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Electric Field Stimulation Integrated into Perfusion Bioreactor for Cardiac Tissue Engineering

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We describe herein the features of a novel cultivation system, combining electrical stimulation with medium perfusion for producing thick, functional cardiac patches. A custom-made electrical stimulator was integrated via inserting two carbon rod electrodes into a perfusion bioreactor, housing multiple neonatal Sprague-Dawley rat cardiac cell constructs between two 96% open-pore-area fixing nets. The stimulator produced adjustable stimulation waveform (i.e., duty cycle, number of stimulating channels, maximum stimulation amplitude, etc.), specially designed for cardiac cell stimulation. The cell constructs were subjected to a homogenous fluid flow regime and electrical stimulation under conditions optimal for cell excitation. The stimulation threshold in the bioreactor was set by first determining its value in a Petri dish under a microscope, and then matching the current density in the two cultivation systems by constructing electric field models. The models were built by Comsol Multiphysics software using the exact three-dimensional geometry of the two cultivation systems. These models illustrate, for the first time, the local electric conditions required for cardiomyocyte field excitation and they confirmed the uniformity of the electrical field around the cell constructs. Bioreactor cultivation for only 4 days under perfusion and continuous electrical stimulus (74.4 mA/cm^2, 2 ms, bipolar, 1 Hz) promoted cell elongation and striation in the cell constructs and enhanced the expression level of Connexin-43, the gap junction protein responsible for cell–cell coupling. These results thus confirm the validity of the electrical field model in predicting the optimal electrical stimulation in a rather complex cultivation system, a perfusion bioreactor.

Introduction

Ex vivo cardiac tissue engineering is a promising approach for regenerative therapy of damaged myocardial tissue. Here, the cardiac patch is constructed by seeding donor cells within polymeric scaffolds followed by cultivation of this cell construct under controlled conditions to induce and direct the generation of new, functional tissue. However several hurdles still remain to be overcome before the engineered tissue is ready to be implanted in patients as replacement for diseased tissue. Among these is finding the best culture conditions to grow the cells so that they form thick viable cardiac tissue that is long lasting and structured at a cellular level like natural tissue.

Perfusion bioreactors enable the development of avascular tissues thicker than 100 μm, that is, the oxygen diffusion distance. In a perfusion bioreactor, the oxygen transport from the medium to the constituting cells occurs via diffusion and convection and the medium is continuously reoxygenated by a gas exchanger, via aeration, or by membrane exchange.[1,2] Such a bioreactor has been developed in our group, enabling the modular cultivation of multiple cell constructs in a total cross section of 20 cm^2, while providing homogeneous fluid flow and shear stress along the bioreactor cross section.[3,4] The unique design of the mesh holding the cell constructs in this bioreactor enables maximal exposure of >98% of the cell constructs to the culture medium, leading to the formation of viable tissue. We further showed that providing pulsatile interstitial fluid flow in bioreactor induced the ERK1/2 signaling cascade in neonatal rat cardiac cell constructs, leading to the assembly of thick cardiac tissue (>500 μm) with ultrastructural features approaching those found in adult myocardium.[5]

Electrical stimulation has long been recognized as a critical parameter for the induction of synchronously contracting cardiac cell construct. Studies performed on cardiomyocyte monolayers have revealed that electrical stimulation (80–150 V, pulse duration of 5–10 ms, I <5 mA, 1–5 Hz) induced cell enlargement, the development of more organized myofibrils, and greater expression of cardiac genes (*ANF* and *MLC*-2).[6] Short-term electrical stimulation (60–120 min) of cardiomyocyte monolayers led to upregulation of the gap

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junction protein Connexin-43 (Cx-43), which is responsible for mechanical and electrical communication between adjacent cardiomyocytes.[7] Radisic and colleagues subjected three-dimensional cardiac cell constructs to electrical stimulation (rectangular pulses, 5 V/cm, duration of 2 ms at pacing frequency of 1 Hz). This treatment induced cell alignment and coupling and increased the amplitude of the synchronous contractions of the cell constructs by a factor of 7.[8] The studies were performed under static conditions, limiting the thickness of the engineered tissue to 100 μm.

In the present work, we sought to integrate electric field stimulation into the perfusion bioreactor in aim to produce functional cardiac tissue. For this, a custom-made electrical stimulator was integrated into the perfusion bioreactor by insertion of carbon rod electrodes. Electric field models were created to illustrate the stimulation conditions (current density and electric field) in the bioreactor, and then the optimal stimulation conditions were employed during bioreactor cultivation of the cardiac cell constructs. By employing perfusion combined with electrical stimulation, the regenerated cell constructs revealed enhanced levels of Cx-43, and promoted cell elongation and striation compared to static-cultivated constructs.

Materials and Methods

Custom-made electric stimulator

We fabricated an electrical stimulator with adjustable stimulation waveform (i.e., duty cycle, number of stimulating channels, maximum stimulation amplitude, etc.) specially designed for cardiac cell construct stimulation. The electrical waveform output (Fig. 1) is defined by several parameters specifying the stimulation conditions (Table 1). The determination of the different positive and negative pulse duration (T1 and T2, respectively, in the waveform) and the option to define the duty cycle for a given stimulation period (WT in the waveform) are unique features missing in commercially available function generators. In addition, different waveform parameters can be defined in parallel in two output channels, allowing the simultaneous execution of two different experiments.

Experimental setup of the cultivation system

Figure 2 depicts the setup of the electrical-stimulated cultivation systems in a Petri dish or in the perfusion bioreactor. The electric stimulator is controlled by a Matlab graphical user interface in the PC and is connected to a direct current (DC) power supplier. The graphical user interface allows the user to enter the desired stimulation parameters and to follow the stimulation progress (period state and elapsed time). The stimulator output is connected to two carbon electrodes (ground and reference potential) that are integrated into the perfusion bioreactor.

