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Patterned Cardiomyocytes on Microelectrode Arrays as a Functional, High Information Content Drug Screening Platform

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Abstract

Cardiac side effects are one of the major causes of drug candidate failures in preclinical drug development or in clinical trials and are responsible for the retraction of several already marketed therapeutics. Thus, the development of a relatively high-throughput, high-information content tool to screen drugs and toxins would be important in the field of cardiac research and drug development. In this study, recordings from commercial multielectrode arrays were combined with surface patterning of cardiac myocyte monolayers to enhance the information content of the method; specifically, to enable the measurement of conduction velocity, refractory period after action potentials and to create a functional reentry model. Two drugs, 1-Heptanol, a gap junction blocker, and Sparfloxacin, a fluoroquinone antibiotic, were tested in this system. 1-Heptanol administration resulted in a marked reduction in conduction velocity, whereas Sparfloxacin caused rapid, irregular and unsynchronized activity, indicating fibrillation. As shown in these experiments, patterning of cardiac myocyte monolayers solved several inherent problems of multielectrode recordings, increased the temporal resolution of conduction velocity measurements, and made the synchronization of external stimulation with action potential propagation possible for refractory period measurements. This method could be further developed as a cardiac side effect screening platform after combination with human cardiomyocytes.

Keywords

Biosensor; Cardiac tissue engineering; Cardiomyocyte; Electrode; In vitro test; Micropatterning

INTRODUCTION

The development of a high-throughput, high-information content device to study and understand cardiac electrophysiology would be important for the fields of cardiac

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physiology, tissue engineering and drug research. More than 850,000 people are hospitalized for arrhythmias each year and ventricular fibrillation (VF) is a leading cause of cardiac death[1]. Despite the intensive research in this area, the mechanism of VF is still poorly understood[2–5].

Arrhythmia is a known side effect of commercial drugs. One of the mechanisms by which drugs can cause a potentially fatal form of ventricular tachy arrhythmia, called Torsades de pointes (Tdp), is through the prolongation of the QT interval (in an ECG the length of the ventricular action potential). It has been reported that approximately 2–3% of all prescribed drugs can cause long QT syndrome[6, 7]. A broad range of cardiovascular drugs and antibiotics also have the potential risk of causing drug induced Tdp[8, 9]. At the same time, prolongation of the QT interval does not necessarily lead to Tdp; lengthening of the QT interval could even be anti-arrhythmogenic, as it is considered a mechanism of action of the class III anti-arrhythmics[8, 9]. Thus, a relatively high-throughput method to identify cardiac side effects and differentiate between arrhythmic and anti-arrhythmic effects at an early stage of drug development would have a significant impact on the field.

Gap junctions play an important role in the propagation of excitation in cardiac tissue. Changes in gap junction function affect major cardiac parameters, such as conduction velocity (CV). It has been observed in several cardiovascular diseases that the expression of connexins (protein molecules that form gap junction channels) is decreased or their distribution is changed, leading to a malfunction in gap junction coupling[10]. Understanding the pharmacological modulation of cardiac gap junction channels would further aid the drug development enterprise.

Introduction of an in vitro method for cardiac side effect testing, which has high predictive value, would have a significant impact on drug development as it could also reduce the cost, time and the number of drugs failing in clinical trials[11]. In vitro testing would also reduce the need for animal testing and could be used to study drug effects with a functional assay, but at the cellular level. Other in vitro methods, such as whole heart experiments (Langendorff heart model) or the Purkinje fiber preparation are difficult and time consuming[11]. Traditional methods used to study QT interval prolongation at the cellular level include patch-clamp experiments. However, these experiments are time intensive, require a skilled operator and cannot be used to study action potential (AP) propagation or parameters such as CV and re-entry. Moreover, evidence suggests that prolongation of QT intervals is not the best predictor of Torsades de pointes. The measurement of the length, or the variability in the length, of the refractory period after a cardiac action potential may have more relevance for predicting arrhythmic behavior[9].

Cardiac myocytes cultured on microelectrode arrays (MEA) have several benefits compared to either traditional patch clamp electrophysiology or isolated organ methods. The use of MEA's in the investigation of cardiac side effects would provide information in a relatively high-throughput and low cost manner compared to standard patch-clamp electrophysiology. However, at this time, it is still a low information content method and this has limited its use. Cardiac myocytes on MEAs have been used in a number of studies to investigate the effect of toxins, such as pesticides[12] and cardioactive drugs[13] on cardiac field potentials. A commercial system has also been introduced to measure QT intervals in a relatively high-throughput fashion[14], but, to date, it has only limited applications. However, cardiac myocytes can now be maintained over longer periods of time[15], thus chronic experiments, such as the monitoring of network remodeling for specific diseases, is now feasible. In addition, serum-free formulations for cardiac culture have also been introduced, which would increase the reproducibility of such a system[16].

All of the above mentioned studies utilized unorganized monolayers of cardiomyocytes on the MEAs. Development of a patterned cardiac myocyte layer that is aligned with the electrodes of a MEA could solve several problems associated with the random spread of excitation in a cardiac monolayer, which makes evaluation of the obtained data, such as CV, difficult. It would also enable the development of specific open-loop or closed-loop stimulation protocols to measure critical parameters, such as the length of the refractory period after the action potential. It could also be used to create a high-throughput, low-cost functional reentry model.

