for between 12 and 72 hours before coculture with hMSCs. Measurement of coupling occurred 6 to 12 hours after adding hMSCs to the myocyte culture. Our preliminary observations reveal that stem cells couple to cardiac cells. Figure 8A illustrates one example of an hMSC-myocyte pair in coculture; it is one of four so far observed. For heterologous pairs identification the hMSCs were tagged with Cell Tracker green (Molecular Probes).11 A bipolar voltage-ramp protocol was used to alter transjunctional voltage $V_j (V_2 - V_1)$ over ±100 mV range at 200 mV/15-second rate (see $V_1$ and $V_2$) and is shown in Figure 8B. The ramp pulse was applied to the myocyte ($V_1$) while membrane potential of the hMSC was kept at 0 mV ($V_2$). The associated sister currents, $I_1$ and $I_2$, were recorded from the myocyte and hMSC, respectively.

Figure 5. Pacemaker function in in vitro model. Spontaneous electrical activity of neonatal rat ventricular myocytes cocultured for 4 to 5 days with hMSCs transfected with EGFP alone (A) or mHCN2 and EGFP (B). Experiments were conducted at 35°C.

Figure 6. Pacemaker function in canine heart in situ. Top to bottom, ECG leads I, II, III, AVR, AVL, and AVF. Left, Last two beats in sinus rhythm and onset of vagal stimulation (arrow) causing sinus arrest in a dog studied 7 days after implanting mHCN2-transfected hMSCs in LV anterior wall epicardium. Middle, During continued vagal stimulation, an idioventricular escape focus emerges, having a regular rhythm. Right, On cessation of vagal stimulation (arrow), there is a postvagal sinus tachycardia.
The currents followed the voltage-ramp profile demonstrating gap junction coupling of the heterologous hMSC-myocyte pair. The current, $I_2$, obtained from the nonstepped hMSC, reflects a coupling current, $I_c$. This record demonstrates effective coupling of the hMSC to the ventricular myocyte. Figure 8C shows immunohistochemical staining with anti-Cx43 antibodies of the site of the injection of hMSCs into the canine heart. Intercalated discs are revealed in the myocardium (see purple arrow), whereas small punctate staining for Cx43 is seen between hMSCs (white arrows). There is also Cx43 staining at interfaces between hMSCs and myocytes (red arrows). The inset of Figure 8C shows a section from a piece of myocardium (fixed in 4% paraformaldehyde in 0.1 mol/L phosphate buffer at pH of 7.4 at 4°C and subsequently treated as described by Walcott et al.19) injected with hMSCs expressing EGFP plus HCN2. The red staining from the secondary antibody to EGFP illustrates localization of hMSCs, whereas the blue staining illustrates cell nuclei. A significant majority of the clustered cells are hMSCs.

**Discussion**

Pacemaker implantation is a primary treatment for complete heart block or sinus node dysfunction. The current therapy uses electronic devices with high reliability and low morbidity. Nevertheless, such devices are not optimal because they lack the biological responsiveness of native tissues. Recently several approaches have been attempted to provide biological pacemaker function. Included among these attempts have been an upregulation of $\beta_2$-adrenergic receptors, a downregulation of the background $K^+$ current $I_K$, and our own previous studies with overexpression of the HCN2 gene, the molecular correlate of the endogenous cardiac pacemaker current $I_f$.2-6 In these latter studies, we showed that HCN2 overexpression locally in left atrium or in the proximal bundle-branch system induces both $I_f$-like currents and in situ pacemaker function in the recipient myocytes. The unique voltage dependence of the $I_f$ conductance results in current flow during diastole but not during the action potential plateau, limiting possible complications attendant to significant alterations of the action potential waveform. Although an adenoviral construct has been used to deliver the HCN2 gene to the heart,5,6 this approach is not optimal because adenoviruses are episomal and the nucleic acids they deliver do not integrate into genome. Other viral systems are accompanied by a number of serious drawbacks that hinder their use in vivo.

An alternative means for fabricating biological pacemakers is via embryonic stem cells, which can be differentiated along a cardiac lineage and might provide a platform for cell-based control of cardiac rhythm. Embryonic stem cells can make functional gap junctions and generate spontaneous electrical activity.20 However because of their immunogenicity, rejection is a serious consideration. Moreover, as with hMSCs, embryonic stem cell preparations are not spatially uniform and the proper engineering of both cell-based systems presents a challenge in designing in vivo biological pacemakers.

