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Modeling Conduction in Host-Graft Interactions Between Stem Cell Grafts and Cardiomyocytes

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***Abstract*—Cell therapy has recently made great strides towards aiding heart failure. However, while transplanted cells may electromechanically integrate into host tissue, there may not be a uniform propagation of a depolarization wave between the heterogeneous tissue boundaries. A model using microelectrode array technology that maps the electrical interactions between host and graft tissues in co-culture is presented and sheds light on the effects of having a mismatch of conduction properties at the boundary. Skeletal myoblasts co-cultured with cardiomyocytes demonstrated that conduction velocity significantly decreases at the boundary despite electromechanical coupling. In an attempt to improve the uniformity of conduction with host cells, differentiating human embryonic stem cells (hESC) were used in co-culture. Over the course of four to seven days, synchronous electrical activity was observed at the hESC boundary, implying differentiation and integration. Activity did not extend far past the boundary, and conduction velocity was significantly greater than that of the host tissue, implying the need for other external measures to properly match the conduction properties between host and graft tissue.**

I. Introduction

THE limited ability of the heart to repair itself following myocardial infarction has led to the development of new therapies aimed at regenerating tissue and regaining lost cardiac function. At the forefront of this is cellular cardiomyoplasty, a process in which exogenous cells are transplanted into a damaged heart [1]. The transplantation of a number of different stem cells is being pursued as a regenerative therapy, including those derived from bone marrow [2], skeletal muscle [3],[4], or embryonic tissues [5]. Understanding how these cells interact with the host scaffold of myocardium with regard to electrical communication and propagation of depolarization is a critical step in such regenerative therapies. While clinical studies are proceeding, there is a dearth of information regarding the integration properties of transplanted cells and ability of the graft to conduct electrical signals. To study these interactions, various cell types have been cultured together in numerous experimental systems. Utilizing microfabrication and more recently, micropatterning, investigators have achieved defined co-cultures by patterning biologically active molecules on the culture surface prior to cell plating. Such techniques allow the creation of stripes [6], islands [7], and other patterns of tissue for the investigation of cell-cell interactions or geometrical dependencies. A limitation of these methods is that patterning is often permanent and irreversible for the duration of an experiment. These methods of co-culture also involve a modified surface that results in changes to the cell-substrate interaction, possibly altering cell motility and cell function.

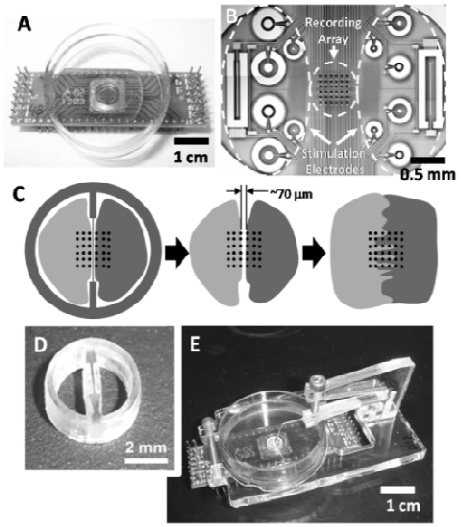

Figure 1. Co-culture instrumentation. (A) Photograph of MEA designed for use with a standard 35 mm petri dish and (B) micrograph of the center well with the recording electrode array and larger auxiliary electrodes used for stimulation or additional recording electrodes over a larger area. (C) The co-culture wall divides the center recording array into 6 x 3 sub-arrays and allows analysis of the boundary between cultures. (D) The reusable acrylic barrier bisects the ring and defines two chambers. (E) The ring is held face-down in place with an accompanying support structure, consisting of base that clamps around the petri dish, and an overhanging arm that contacts the ring through a 20 gauge needle. The position of the arm is held steady with a screw adjustable by hand.

A novel system for culturing two populations of cells on the same surface has been developed that allows the merging

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of independent cultures over time using a laser etched acrylic barrier between 50 and 100 μm in width, achieving a narrow separation of the two cell chambers (Fig. 1c-e). The contact surface is planarized and allows isolation of the cell populations without the addition of sealant or grease that might leave residue on the substrate and impede cell migration or impair cell function. Isolated chambers can be seeded with cell populations individually, and the barrier can be removed without disturbing the specific pattern of cultures, allowing the two cell populations to interact either indirectly, through diffusible factors, or directly by establishing cell-cell contacts over time to form a connected, heterogeneous culture (Fig 1c).

Combining the co-culture technique with microelectrode array (MEA) technology [8] provides constant, non-invasive electrical monitoring of the two distinct populations (Fig 1a). MEAs allow measurement of conduction properties in the two cultures before, during, and after integration, further validating the technique as a cell transplantation model.

