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Cardiac Cell Therapy In Vitro: Reproducible Assays for Comparing the Efficacy of Different Donor Cells

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Introduction

Cardiovascular disease remains a pervasive and significant clinical problem, making it the target of extensive research for novel therapeutic strategies. Due to the limited endogenous potential of the heart to repair itself, significant loss of myocytes will often lead to congestive heart failure, which currently afflicts more than five million Americans and is associated with an annual mortality of more than 20% [1]. Cellular therapies are emerging rapidly as one of the more promising methodologies to repair injured myocardium (for a recent review, see Boyle et al. [2]). Theoretically, cell transplantation offers the potential to correct the loss of myocytes that occurs with infarction and other cardiac pathologies, thereby preventing the onset of or reversing the heart failure.

Although several cellular therapies have rapidly progressed to clinical trials with mixed, yet promising, results, there remain several fundamental, unanswered questions. These include: 1) To what extent donor and host cardiac cells interact? 2) How do these interactions affect the propagation of electrical activity and the mechanical functioning of the myocardium? 3) How does the integration of cells into the myocardium affect the susceptibility to potentially lethal arrhythmic activity? and 4) Which donor cell type(s) will offer the most benefit with the least risk? Furthermore, the mechanisms underlying the functional improvements observed in clinical trials have yet to be defined and may be the result of transdifferentiation of the injected cells, direct functional integration of the donor cells within the host myocardium, paracrine effects that may be reproduced without the need for cell implantation [3], or even passive contributions [4] that could potentially be recapitulated by cell-free materials [5].

One of the primary obstacles to designing safe and efficient cellular therapies is the difficulty in systematically studying the structural and functional interactions between the implanted cells and host cardiomyocytes within the complex three-dimensional heart tissue in situ. Rapid advancements in the field of stem cell biology have identified several novel cell types, including embryonic stem cells [6], [7], resident cardiac stem cells [8], [9], epicardium-derived cells [10], pericardium-derived cells [11], umbilical cord blood stem cells [12], amniotic fluid-derived stem cells [13], [14], liver stem cells [15], and adipose stem cells [16], [17]. All of these cell types are being considered for future clinical trials without any standardized, well-controlled in vitro or in vivo assays available to compare their efficacy to integrate and repair damaged cardiac tissue. Thus, it is becoming increasingly important to develop reproducible cell culture models in which the structural and functional interactions between donor and host cells can be well-controlled and systematically studied (possibly in a high-throughput fashion) at the cellular and tissue levels.

A few investigators have used in vitro coculture model systems [18]–[20] to study the interaction of donor cells with cardiomyocytes. Although some valuable insights were gained through these systems, the applied culture methods lacked reproducibility in the spatial distribution of cocultured cells or potentially introduced cell injury, both of which may render the systematic studies and interpretation of the results difficult. In this report, we describe in

vitro assays with reproducible cellular and cell network geometries for systematic studies of donor and host cell interactions on different spatial scales. In particular, micropatterning and stencil techniques are used in our laboratory to fabricate 1) individual cell pairs with defined shape, size, and area for cell contact, which allow for the quantitative comparison of the potential of different donor cells to couple with cardiomyocytes [Figure 1(a) and (b)], 2) long (pseudo one-dimensional) cardiomyocyte strands with bridges (inserts) made of donor cells, which are used to compare the potential of different donor cells to conduct electrical activity within cardiac tissue [Figure 1(c) and (d)], and 3) two-dimensional cardiomyocyte monolayers containing donor cell implants of different shapes, sizes, and distribution, which are used to compare the potential of different donor cells to yield arrhythmias within cardiac tissue [Figure 1(e) and (f)]. In this report, we focus on describing the methods for the fabrication of cardiac strands with donor inserts and the application of this assay to test the ability of a few selected donor cell types to bridge and propagate electrical impulses from one cardiomyocyte area to another.