The perfusion bioreactor system was previously described in detail.[3] Briefly, it consists of a cultivation vessel (length 12 cm, inner diameter = 5 cm, volume = 100 mL) fabricated from two identical halves that when connected to each other function as the inlet and outlet of the cell construct compartment. Before use, the Plexiglas vessel was sterilized using ethylene oxide. The cell constructs are fixed in place by using two 96% open-pore-area fixing nets. The vessel body is connected to a medium reservoir, gas exchanger, and computerized peristaltic pump (Manostat, CompuLab; Cole-Parmer) that continuously circulates the perfused medium through the cell constructs, as previously shown.[3] The perfused medium is transferred to the reservoir, where it is heated to 37°C using a temperature controller (Cole-Parmer digi-sense EW-89000), is oxygenated to a pO_2 level of 160 mmHg, and then is pumped back to the bioreactor vessel. Throughout the cultivation, the pH, pO_2, and pCO_2 of the culture medium are monitored using a blood gas analyzer (Rapidlab 860; Bayer) and the values are adjusted, using a continuous flow (20 mL/min) of 5% CO_2 gas (95% air) into the reservoir.

For electrical stimulation, two carbon rod electrodes were placed in the bioreactor, 1 cm apart (Fig. 3A). The electrodes were placed in grooves made in the fixing nets to hold them in place and to allow bioreactor assembly. The carbon electrodes occupied <10% of the total bioreactor area and their placement did not interfere with the laminar medium flow. The carbon electrodes were connected to a stainless steel rod by a metal wire covered with standard heat-shrink tubes on both electrodes (Fig. 3B). The metal rod was fixed to the

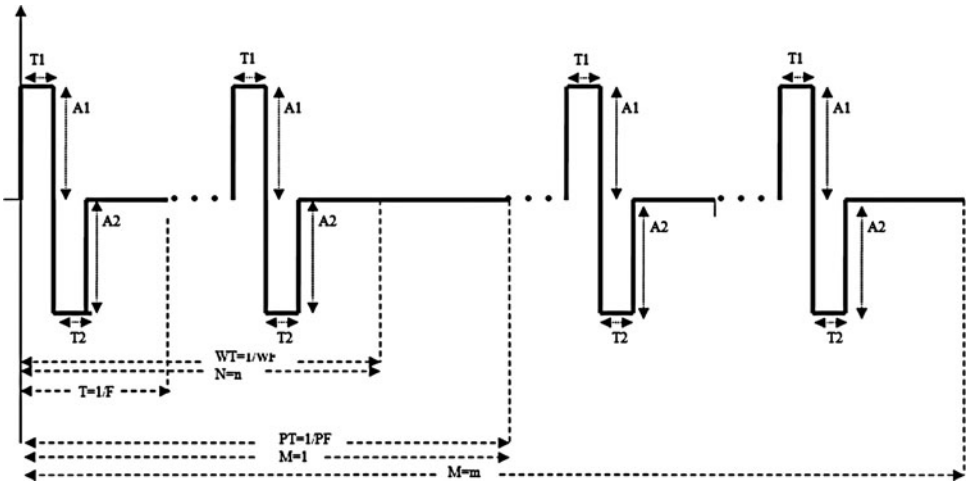

FIG. 1. Stimulation waveform. Parameters are described in Table 1.

Table 1. Description of the Waveform Parameters and the Attainable Parameter Range

Value	*Implication*	*Attainable range*
PT	Period time	1–1024 min
T1, T2	Positive and negative pulse durations	0.1–40 ms
WT	Working time—duty cycle of one burst period time	0%–100%
A1, A2	Positive and negative pulse amplitudes	0–15 V
T	Reciprocal frequency—time between pulses	0.1–40 Hz
M	Number of periods (PTs) in the process	1–1024

bioreactor and sealed through a Teflon cone and stainless steel conic screw (Fig. 3C).

Cell seeding and cultivation

Ventricular cardiomyocytes were isolated from 1- to 4-day-old neonatal Sprague-Dawley rats, using six cycles of enzyme digestion, as previously described in detail.[3] The cardiac cells were seeded onto alginate (LVG; FMC Biopolymers) scaffolds, with a diameter of 5 mm and a thickness of 2 mm, prepared using the freeze–dry technique.[9] The scaffolds were >90% porous, with interconnected pores and pore sizes in the range of 50 to 200 μm in diameter, according to scanning electron microscopy. The cells were suspended in an ice-cold mixture of 30% Matrigel (BD Biosciences)/M-199 (Biological Industries) and were seeded onto the scaffolds, at a density of 1×10^8 cells/cm^3 by dropping 20 μL of the cell suspension on top of the dry scaffolds, in 96-well plates. After seeding, the plates were placed in a plate-holder-type rotor (Labofuge GL) and centrifuged (150 *g*, 2 min, 4°C). After 10 min of incubation (37°C, 5% CO_2) 200 μL of the culture medium (cold M-199 supplemented with 0.6 mM $CuSO_4$•$5H_2O$ [Sigma-Aldrich], 0.5 mM $ZnSO_4$•$7H_2O$ [Sigma-Aldrich], 500 U/mL penicillin and 100 μg/mL streptomycin [Biological Industries], and containing 5% [v/v] fetal calf serum [Biological Industries]) (CM+) was added to each well, and after 2 h of incubation, the cell constructs were transferred to 12-well plates, supplemented with 2.5 mL CM+, and incubated for 48 h under static conditions and with no electrical stimulation.