The utility of the co-culture device is first demonstrated by examining the integration between cardiomyocytes and skeletal myoblasts in a co-culture model for host-graft interactions. Skeletal myoblasts have been proposed as a possible candidate for cell therapy due to its autologous origin, exclusive differentiation into muscle-fiber cells, and high resistance to ischemia [9]. Although transdifferentiation into cardiomyocytes does not take place [10], skeletal myoblasts have been shown to properly integrate [11], electromechanically couple [12], and even fuse [13] with cardiac tissue, making them an ideal candidate to demonstrate the co-culture device as a model for cell transplantation between different cell types.

Second, cardiomyocytes were co-cultured with differentiating human embryonic stem cells (hESC) and observed over time to examine their potential as a candidate for cell therapy. Using a pluripotent cell type instead of skeletal myoblasts as a cardiac graft avoids the issues of tissue disparity and offers the potential to fully match the electrical characteristics of host tissue.

II. Methods

A. Co-culture Device

The co-culture device was designed with AutoCAD software (Autodesk, San Rafael, CA), and machined by etching and cutting a cast acrylic sheet (Chemcast GP; Plastiglas de Mexico) 2.8 mm thick with a CO_2 laser ablation system (V-460 Laser Platform; Universal Laser Systems, Scottsdale, AZ). The device consisted of three parts: the ring (which is bisected by the barrier), the base, and the contact arm (Fig. 1e). The thickness of the wall separating cell cultures averaged 70±30 μm, measured from micrographs acquired following planarization.

The co-culture contact arm is attached to the base of the device through screw holes on the underside of the arm. A 20-gauge needle was attached to the end of the arm with epoxy and contacts the ring to apply pressure, maintain a seal, and prevents movement. The flexible arm and needle are applied to the co-culture ring by applying pressure from the top using a finely-threaded screw with a 0.5 mm pitch. Once the ring is placed, the needle from the over-hanging arm is attached to the co-culture ring using an RTV sealant (Dow Corning, Midland, MI). To remove the ring, the top screw is removed, relieving pressure from the arm, and returning it to its original position while lifting up the attached co-culture ring without lateral motion.

B. Cell Culture

The integration model between host and graft tissue was developed using the murine atrial tumorgenic cell line HL-1 cardiomyocytes [14] as the host, and C2C12 murine skeletal myoblasts or hESC as grafts. HL-1 cells were cultured in Claycomb media (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 100 mM norepinephrine (Sigma-Aldrich), 100 units/ml penicillin-streptomycin (Invitrogen, Carlsbad, CA), and 4 mM L-glutamine (Invitrogen). C2C12 skeletal myoblasts were cultured in a solution containing 89% DMEM (Invitrogen), 10% FBS (Hyclone), and 1% penicillin–streptomycin (Invitrogen). HL-1 cardiomyocytes and C2C12 skeletal myoblasts were plated on the same day (Day 0) at equal densities of 3×10^4 cells per chamber. The barrier was removed the following day (Day 1).

hES from the H9 lineage (WiCell Research Institute, Madison,WI) underwent the differentiating process Iscove's modified Dulbecco's medium and 20% defined fetal bovine serum (FBS; HyClone), 0.1 mM nonessential amino acids, 2 mM L-glutamine, 450 M monothioglycerol (Sigma-Aldrich), 50 U/ml penicillin, and 50 g/ml streptomycin, in ultralow-attachment plates for the formation of suspended embryoid bodies (EB) for six days as previously described [15]. At Day 7, floating EBs were gathered and seeded to 0.1% gelating coated 10cm Petri dishes for further differentiation. At Day 14, EBs were dissociated in collagenase IV (Sigma-Aldrich) and seeded onto the co-culture devices in monolayer with 4×10^4 cells per chamber.

C. Microelectrode Array Instrumentation

The MEA consisted of a 6 × 6 array of platinum electrodes with 22 μm diameters spaced on 100 μm centers, with additional larger electrodes on each side, as discussed in [16]. Data was acquired through 32 channels, with the four corner electrodes excluded. Petri dishes with 35 mm-diameters and drilled through-hole 1 cm. in diameter were fixed to the package using bio-compatible epoxy (EP42HT, Master Bond; Hackensack, NJ). Signals from the MEA were processed by a custom recording system consisting of a 32-channel amplifier with two-stage gain of 60 dB, 7 Hz 1st-order high-pass cutoff, and 8th-order low-pass cutoff at 3 kHz, as previously reported in [17]. The signals were then digitized with 16-bit resolution at 10 kHz and acquired by a custom-designed visualization and analysis tool, written in Matlab™ (The MathWorks; Natick, MA) [18].