Methods

Cell Cultures

Cardiomyocytes—Ventricular cardiomyocytes were derived by dissociation from 2-day-old neonatal Sprague Dawley rats using trypsin and collagenase. Cells were suspended in Dulbecco's modified Eagle's medium (DMEM/F12) supplemented with 10% calf serum and 10% horse serum, as previously described [21], and preplated to enrich the proportion of cardiomyocytes by removing faster adhering nonmyocytes. A high purity of cardiomyocytes (>93%) was confirmed by positive staining for sarcomeric α -actinin.

Cardiac Fibroblasts—Cardiac fibroblasts (CFs) were recovered from the cardiomyocyte isolation during the preplating step. Cells were maintained in M199 medium supplemented with 10% fetal bovine serum (FBS) and HEPES (10 mM), nonessential amino acids solution (1 $\times$), L-glutamine (2 mM), dextrose (3.5 mg/mL), cobalamin (4 μ g/mL), and penicillin G (100 U/mL). Upon reaching confluence, the fibroblasts were split and passaged at a 1:2 ratio and were used at passage 1. A high purity of cells (>99%) was confirmed by positive staining for vimentin.

Rat Mesenchymal Stem Cells—Adult rat mesenchymal stem cells (rMSCs) were isolated from the bone marrow of male Sprague Dawley rats, as previously described [22]. Briefly, whole bone marrow was collected from the femur and tibia in MSC growth medium (α -modified Eagle's medium supplemented with 20% FBS, 50,000 U penicillin, and 50,000 μ g streptomycin). Mononuclear cells were isolated by a ficoll gradient and plated in tissue culture-treated dishes. Nonadherent cells were discarded, and adhered cells formed colonies that were identified as MSCs by positive staining for the cell surface marker CD90 and negative staining for the hematopoietic stem cell marker CD45.

A subpopulation of MSCs that had been transfected with green fluorescent protein (GFP) using IRES-GFP, which had been cloned into the murine stem cell virus vector (pMSCV), was obtained as a kind gift from Dr. Victor Dzau of Duke University [22].

C2C12 Myoblasts—Mouse C2C12 myoblasts were purchased from the American Type Culture Collection (ATCC). Cells were maintained in growth medium (DMEM supplemented with 10% FBS, 10% calf serum, 2.8-mL chick embryo extract, and 0.1% gentamicin) and kept below 70% confluence to prevent the formation of myotubes.

Wild-Type and Connexin-43 Transfected HEK-293 Cells—Wild-type HEK-293 cells were purchased from ATCC. Cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin solution. The rat connexin-43 (Cx43) gene (GJA1) was cloned from neonatal rat cardiac myocytes using primers based on the published rat connexin-43 sequence (PubMed NM_012567) and amplified using the polymerase chain reaction. The connexin-43 gene was modified during the polymerase chain reaction to include a 5' Kozak sequence and was subcloned into a custom mammalian expression plasmid, pCMV-IRES-mCherry. Transfection was performed using the GenJet transfection reagent. Positively transfected cells were identified by mCherry expression and selected using puromycin. Stable monoclonal cell lines were isolated from the polyclonal population and used for experimental studies.

Mouse Cardiac Progenitor Cells—Mouse embryonic stem cell-derived cardiomyocytes (mES-CM) were obtained as a kind gift from Dr. Nicolas Christoforou of Duke University. Cardiomyocytes were selected using puromycin resistance under ventricular α -myosin heavy chain promoter expression, as previously described [23], and were engineered to express either DsRed or GFP. After 7 days of embryoid body differentiation, puromycin selection was applied for 2 days, and on day 9 cells were enzymatically dissociated and used for coculture.

Micropatterning

Fibronectin micropatterns were prepared using our methods described previously [21]. Briefly, silicon wafers were coated with a 10- μ m thick layer of photoresist (SU8-10), exposed to ultraviolet (UV) light through a photomask, and developed to produce a negative of the desired pattern on the silicon wafer. Poly(dimethylsiloxane) (PDMS) stamps were cast against the wafer, coated with a 50 μ g/mL fibronectin solution and used to transfer the 50–100 μ m wide and 10-mm long fibronectin parallel lines onto 25-mm diameter PDMS-coated glass coverslips [Figure 2(a, sequences 1–3) and (b)]. Unstamped areas (200- μ m spacings in between the lines) were coated with 0.2% w/v Pluronic F-127 to prevent cell bridging between adjacent lines. Surrounding the lines on three sides (with one end of each strand being free) was a common area used to coordinate the synchronous beating of all cardiac strands.