Electrical stimulation

Electrical stimulation under static cultivation conditions in Petri dish. The carbon rod electrodes (Graphite 3.05 mm; Alfa Aesar) were inserted into Delrin holder (Fig. 3D, E), equipped with needles to immobilize the cell construct in place (Fig. 3F). Carbon electrodes were chosen due to the material high resistance to corrosion.[10] For cultivation under electrical stimulation, the cardiac cell constructs were transferred into a 100 mm Petri dish containing 30 mL of the culture medium and immobilized onto the Delrin holder. The cell constructs were cultivated in a cell incubator (37°C, 5% CO_2), under electrical stimulation (5 V, bipolar, 2 ms pulse, 1 Hz).

Electrical stimulation in perfusion bioreactor. The cell constructs were placed in the bioreactor following 48 h of static cultivation; 35 scaffolds per 500 mL medium. The perfusion bioreactor was operated at the specified flow rate of 25 mL/min. Electrical-stimulated cell constructs were considered those constructs that are placed in-between the two carbon electrodes.

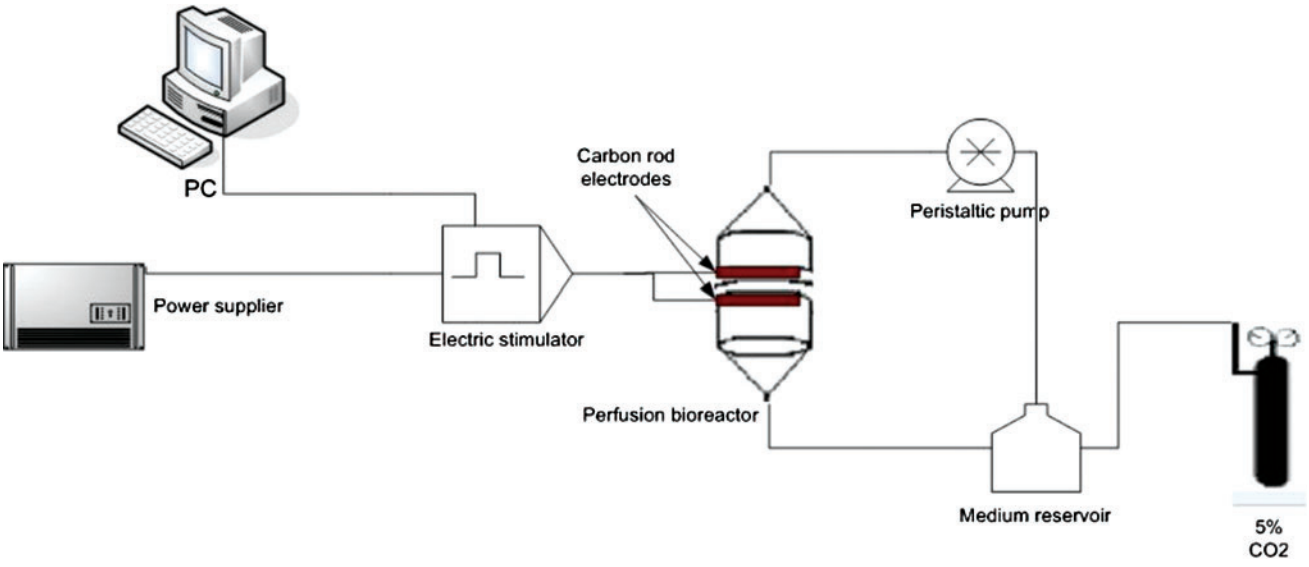

FIG. 2. Bioreactor setup. Color images available online at www.liebertonline.com/ten.