For several reasons, hMSCs are an attractive cellular vehicle for gene delivery applications. They can be obtained in relatively large numbers through a standard clinical procedure. hMSCs are easily expanded in culture and capable of long-term transgene expression.21 Their administration can be autologous or via banked stores, given evidence that they may be immunopriviliged.22 Long-term function of such a pacemaker is based on prolonged expression of mHCN2, which in turn requires integration into the genome of hMSCs. Random
The objective of this study was to test the feasibility of using genetically modified hMSCs as a platform for systemic delivery of pacemaker genes into the heart. HCN2 served as the model system for this study. Our genetically engineered hMSCs expressed an \( I_f \)-like current and were capable of increasing the spontaneous beating rate of cocultured rat neonatal myocytes and originating a ventricular rhythm during vagally induced sinus arrest in the canine heart. Control hMSCs expressing only EGFP did not exert these effects either in vitro or in vivo. Thus, the electrical effects of the hMSCs transfected with the mHCN2 gene were similar to the effects of overexpression of the same gene in the myocytes in in vitro and in vivo systems. These findings suggest that hMSCs may serve as an alternative approach for the delivery of pacemaker genes for cardiac implantation.

In sinus node myocytes the HCN gene generates an inward current necessary for cardiac excitation. Unlike sinoatrial node cells, mHCN2-transfected hMSCs are not excitable, because they lack the other currents required to generate an action potential. However, these cells are able to generate a depolarizing current, which spreads to coupled myocytes, driving myocytes to threshold. Our hypothesis is that as long as the hMSCs contain the pacemaker gene and couple to cardiac myocytes via gap junctions, they will function as a cardiac pacemaker in an analogous manner to the normal primary pacemaker the sinoatrial node. We demonstrated using dual patch technique that hMSCs form gap junctions that couple electrically with canine cardiac myocytes. The coupling between engrafted hMSCs and cardiac myocytes was also shown by immunohistochemical staining of the tissues isolated from the site of hMSC injection using anti-connexin 43 antibodies. Within an injection site, the clusters of cells were vimentin and CD44 positive, and we also demonstrated that a significant majority of the cluster of cells were EGFP positive, thereby confirming their identity as hMSCs. A recent report has suggested that mouse MSCs can fuse with mouse myocytes in vivo with a fusion rate of 0.005%. We have not ruled this possibility out in our studies, but at the fusion rate reported by Morimoto et al, only 50 hMSCs of the million cells injected would fuse.

There are limitations to the approach used in this study. First, the hMSCs were delivered to the free wall myocardium, not an optimal site for ordered contraction. However, we have recently used catheter approaches to insert pacemaker genes into the canine left bundle branch system. Such a locus offers the possibility of more ordered and normal activation and contraction than is the case with a pacemaker residing in the free wall. Before this approach is used for hMSCs, catheter modification may need to occur to optimize injection of cells of the size of an hMSC without cell injury or destruction.

Another question relates to the duration of efficacy of these pacemakers. In the present study, we were only concerned with demonstrating the feasibility of using hMSCs as a gene delivery system. Because our studies in vivo lasted only 3 to 10 days, transient transfections were sufficient. Before this approach can be considered clinically relevant, far longer periods of study will be required. In this regard, our transfected cells maintain their green fluorescence for at least 3 months when grown on antibiotic to select stably expressing cells. This indicates that we have selected for stable clones expressing mHCN2, so it is likely that persistence of expression will not pose significant difficulties for more prolonged...
studies. However, it remains to be determined if the differentiation state of the hMSCs is altered in situ in the long term, or whether such differentiation would affect mHCN2 expression or biophysical properties. In addition, a murine gene, which is quite close but not completely identical in sequence to the human gene was used here. Not only would it be most advantageous to use human genes, but the exploration of various mutations to optimize activation and recovery characteristics, as well as neurohumoral response would be desirable. Such approaches are currently being explored.

In closing, the delivery of hMSCs expressing mHCN2 to the canine heart is not only a demonstration of feasibility of preparing hMSC-based biological pacemakers, but is the first concrete example of a general principle: hMSCs can be used to deliver a variety of genes to influence the function of syncytial tissues. One alternative potential cardiovascular application is delivery of K⁺ channel genes to hyperpolarize vascular smooth muscle inducing relaxation. Indeed, the payload delivered by hMSCs need not be restricted to membrane channels: any gene product or small molecule that can permeate gap junctions (MW <1000, minor diameter <1.2 nm) can be incorporated into the hMSCs and delivered to a syncytial tissue as its therapeutic target.

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