Stencils

To obtain differential seeding of cardiac and donor cells on the fibronectin lines, cardiac cells were seeded within a stencil that contained a narrow (200–500 μ m) spacer segment that selectively blocked cardiac cell attachment to the fibronectin lines. Stencils were fabricated as previously described [25]. Briefly, silicon wafers were coated with a thick ($\sim$ 650 μ m) layer of photoresist (SU8-100), exposed to UV light through a photomask, and then developed [Figure 2(c)]. Thin PDMS membranes were cast against the wafer, yielding a window on each half of the stencil to allow for cell seeding and gas exchange. Stencils were plasma etched to increase hydrophilicity and facilitate uniform cell seeding.

Preparation of Cardiac Strands with Defined Donor Cell Inserts

The stencil was adhered to the patterned coverslip with the stencil spacer oriented perpendicular to the stamped fibronectin lines [Figure 2(a, sequence 4)]. Cardiomyocytes were seeded within the stencil at a concentration of 2.5×10^6 /mL and allowed to adhere for 6 h [Figure 2(a, sequence 5)]. Epinephrine (2 μ M) was added to the cell suspension during seeding to promote attachment and spreading of the cardiac cells into strands.

The stencils were then removed, and the unattached cells were discarded with a PBS wash [Figure 2(a, sequence 6) and (e)]. The medium was replaced with cardiac seeding medium supplemented with cytosine arabinoside (1 μ M) to prevent the proliferation of nonmyocytes into the open insert area. After 24 h, the donor cells were resuspended in serum-free medium

[26] and seeded at a density of $1.67 \times 10^5/\text{mL}$ for 15–30 min [Figure 2(a, sequences 7–8)]. During this time, the donor cells attached preferentially to the insert area within the cardiac strands, with few cells attached on top of the cardiomyocytes [Figure 2(e)]. Nonadhered cells were rinsed away, and the medium was replaced with 2% cardiac medium [M199 medium, 2% FBS, HEPES (10 mM), nonessential amino acids (1 $\times$), L-glutamine (2 mM), dextrose (3.5 mg/mL), cobalamin (4 $\mu\text{g}/\text{mL}$), and penicillin G (100 U/mL).] After preparation, the cardiac strands with donor cell inserts [Figure 2(f)] were maintained for 48 h before optical mapping of intracellular calcium transients.

Microscopic Optical Mapping of $(\text{Ca}^{2+})_i$ Transients

Propagation of intracellular Ca^{2+} transients was measured using a fiber optic bundle with 504 hexagonally arranged optical fibers positioned at the side port of an inverted microscope. The mapping resolution and field of view were 37.5 μm and 937.5 μm , respectively, at 20 $\times$ magnification [Figure 2(g)]. Cell cultures were incubated with calcium sensitive dye (2 μM Rhod-2) for 30 min and then gravity perfused with oxygenated Tyrode's solution in a custom-made thermoregulated recording chamber, similar to previously described methods [21], [27]. Excitation light was delivered by a 250-W Tungsten lamp. Recorded fluorescence maps were transferred through the fiber optic bundle, converted to voltage with photodiodes, sampled at 4.8 kHz, and stored on a computer. Data acquisition and shutter and stimulus control were synchronized using custom-designed LabVIEW software.

An extracellular bipolar platinum electrode was used to stimulate the common area of the culture (~ 5 mm distant from the insert) and generate the electrical propagation in cardiac strands at a rate of 2 Hz. Propagation of calcium waves across the insert region was microscopically mapped to scan donor cell types that could sustain impulse propagation at given insert lengths. Conduction time (CT) was measured between the two 638- μm spaced points located in the cardiomyocyte areas of the strand on opposite sides of the insert.