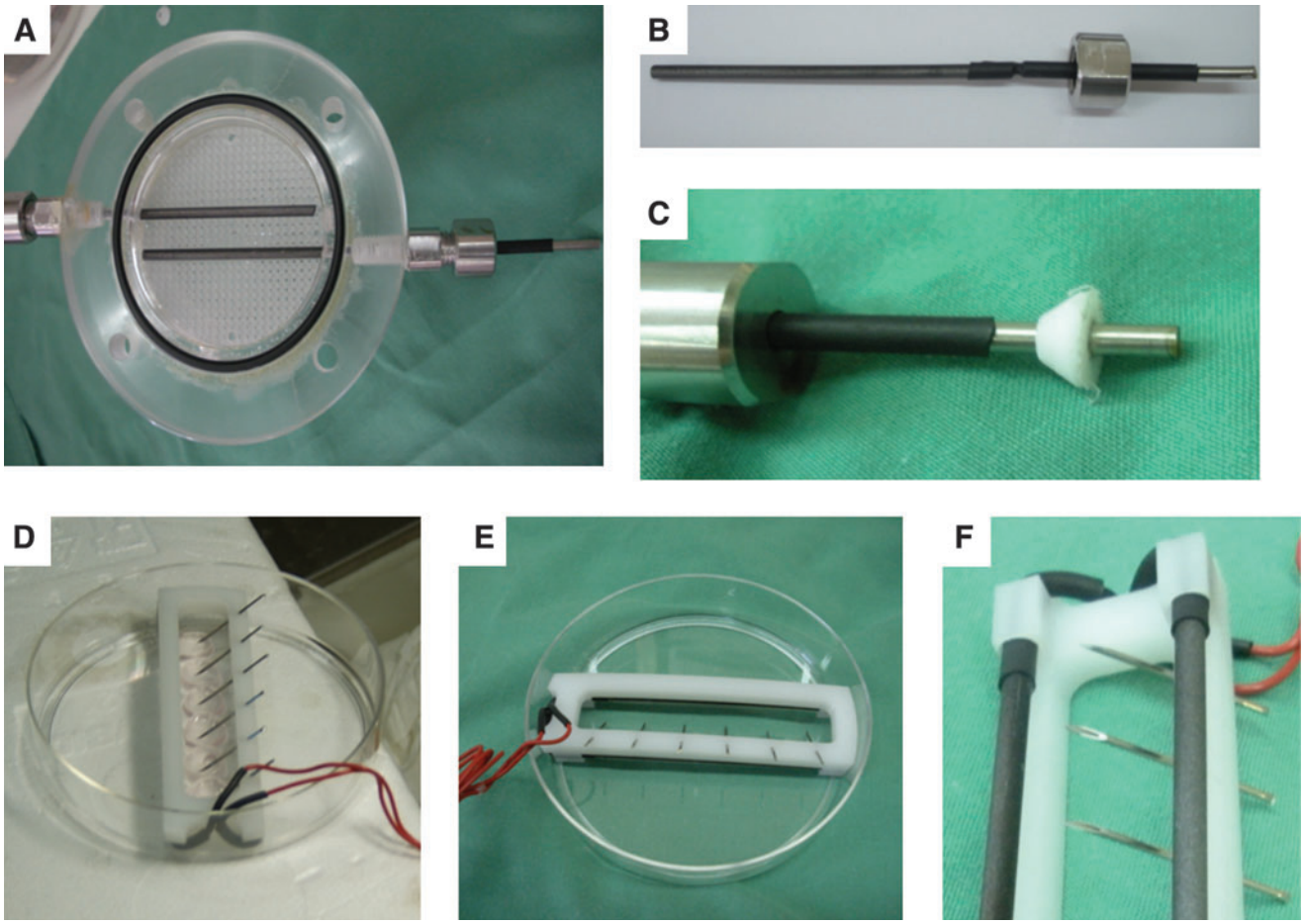

FIG. 3. Configuration of the carbon electrodes in the different cultivation systems. (**A**) The carbon electrodes integrated into the bioreactor. (**B**) Standard heat-shrink tube and SS conic screw for insulation. (**C**) Teflon cone for insulation. (**D**) Electrical stimulation of cell constructs in the Petri dish using the Delrin holders. (**E**) Delrin holders integrated with the carbon electrodes. (**F**) The needles for fixing the cell constructs. Color images available online at www.liebertonline.com/ten.

Cell viability assay

One milliliter of 50% Alamar blue reagent (AlamarBlue; Invitrogen Corp.) diluted in warmed (37°C) CM+ medium was added to the cell construct in a 24-well plate. The cell constructs were incubated for 2 h in the dark, and then a sample of 250 μL of the cell medium was removed and fluorescence was determined using a plate reader (synergy HT Microplate Reader; Biotek Instruments), at 528 nm excitation and 590 nm emission.

Immunostaining and confocal microscope imaging

The cardiac cell constructs were fixed and permeabilized in cold methanol for 10 min, washed three times with Dulbecco's modified Eagle's medium–based buffer ($CaCl_2$•$2H_20$ [1.8 mM], KCl [5.36 mM], $MgSO_4$•$7H_2O$ [0.81 mM], NaCl [0.1 M], $NaHCO_3$ [0.44 mM], NaH_2PO_4 [0.9 mM], pH 7.4), and then blocked for 1 h, at room temperature, in a Dulbecco's modified Eagle's medium–based buffer containing 5% (v/v) fetal calf serum. After three washes with buffer, the samples were incubated overnight with two primary antibodies to detect α-actinin (1:450; Clone EA-53 [mouse]; Sigma) or Cx-43 (1:100, rabbit). The samples were then washed three times and incubated for 1.5 h with goat anti-mouse Alexa 488–conjugated antibodies (1:150; Molecular Probes, Invitrogen) or with goat anti-rabbit Alexa 548–conjugated antibodies (1:150; Molecular Probes, Invitrogen). For nuclei detection, the cell constructs were incubated for 5 min with To-Pro (To-Pro 3 Iodide; Invitrogen) and washed three times. Samples were observed using a laser scanning confocal microscope (Olympus FV1000 Confocal Microscope); optical sections were <1 μm.

Western blotting

For western blot analysis, the cellular constructs were dissolved with citrate buffer (4% [w/v]; pH 7.4), followed by 5 min centrifugation (8000 *g*, 4°C), after which the supernatant was removed. Total protein was extracted from the cell pellets using lysis buffer (10% glycerol, 25 mM NaCl, 50 mM NaF, 10 mM Na pyrophosphate, 2 mM EGTA, 2 mM DTT, 20 mM P-nitro-phenyl-Pi, 25 mM Tris-HCl, 2 mM Na_2VO_4, 100 μM PMSF, 10 μg/mL leopeptin, and 0.1% Triton ×100, all chemicals from Sigma-Aldrich), followed by centrifugation for 20 min (17,000 *g*, 4°C). The total protein concentration in supernatant was evaluated using the Bradford protein assay (Bio-Rad). Total extracted proteins were size-fractionated by

sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Schleicher & Schuell). The blotted membranes were incubated with antibodies against Cx-43 (1:1000; Cell-Signaling) or glyceraldehyde 3-phosphate dehydrogenase (1:1000; Cell-Signaling). Densitometry analysis was carried out using a Phaser 3300 MFP scanner and ImageJ software (National Institutes of Health; http://rsbweb.nih.gov/ij/).

Electric field models

The electric field was modeled using Comsol Multiphysics software (www.comsol.com). The exact geometries of the stimulated cultivation systems were employed, and DC and steady state were assumed. The following assumptions are valid. (a) Steady state can be assumed since the pulse duration for stimulation is much shorter than the time constant of the electrode–electrolyte analog electric circuit[10] (2 ms as compared to 1.9 s[11]), that is, before charging the capacitor of the medium–electrode interface. (b) With the uncharged capacitor, the circuit can be simplified to a source and a resistor (zero-order electric circuit) and thus can be considered at steady state. DC simulation will give the same total current value as a transient simulation with a rectangular voltage wave during stimulation. The governing equation of the model is the electric current analog of the Poisson's equation:

$$-\nabla(\sigma\nabla V - J^e) = Q_j \tag{1}$$

The solution of Equation 1 defines the local electric potential (V in the equation), J^e represents an external current density (which is zero in the model's physical conditions), and Q is the local current source (which is also zero except in the combination of this model with the cell excitation model; see below). The solution is given by applying insulating boundary conditions on all solid parts except the electrodes, and electric potential values (ground and referenced potential) on the electrodes.