Conduction patterns were analyzed by coordinating the local activation time, as defined as the point of maximum negative slope of the extracellular AP, with it spatial location on the MEA. Active electrodes were then grouped together by three, and for each electrode triplet, the magnitude and direction of propagation were calculated as

previously described [19] and represented as a vector. The velocity of the culture presented is the average of all triangulated vectors from the array.

III. Results

A. Co-culture validation

In initial studies HL-1 cardiomyocytes were cultured on both sides of the co-culture divider to demonstrate integration between cell populations (Fig. 2). Over the course of 24 hours, HL-1 cardiomyocytes merged together into a confluent culture. More importantly, the asynchronous electrical activity between separated cultures synchronized once merged together (N=5).

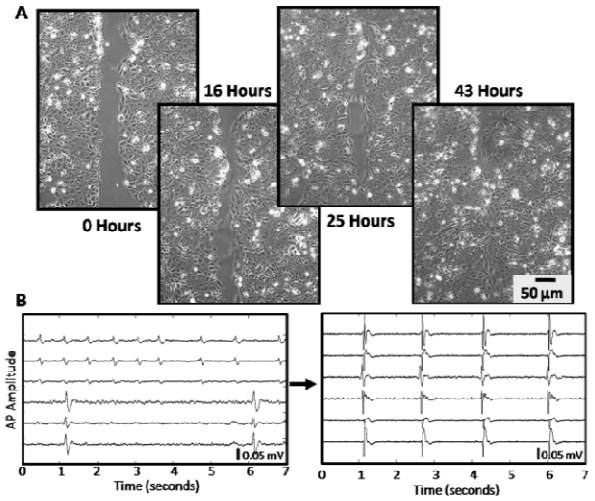

Figure 2. Development of cell transplantation model. (A) HL-1 cardiomyocytes seeded on both sides of the barrier in a standard 35 mm petri dish and allowed to merge to model a host-graft interaction. (B) The same experiment was performed on a MEA, where two asynchronous sets of electrical signal were initially observed, but synchronized after merging.

B. Host-Graft Interactions with Skeletal Myoblasts

HL-1 cardiomyocytes (host) were co-cultured with C2C12 skeletal myoblasts (graft), for 1 day before the barrier was removed. Within 24 hours of barrier removal the two cell populations had physically merged on the MEA, as observed under the microscope. After the host and graft cell populations had merged all channels displaying electrical activity was assumed to be originating from the host, and was validated by noting that the active electrodes were located at the appropriate side of the MEA. In long term culture conditions (four days), electrical activity was also observed on the side of the graft cells, but only under C2C12 cells bordering the cardiomyocytes (N=5; Fig. 3a).

Action potentials originating from the graft side were synchronous with activity from the host side, but carried lower amplitudes than host action potentials, and are likely due to electrically coupled skeletal myoblasts (Fig. 3b). Analysis of conduction patterns within the sample shown in Figure 3c also demonstrates a significant decrease ($P<0.05$) in the velocity of action potential propagation on all samples to 37±26% of the host velocity (N=5).

On all samples, conduction analysis indicated that action potentials originated from the host side and propagated towards and into the graft. This was confirmed by the addition of 10 µM isoproterenol (ISO) which only interacts with cardiomyocytes as myoblasts lack the necessary β-receptors [12]. After ISO addition all samples (N=5) remained synchronous and beat rates increased from 12% to 130%. The amplitudes of the APs on the host side increased as expected from 3% to 30%, while the amplitudes of the graft APs did not change more than 3%.