For data analysis and display, phase contrast images of the cell strand and insert area obtained before and after optical mapping were registered with the recording sites of the fiber optic array using custom-designed MATLAB software.

Immunocytochemistry

Immunostaining was performed as previously described [21]. Briefly, cell cultures were fixed, permeabilized, blocked, and incubated overnight with primary antibody (anti-sarcomeric α -actinin, 1:100; anti-connexin-43, 1:100; anti-vimentin, 1:1,000; anti-CD90, 1:100; anti-desmin, 1:50; Phalloidin-FITC, 1:100) and secondary antibody (anti-rabbit and anti-mouse Alexa Fluor IgGs, 1:200) for 2 h. DAPI was used for visualization of the nuclei. Optically mapped areas in immunostained cultures were imaged using a fluorescent microscope, a CCD camera, and IPLab image acquisition software.

Results

Creation of Cardiac Strands with Donor Inserts

Using standard photolithography techniques, we created stencils containing a spacer to block cardiomyocyte adhesion to patterned fibronectin lines [Figure 2(b)], thereby creating an area available for the insertion of donor cells. By varying the width of the spacer, we were able to create inserts of various lengths. Scanning electron microscope imaging of the master wafer used to create the stencil demonstrates the precision with which the spacer can be produced [300- μm photomask pattern produced a 312- μm wide photoresist channel on wafer, Figure 2(c)]. Furthermore, varying the geometry of the perpendicular spacer enables the production of

many cardiac strands with donor inserts of the same [Figure 2(f)] or variable lengths (not shown) on a single coverslip.

Control Cardiomyocyte Strands

Continuous cardiomyocyte strands with no donor inserts [Figure 3(a)–(c)] were used as a control. When stimulated, cardiac cells in the strand rapidly propagated electrical activity [demonstrated by colored isochrone lines, Figure 3(a)] with calcium transients produced in all recording sites. Figure 3(b) shows Ca^{2+} transient upstrokes from three channels along the length of the cardiac strand. The CT between channels 1 and 3 was ~ 2 ms. The overall conduction velocity in this representative cardiac strand was 29 cm/s. As expected, immunostaining showed extensive connexin-43 expression between the cells in the strand [Figure 3(c)].

C2C12 Myoblasts

We further investigated whether skeletal myoblasts in an insert area were capable of conducting electrical impulses within the cardiac strand. Impulse propagation in cardiac strands containing inserts of C2C12 myoblasts ranging in length from 350–400 μm resulted in conduction block within the insert. For example, the length of the myoblast insert in Figure 3(d) was determined to be 375 μm by positive staining for desmin and relatively weak staining for F-actin [Figure 3(f)]. Impulses at a rate of 2 Hz that were initiated 5 mm to the right of the insert [Figure 3(d)] propagated through the proximal cardiac portion of the strand [Figure 3(e), channel 1] and then failed to propagate through the myoblast insert (channel 2) and into the remainder of the cardiac strand (channel 3).

Wild-Type and Genetically Modified HEK-293 Cells

Genetic modifications hold the potential to improve the functional integration of certain donor cell types with cardiomyocytes. We tested this paradigm using wild-type and connexin-43 transfected HEK-293 cells developed in our lab. A representative conduction block in the 260- μm long insert [Figure 3(a) and (c)] made of wild-type HEK cells (inserts ranged from 250–400 μm) is shown in Figure 3(b). Upon electrical stimulation, impulses were propagated through the proximal part of the cardiac strand (channel 1) but not in the insert (channel 2) or distal portion of the strand (channel 3), indicating conduction failure through the insert. Immunostaining for connexin-43 showed only few positive spots in the wild-type HEK cells within the insert [Figure 4(c)].