The unknown variable in this simulation is the electric potential throughout the medium volume. From this variable, the electric field (E in Eqs. 2 and 3) and current density (J in Eq. 3) are derived. The local electric field is the gradient of the potential (V in Eq. 2), while the current density is linearly correlated to the electric field by Ohm's law (Eq. 3).[12]

$$\vec{E} = -\nabla V \tag{2}$$

$$\vec{J} = \sigma \cdot \vec{E} \tag{3}$$

The media conductivity (σ in Eq. 3) value of 1.4 S/m (72 Ωcm) was taken from formerly published values.[13]

Results

Electric field models

We formulated an electric field model to enable the determination of the optimal stimulation parameters to be applied in a perfusion bioreactor for inducing cardiac cell contraction. These parameters could not be directly determined experimentally due to the translucent texture and the complex structure of the bioreactor vessel. The model enabled simulation of the exact stimulation conditions (i.e., current density and electric field), under flow and static conditions in the bioreactor and the Petri dish, respectively.

Petri dish modeling. At first, the parameters of the electric field applied to the cell constructs located between two electrodes were evaluated under static conditions in a Petri dish. In this system, cell stimulation by the electrical field and the consequent contraction response were directly viewed under a microscope, enabling determination of the capture threshold.

When modeling, the configuration of the electrical stimulation in the cultivation vessel (Fig. 4A) was simulated using Comsol software (Fig. 4B). The cell construct was modeled as part of the medium due to its high, over 95%, medium content. Results of solving the model (Fig. 4C–E) describe the current density in the area of cell construct location and confirm the uniformity of the electric field, as revealed by comparing the three sections of the orthogonal current components. The color bars define the magnitude of the current density in the specified direction; the current density in the x (Fig. 4C) and z (Fig. 4D) directions have zero value where the cell constructs are located, and the current density in the y direction (Fig. 4E) has a uniform value between the electrodes in this area. As a consequence, the sum vector of these three components, as illustrated by the total electric field (Fig. 4F), is uniform in the direction between the electrodes. The current density value in the model results was 12.4 mA/cm^2 for 1 V between the electrodes and 74.4 mA/cm^2 when the applied voltage in the model was 6 V, which is the experimental threshold voltage in the Petri dish configuration.

Perfusion bioreactor modeling. The electric field in the perfusion bioreactor was modeled by applying the same assumptions and governing equations as for the Petri dish, while taking into account the configuration of the electrode and bioreactor setup. The exact configuration of the bioreactor and the carbon rod electrodes were built in Comsol software (Fig. 5A). Solving the model gave the value of the current density inside the cell constructs and further confirmed its uniformity. Figure 5B, C presents the results of the mathematical modeling of the electrical field in the bioreactor, as a two-dimensional section showing no field in the y and z axes. The x component of the model (Fig. 5D) shows a uniform electric field and current density of 14.5 mA/cm^2 for 1 V between the electrodes. Thus, cell constructs that are placed in between the two carbon electrodes are stimulated by a uniform electrical field, while those in the outer regions of the bioreactor are not subjected to electrical stimulation and can serve as nonstimulated internal control.

Validation of the models. To evaluate the potential of the two models in predicting the total current and by that to confirm the magnitude of the current density in the model, current–voltage measurements were conducted for different stimulation setups while the current value being measured on 1 ms from the start of the 2 ms rectangular pulse. The applied voltage was varied (0.5, 1, 2, and 5 V) in both cultivation vessels. In addition, the distance between the electrodes (0.6, 1, and 1.5 cm) and the medium volume (12.5, 25, and 50 mL) were changed between measurements in the Petri dish. These stimulation conditions were all simulated in the computer models and compared to the measured values (correlation coefficient $\rho = 0.992$) (Fig. 6).