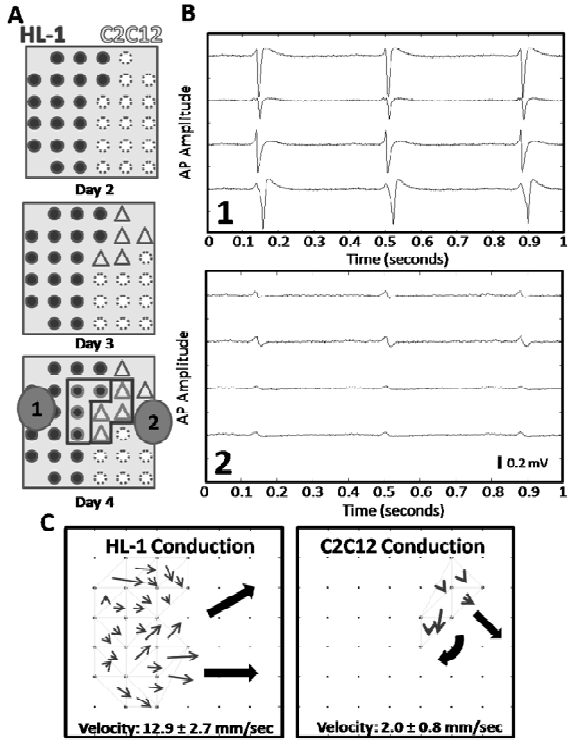

Figure 3. Host-graft model with heterogeneous cultures. HL-1 cardiomyocytes (host) were co-cultured with C2C12 skeletal myoblasts (graft). (A) A representation of the MEA displays electrodes after cultures have merged on Day 2. Electrodes displaying electrical activity on Day 2 are assumed to originate from the host, and are represented by solid circles. Additional electrodes on the graft side began exhibiting action potentials in subsequent days and are represented by triangles. (B) Activity from the boxed electrodes are displayed for Day 4, showing a difference in amplitude between cultures, but still synchronous behavior (N=5). (C) Conduction analysis on both sides on Day 4 indicated that electrical activity originated from the host, and experienced a significant ($P<0.05$) decrease in conduction velocity.

C. Host-Graft Interactions with hESC

To explore the advantages of using pluirpotent stem cells in transplantation, HL-1 cardiomyocytes (host) were co-cultured with hESCs (graft). Following the removal of the barrier on Day 1, both populations of cell cultures merged together within 24 hours. Electrical signals observed at this point were confirmed as originating from the host side. Between Days 5 to 7, additional action potentials were observed on the graft side (N=3), beating synchronously with the host, and exhibited a lower AP amplitude. Propagation velocity significantly increased ($P<0.05$) from 9.0±1.0 mm/sec on the host side to 33.8±12.4 mm/sec in the graft (Fig. 4). The propagation velocity of HL-1 cardiomyocytes is consistently observed in homogeneous HL-1 cultures, while the propagation velocity of the differentiating hESC cells more closely resembles primary neonatal cardiac myocytes.

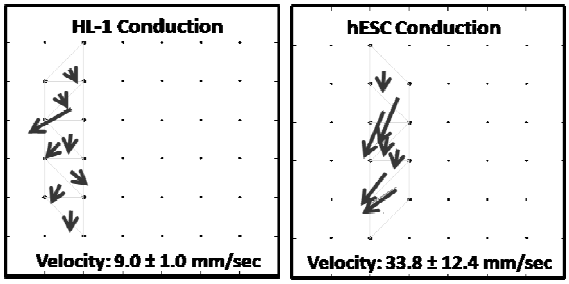

Figure 4. Host-graft model with hESC on Day 6. HL-1 cardiomyocytes (host) were co-cultured with differentiating hESC (graft) on a representation of the MEA. Cultures have merged by Day 2, and by Day 6, additional electrodes on the graft side which previously did not display activity began exhibiting synchronous action potentials with host tissue, with a propagation velocity significantly greater than host tissue. Activity on the graft was confined to the boundary and did not extend further.

IV. Conclusion

The described co-culture device allows the definition of two distinct cell populations from different origins, and the study of their subsequent interaction following removal of the co-culture barrier. During integration, mechanical and electrical junctions are established between cells and propagation of depolarization commences. The boundary between cell types is further analyzed as a mismatch of conduction properties between two cell cultures, as exhibited in the differences seen in the conduction properties. This scenario can be analogous to an impedance mismatch found in transmission lines.

This experimental paradigm is applied towards the study of interactions between different cell types, first attempted using HL-1 cardiomyocytes (host) and C2C12 skeletal myoblasts (graft) as a model of cellular cardiomyoplasty. The use of MEAs allows the examination of the interface between host and graft cells with regard to the development of electrically integrated cell populations and its spatial limitations beyond that boundary. In this case, only the graft cells at the host boundary displayed signals which did not extend past more than two electrode lengths (~200 μm). It has been shown that early stage C2C12 skeletal myoblasts develop proteins for electrical connections (connexin43) and mechanical coupling (N-cadherin) within days of plating, but down-regulate them as they differentiate unless coupled with cardiomyocytes [12].

The limited response at the boundary was also present in differentiating hESCs used as a model graft. Because the hESCs were spontaneously differentiating, most of the cells were not electrically conductive, and despite its pluripotent properties, was unable to spontaneously match the conduction properties of host tissue.

The boundary between cell types is clearly mapped by the presence of a mismatch of conduction properties between two cell cultures as seen by differences in propagation velocity. Changes in propagation characteristics could lead to an otherwise abnormal rhythm with unknown consequences. While cells may survive transplantation *in-vivo*, some studies suggest certain methods of transplantation may be pro-arrhythmic [20]. The observed change in propagation velocities allows this model to address the affects of electrical connectivity at, and propagation through, heterogeneous tissue boundaries. Indeed, the implications that cell transplantation may adversely modulate the host system acts as a reminder that successful myoplasty must not only include functional integration but also evidence of the restoration of proper electrical conduction and intracardiac propagation.

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