Inserts ranging from 250–350 μm in length were also made using connexin-43 transfected HEK-293 cells. For example, impulses delivered at 2 Hz propagated through the ~ 330 - μm long insert area [Figure 4(d) and (f)] from the proximal portion of the cardiac strand (channel 1) through the insert (channel 2) and into the distal portion (channel 3) [Figure 4(e)]. The CT between channels 1 and 3 was 25 ms. As expected, no Ca^{2+} transients were recorded within the insert, as HEK cells lack the intracellular Ca^{2+} store and release processes characteristic of muscle cells. Therefore, the impulse propagation in the strand was passive. Staining for connexin-43 [Figure 4(f)] demonstrated the extensive gap junctions between the cells in the insert.

MSCs and ES-CM Cells

As an example of adult stem cell and embryonic stem cell-derived donors, we studied the ability of rMSCs and mES-CMs to functionally couple with cardiomyocytes in inserts ranging from 400–500 μm in length. A representative cardiac strand with a 410- μm long CD90-positive rMSC insert is shown in Figure 5(a) and (c). Similar to connexin-43 transfected HEK-293 cells, Ca^{2+} waves propagated at a 2-Hz rate from the proximal portion of the cardiac strand (channel

1) across the rMSC insert (channel 2) to the distal portion (channel 3) in a 1:1 fashion [Figure 5(b)]. The CT between channels 1 and 3 was 18 ms. No Ca^{2+} transients were observed in the insert area, as the propagation through the rMSCs was passive.

Similarly, mES-CMs were also able to sustain electrical propagation over a relatively long insert within the cardiac strand [e.g., 475 μm in Figure 5(d) and (f)]. Impulses were propagated at a rate of 2 Hz across the ES-CM inserts [Figure 5(e)] with a CT of 40 ms between channels 1 and 3. In contrast to the other donor cell types, the impulse conduction was active, as Ca^{2+} transients were generated by the cells within the insert [channel 2, Figure 5(e)]. Immunostaining for connexin-43 [Figure 5(f)] showed very little expression in ES-CMs within the insert as compared with the surrounding cardiac strand.

Discussion

One of the road blocks to the further advancement of stem cell therapies is the lack of rigorous in vitro assays to bridge the knowledge gap between clinical outcomes and basic studies of cellular function. In particular, cellular therapies for cardiovascular disease have been predominantly tested in the clinical and in vivo settings, without developing the in vitro assays that would allow for 1) systematic studies of the basic mechanisms behind the observed functional improvements or occurrence of arrhythmias, 2) meaningful and quantitative comparisons of different donor cells for their efficacy to improve cardiac function, and 3) a predictive and high-throughput prescreening of novel cell types, pharmaceuticals, or genetic manipulations aimed to improve already existing therapies. Here, we showed a set of reproducible, well-controlled in vitro assays that allow for comparative and quantitative studies of cardiomyocyte and donor cell interactions on several functional levels. This report focuses specifically on the methodology for the assessment of electrical propagation between cardiomyocytes and several different donor cell types using micropatterned cardiac strands with donor cell inserts.

Comparison of the Various Donor Cell Types for Their Potential to Functionally Integrate with Cardiomyocytes

To demonstrate the utility of the developed in vitro assay, we preliminarily compared various donor cell types for their potential to functionally integrate with cardiomyocytes and propagate electrical activity. In particular, we optically mapped at a spatial resolution of 37.5 μm (i.e., subcardiomyocyte length) the propagation of calcium waves in micropatterned strands composed only of cardiomyocytes (as a control) and strands containing donor cell inserts made of skeletal myoblasts, MSCs, wild-type or genetically modified HEK-293 cells, or ES-CMs. Similar to studies by others [19], [28], positions of optical channels were overlaid on a phase contrast image of the strand, allowing the monitoring of impulse propagation along the strand by viewing impulse traces in successive channels. Importantly, the use of a pseudo one-dimensional geometry circumvented potential ambiguities in the pattern of electrical propagation, a situation occasionally encountered in two-dimensional and, in particular, three-dimensional tissue settings. The ability to conduct and the CT between the two channels proximal and distal to the insert were used as a measure of the functional integration between donor cells and cardiomyocytes.