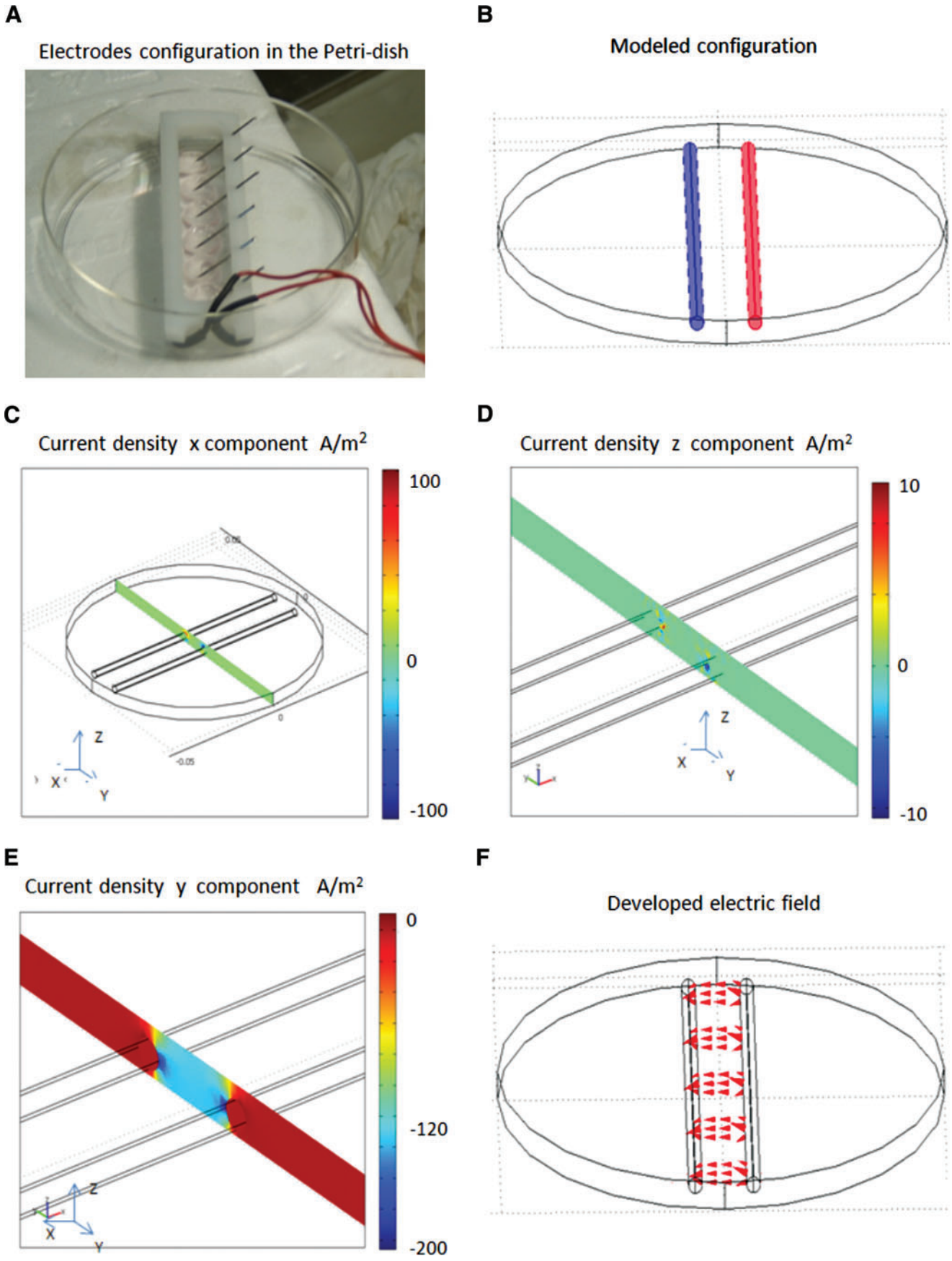

FIG. 4. The electrode configuration and 3D electric field model in the Petri dish. (**A**) The system configuration. (**B**) The system simulation as seen in the software. (**C–E**) The current density on a representative cross section in the x (**C**), z (**D**), and y (**E**) directions (1 V between electrodes). (**F**) The uniform electric field between the electrodes. 3D, three-dimensional. Color images available online at www.liebertonline.com/ten.

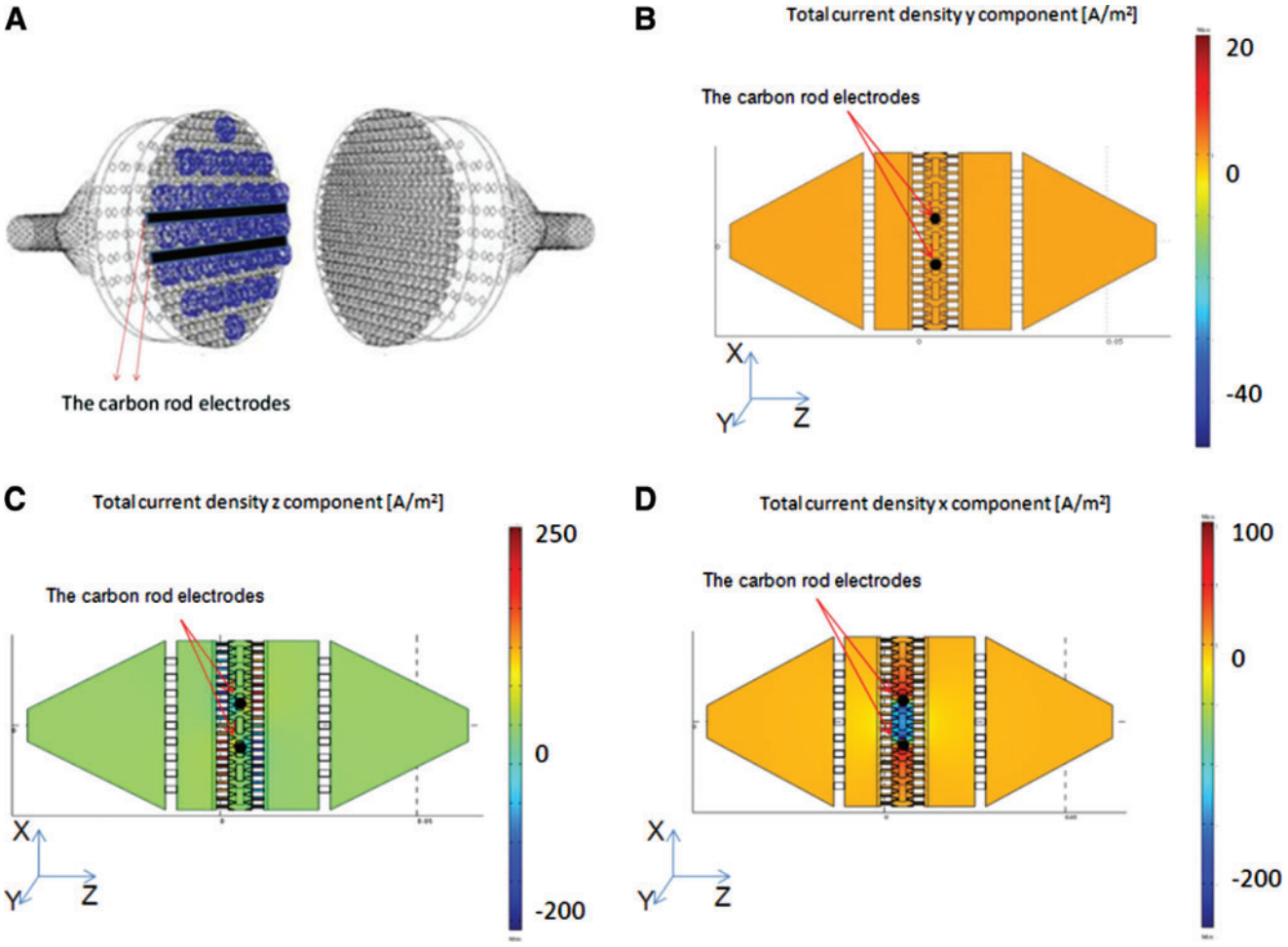

FIG. 5. The electrode configuration and 3D electric field model in bioreactor. (**A**) An illustration of the 3D configuration with the carbon electrodes in black and the cell constructs in blue. (**B–D**) The current density in the y (**B**), z (**C**), and x (**D**) directions (1 V between the electrodes). Color images available online at www.liebertonline.com/ten.

The total current between the electrodes was evaluated in the model by the integral of the x component of the current density on an x normal plane between the two electrodes. The feature of boundary integration on the electrode for evaluating total current was found to be much less accurate and more sensitive to small changes in geometry. This can be explained by the fact that the current density relates to the voltage derivative, and thus small changes in the voltage or geometry near the electrode can have a significant effect on the derivative.

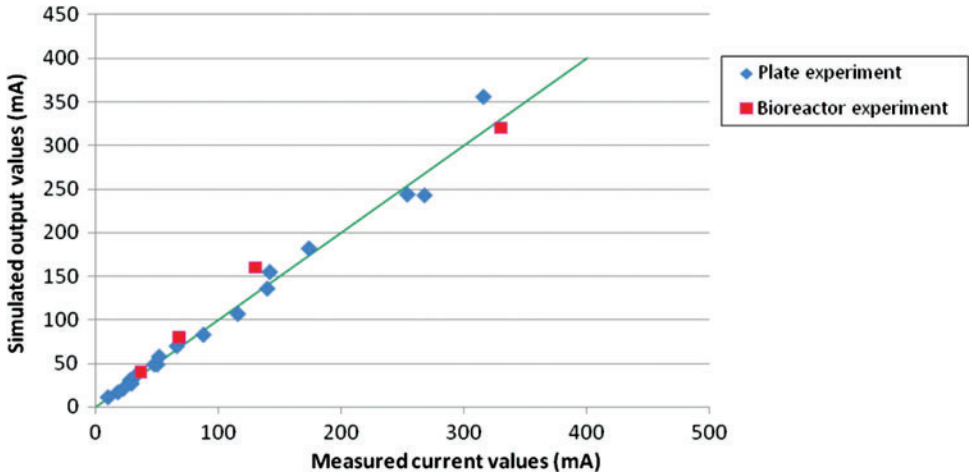

FIG. 6. Comparison between modeled and measured current values on different stimulation amplitudes in the bioreactor and different stimulation amplitude, electrode distance, and medium volume in the Petri dish. The straight line represents perfect correlation. Color images available online at www.liebertonline.com/ten.

Cell morphology and Cx-43 expression in stimulated cell constructs

The effect of a homogenous electric field in the perfusion bioreactor on the outcome of cardiac cell constructs was evaluated in terms of cell elongation and Cx-43 expression level. These two parameters have been previously shown to be affected by electrical stimulation and are important for cardiac tissue assembly and synchronous contraction.[8] Thus, electrical stimulation was applied for 4 days (74.4 mA/cm^2, 1 Hz, bi-polar, 2 ms, 80% duty cycle) on cardiac cell constructs in a perfusion bioreactor. As revealed by immunostaining for α-sarcomeric actinin, cells subjected to both electrical and mechanical stimulation (applied by medium perfusion in the bioreactor) present a more elongated and striated morphology than the cells cultivated in the bioreactor with no electric field stimulation (Fig. 7A, B).

Immunostaining of anti-Cx-43 (Fig. 7C, D) revealed higher levels of the protein in the cells that were subjected to both mechanical and electrical stimuli in the bioreactor, as compared to cultivation in the bioreactor with no electrical stimulation. These results were later supported by semi-quantitative analysis assessed by Western blot, which showed twofold higher expression of Cx-43 in cultures subjected to both mechanical and electrical stimuli (Fig. 7E) in comparison to cultures receiving mechanical stimuli alone.

Discussion

Various *ex vivo* approaches have been proposed for engineering of cardiac patches as scar replacements following myocardial infarction.[14–18] With the great progress made in recent years, there are still two major challenges: obtaining synchronously contracting myocardial tissue and increasing the graft thickness. In this article, electrical field stimulation and medium perfusion were combined into one cultivation vessel to produce a functional cardiac patch.

We first designed and built an electrical stimulation system that enabled greater degrees of freedom over the stimulation waveform parameters (duty cycle, different pulse duration for positive and negative amplitudes, adding more independent channels, etc.) in comparison to existing systems. Then, it was needed to determine the stimulation threshold required to induce cell contraction, which is dictated by the extent of the electric field outside the cell.[19] This information could not be simply obtained from literature. Although previous studies have reported the value of stimulation amplitude in terms of electrical field units (V/cm), this value, in fact, described the stimulation amplitude divided by the electrode distance. Thus, unless using the exact system configuration, the current density, that is, the parameter that should be kept constant between different experiments, could not be extrapolated from these studies.