We first optically mapped normal propagation in 100- μm wide cardiac-only strands and measured the control conduction velocity of ~ 30 cm/s. We then tested propagation in inserts made of different donor cells including mouse C2C12 skeletal myoblasts and rat bone-marrow derived MSCs, i.e., cell types similar to those currently used in clinical trials of cellular cardiomyoplasty [2]. Both C2C12 cells and primary skeletal myoblasts have been previously shown to electrically couple with cardiomyocytes [29], [30]. However, in our system, myoblasts were not able to propagate impulses within cardiac strands over a length of ~ 375

μm . Other groups have similarly observed the failure of myoblasts to resynchronize two separated cardiomyocyte fields [18], the absence of synchronous beating between adjoining cardiomyocyte and myoblast cell sheets [31], and blocked propagation within cardiac tissue [32]. These results highlight the importance of characterizing the functional integration of donor and host cells not only by immunostaining, dye transfer, and other studies in isolated cell pairs but also by mapping electrical propagation within the cardiac network. In this realistic setting, cell size and shape, spatial relationships between donor cells and cardiomyocytes, as well as the frequency and strength with which donor cells couple, both among themselves and with cardiomyocytes, all play important roles in the ability of the donor implant to sustain electrical propagation.

Bone marrow-derived MSCs have been shown previously to form functional gap junctions with cardiomyocytes [33], [34]. In our system, an MSC insert was able to sustain impulse propagation and electrically connect cardiac strands proximal and distal to the insert. The effective conduction velocity of 2.5 cm/s, measured in 410- μm long MSC inserts 2 days postseeding, is in agreement with that found in human MSCs, bridging 400- μm wide gaps in studies by Beeres et al. [35]. Note, however, that for passive (i.e., detrimental) propagation within the insert, the conduction velocity decreases (likely in a nonlinear fashion) with the increase in the insert length. Therefore, it is meaningful to directly compare only the effective conduction velocities of propagation between two inserts with identical lengths.

We further tested the coupling potential of genetically modified donor cells, as genetic modifications are an emerging strategy for improving the integration between donor cells and cardiomyocytes [30], [36]–[38]. In particular, HEK-293 cells were genetically modified to overexpress connexin-43, the predominant gap junction protein in the ventricles of different species [39]. While wild-type HEK-293 cells have been shown to express some amount of connexin-43 [40], this amount in our studies did not appear sufficient to support impulse propagation within the cardiac strands, at least for the insert lengths of 260 μm or longer. Unlike wild-type cells, genetically modified cells were able to propagate impulses over the 330- μm long inserts within cardiac strands. The results obtained are consistent with studies on wild-type and genetically modified HeLa cells by Gaudesius et al. [19].

Finally, we tested the ability of mES-CMs (after 9 days of differentiation in embryoid bodies) to propagate electrical activity within a cardiac strand. Unlike other cell types used in this study, these cells have inward sodium and calcium currents able to support action potential generation and active impulse propagation [41]. Optical mapping of Ca^{2+} waves revealed generated Ca^{2+} transients inside the insert but relatively slow propagation, with an average velocity of 1.6 cm/s for a 475- μm long insert. We also optically mapped propagation in pure monolayer cultures made of the same ES-CMs (not shown). The velocity of propagation was similar to that obtained in the inserts, suggesting that ES-CMs could support propagation in an insert of any length, albeit with a slow velocity. Based on the connexin-43 staining, the low velocity could be at least in part attributed to the scarcity of gap junctional expression. It is expected that further ES-CM differentiation will yield a faster velocity of propagation due to higher expression of connexins and maturation of different ion currents involved in propagation [42].

Reproducible, High-Throughput In Vitro Assay for Studies of Functional Integration between Donor Cells and Cardiomyocytes

Development of standardized assays for comparing the ability of different donor cells to structurally and functionally integrate with cardiomyocytes is essential for the rational design of more successful cardiac cell therapies. The main advantage of the described in vitro assay is the ability to create empty areas of a defined length in a cardiac strand (without cardiac cell damage) within which different donor cells can be inserted and compared under identical

geometric conditions for their ability to synchronize impulse propagation across the strand. Using this approach, inserts of identical length can be produced in closely spaced cardiac strands [Figure 2(f)], simultaneously excited from the common area, and optically mapped (at lower magnification). This would allow for a relatively high-throughput assessment of the probability of successful conduction for a given insert length. In addition, the use of staircase-like spacers would allow for the creation of adjacent strands with progressively longer inserts, which would consequently enable high-throughput measurements of the maximum insert length supportive of successful propagation for a given donor cell type.