In the complex configuration of the perfusion bioreactor, direct measurements of the electric field and observation of cell contraction are difficult to achieve, and thus optimization could not be performed. Thus, a new strategy was employed, consisting of two stages. At first, the stimulation threshold for inducing a synchronous contraction in the cell constructs was determined in a Petri dish under a microscope, by trial and error. Then, computer models of the electric fields (and current density) inside the bioreactor and in the Petri dish were created. The models were validated by voltage–current measurements and confirmed the uniformity of the electric field in the two cultivation vessels. The model enabled matching the stimulation conditions in bioreactor to those in

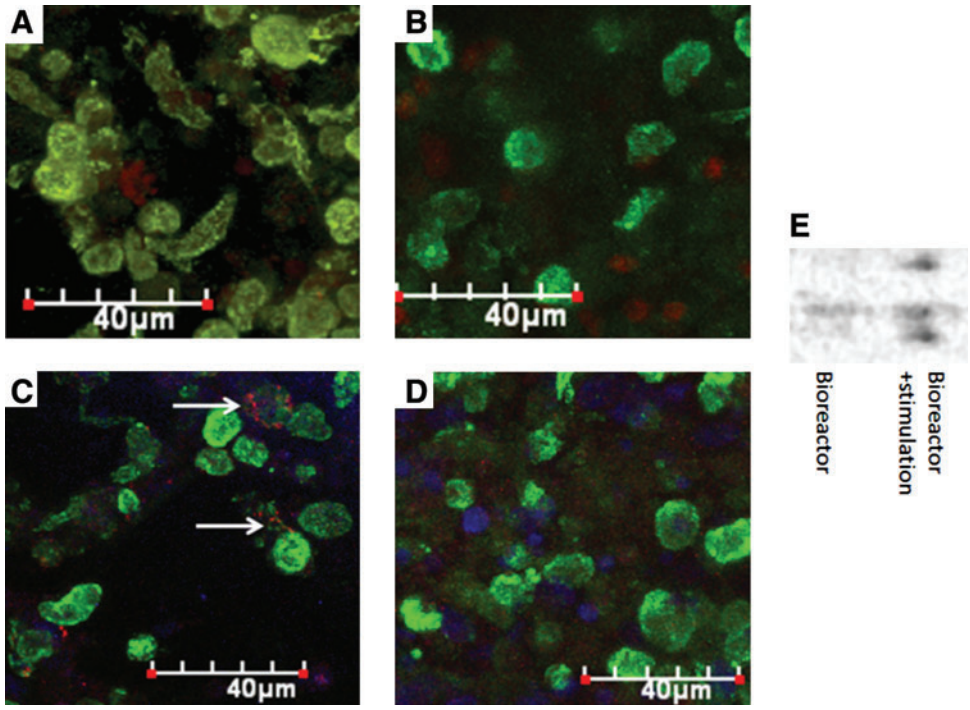

FIG. 7. The effect of the electrical stimulation on cell morphology and Cx-43 levels. (**A, B**) Confocal microscopy images of anti-α-sarcomeric actinin immunofluorescence (green) of the cell constructs in the bioreactor with (**A**) or without (**B**) electrical stimulation. To-Pro (To-Pro 3 Iodide) was used for nuclear staining (red). The electrical-stimulated cell constructs are those placed in between the carbon electrode. (**C, D**) Confocal microscopy images of anti-Cx-43 (red), anti-α-sarcomeric actinin (green) immunofluorescence of the cell constructs in a bioreactor with (**C**) or without (**D**) electrical stimulation. The white arrows indicate positive staining of Cx-43 between adjacent cells. To-Pro was used for nuclear staining (blue). The electrical-stimulated cell constructs are those placed in between the carbon electrode. (**E**) Representative Western blot analysis for Cx-43 expression after 4 days of cultivation in the bioreactor with or without electrical stimulation. Cx-43, Connexin-43. Color images available online at www.liebertonline.com/ten.

the Petri dish, that is, by adjusting the voltage amplitude in the bioreactor for achieving the same current density around the cardiac cell constructs. Current density should be preserved for achieving similar stimulation conditions from one experiment to another. In the Petri dish, a successful stimulation of the cell construct was achieved at 6 V with a current density of 74.4 mA/cm^2, whereas in the bioreactor, with the carbon rod electrodes, 5 V was sufficient to achieve the same current density. The successful electrical stimulation of the cell constructs in the perfusion bioreactor was confirmed by the presence of elongated/striated cells as well as the greater Cx-43 expression level compared to constructs cultivated in perfusion bioreactor without electrical stimulation.

The native heart consists of elongated and well-striated cardiomyocytes that are aligned in the same direction, contributing to the heart anisotropic (direction-related) structure and facilitating efficient electric and mechanical activation of the ventricles.[20] The minimum stimulation threshold is reduced for cells that are situated in parallel to the electric field; thus, cardiac cell elongation induced by electrical stimulation should be accompanied by cell alignment.[21] Radisic *et al*. adopted this concept for cardiac tissue engineering performed under static cultivation conditions, showing that stimulation with a uniform electric field can induce an alignment in the engineered tissue. We believe that our combined perfusion/electrical stimulation bioreactor offers an advanced cultivation tool with a potential to create thick, functional cardiac tissue. In addition, the electric field models formulated herein are beneficial to illustrate the local stimulation conditions in vessel and to enable the same threshold conditions in cultivation systems with different geometry. The model can be then confirmed for any new configuration by external current–voltage measurements.

Conclusions

We describe herein the features of a novel cultivation system, combining electrical stimulation with medium perfusion with the potential of producing thick, functional cardiac patches. The cell constructs were subjected to a homogenous fluid flow regime and electrical stimulation under conditions optimal for cell excitation, provided by a custom-made stimulator. The stimulation threshold in bioreactor was set up by a new strategy including the construction of electrical field models. The models illustrate, for the first time, the local electric conditions required for cardiomyocyte field excitation and they confirmed the uniformity of the electrical field around the cell constructs. The models developed herein can be applied to different cultivation vessels, with different geometries, while maintaining the current density constant from one vessel to another.

Acknowledgments

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Disclosure Statement

No competing financial interests exist.

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