Finally, the described in vitro assay can allow for systematic studies to predict the pharmacological and genetic factors that can improve the structural and functional integration of transplanted cells/tissues into the heart. We believe that through control of structural and functional interactions of donor cells and host cardiomyocytes we may be able to promote donor cell survival, differentiation, and the ability to exert functional cardiac repair without arrhythmogenic risks, thus aiding the design of safer and more efficient cardiac cell therapies.

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Fig. 1.

In vitro assays for well-controlled studies of donor-cardiac cell interactions. (a) Schematic diagram of micropatterned cell pair system for studying homotypic and heterotypic cell coupling. (b) Example of cardiac-cardiac and cardiac-fibroblast cell pairs. Fibroblast is outlined by a white dashed line. (c) Schematic representation of a cardiac strand with donor cell insert, for studying electrical propagation between cardiac and donor cells. (d) Example of a cardiac strand with a 350-μm long fibroblast insert; inset, close-up of cardiac/fibroblast border. (e) Schematic of cardiac monolayer with donor cell island for studying propensity for arrhythmias. (f) Example of a cardiac monolayer with central island made of GFP mES-CMs.

Fig. 2.

Methods for creation and assessment of donor inserts in cardiac strands. (a) Fabrication of strands with inserts: sequences 1–3, microcontact printing of fibronectin lines; sequences 4–5, application of stencil and seeding of cardiac cells; sequences 6–8, removal of stencil and seeding of donor cells in the insert area. (b) Microcontact printed fibronectin lines (red) with common area shown to the left. (c) SEM image of a portion of microfabricated wafer used to cast the 300-μm wide spacer of a PDMS stencil. Cardiac strand (d) before and (e) after GFP rMSCs were seeded into the 200-μm insert area. (f) Several closely spaced cardiac strands with fibroblast inserts of the same length. (g) Left, setup for optical micromapping of impulse

propagation in strands. Right, field of view of the photodiode array with overlaid cardiac strands containing donor cell inserts.

Fig. 3.

Propagation in a control cardiac strand and myoblast insert. (a) Phase contrast image of a control cardiac strand with overlaid optical recording sites. Twenty-five microsecond spaced colored isochrones show uniform propagation of impulse across the strand. Yellow numbered recording sites correspond to impulse upstrokes shown in (b). Impulse propagates with a CT of 2 ms between sites 1 and 3. (c) Immunostaining of the strand in (a). (d) Phase contrast image of a cardiac strand with C2C12 myoblast insert and overlaid recording sites. Dashed lines indicate the insert borders. Yellow numbered sites correspond to Ca transients shown in (e). Propagation fails to continue to site 3. (f) Immunostaining of the recorded strand in (d). Length of the insert is estimated as 375 μm.

Fig. 4.

Propagation in wild-type and genetically modified HEK-293 cell inserts. (a) Phase contrast image of a cardiac strand with wt HEK-293 insert and overlaid recording sites. Dashed lines indicate the insert borders. (b) Propagation fails to continue to site 3. (c) Immunostaining of the recorded strand in (a). Length of the insert is estimated as 260 μ m. (d)–(f) Impulses propagate through a 330- μ m long insert made of connexin-43 transfected HEK cells with a CT of 25 ms between sites 1 and 3. The rest of the nomenclature is the same as in Figure 3.

Fig. 5. Propagation in rMSC and mES-CM inserts. Impulses propagate through (a)–(c) a 410- μ m long insert made of rMSCs with CT = 18 ms and (d)–(f) a 475- μ m insert made of mES-CMs with CT = 40 ms. The rest of the nomenclature is the same as in Figure 3.