There are several lines of evidence indicating that not only contact interaction with the surface but the shape of the attachment area determines the physiology of cardiac myocytes[17]. Pattern geometries determine the extent of the alignment of the long axis of cardiac myocytes, alignment determines CV[18] and other physiological and pharmacological properties of cardiac tissues[19, 20].

Several different methods have been developed for cell patterning. One category of this technique is based on direct placement of cells or extracellular matrix molecules on desired locations and includes patterning through microfluidic channels[21–23], microcontact printing[24, 25] and inkjet printing[26]. Cardiac myocytes have previously been patterned on glass using photoresist[27] as well as other techniques[15, 17, 19, 20, 25]. Another method utilized photolithography following surface modification with self-assembled monolayers (SAMs) for neurons[28–30] as well as myocytes[15, 31]. The benefit of this method is the compatibility of the technique with cheap automated silicon manufacturing steps and the ability of the cells to self-assemble after random plating.

SAMs are one molecule thick monolayers attached to a surface composed of organic molecules, which have been extensively used for surface patterning[31–33]. Surface modification with SAMs is also compatible with advanced photolithography methods[30, 34]. Studies have also shown that cells survive on these surfaces for extended periods of time[35, 36], do not migrate off the patterned areas[34] and exhibit the typical morphology and physiology of the specific cell type[16, 37].

The goal of this study was the development of patterned, rat, cardiomyocyte cultures on MEAs in a serum-free medium for the study of cardiac physiology and pharmacology utilizing a high-throughput technique, but with high information content. An adsorbed fibronectin layer was used as the foreground because it supported cardiac myocyte attachment and growth and a 2-[Methoxy(Polyethyleneoxy) Propyl]TrimethoxySilane (SiPEG) SAM was used as the cell repellent background because of its excellent protein adsorption resistant properties[38]. The measurement of CV with the patterned cardiac myocyte monolayers and the feasibility to apply different stimulation protocols to the MEA/cardiac system was demonstrated. The action of 1-Heptanol and Sparfloxacin was also assessed.

MATERIALS AND METHODS

Experimental

Surface modification of microelectrode arrays with PEG silanes—MEA's containing 60, 10 μm diameter electrodes (Multichannel Systems, Germany) were cleaned by soaking the arrays in a detergent solution for 2 hours followed by sonication for 10 minutes. The arrays were then oxygen plasma cleaned for 20 minutes. Surface modification was completed by incubation of the MEAs in a 3 mM PEG silane, 2-[Methoxypoly(ethyleneoxy)propyl]trimethoxysilane (MW = 460–590, Gelest), solution in toluene, with 37% concentrated HCL added to achieve a final value of 0.08% (0.8 ml HCL/

L), for 45 minutes at room temperature. The arrays were then rinsed once in toluene, twice in ethanol, twice in water and sonicated in water for 2 minutes to remove the non-covalently linked material[39]. The arrays were air dried with nitrogen and stored in a dessicator overnight.

Laser ablation and patterning of the microelectrode arrays—The MEAs were patterned using a deep UV (193 nm) excimer laser (Lambda Physik) at a pulse power of 230 mW and a frequency of 10 Hz for 45 seconds through a quartz photomask (Bandwidth foundry, Eveleigh, Australia). The arrays were sterilized using 70% isopropanol and then incubated with 5 μg/ml of fibronectin in a Phosphate buffered solution (Invitrogen) for 20 minutes at room temperature. The solution was removed and the surface was first rinsed with PBS, followed by the plating medium, and then dried before the cells were plated.

Neonatal rat cardiomyocyte culture—The neonatal rat cardiomyocyte culture was prepared using the cardiac isolation kit from Worthington[40]. All animal work was approved by the UCF IACUC and followed NIH guidelines. Briefly, two day-old rat pups were euthanized in a precharged CO_2 chamber. Hearts were dissected and minced in ice cold Hanks balanced salt solution (HBSS). Cardiac myocytes were dissociated by incubation of the hearts in trypsin (100 μg/ml in HBSS) for 16 hours at 2–8°C. The hearts in the trypsin solution were briefly warmed with a trypsin inhibitor before adding collegenase (300 units/ml in L-15 medium) for 45 minutes in a water bath at 37°C followed by mechanical trituration. The cell solution was filtered to remove any remaining tissue and centrifuged at 50g for 5 minutes at 22°C. The cells were resuspended in high glucose Dulbecco's modified eagle medium (DMEM, (Gibco/Invitrogen) supplemented with 10% Fetal Bovine Serum (Gibco/Invitrogen) and 1% penicillin streptomycin (Gibco/Invitrogen), preplated in Petri dishes and incubated at 37°C and in 5% CO_2 for 45 minutes. This was necessary to eliminate the fibroblasts. The supernatant from the Petri dishes was centrifuged at 50g for 5 min at 22°C. The cells were then resuspended in the plating medium. The serum-free plating medium consisted of: 100 ml Ultraculture medium (Bio Whittaker Cambrex) supplemented with 10 ml B27, 1 ml L-glutamine (Gibco/Invitrogen), 1 ml Penicillin Streptomycin, 0.375 g dextrose (Fisher Scientific) in 800 μl water, 1 ml non-essential amino acids and 1 ml of Hepes buffer (Gibco/Invitrogen)[41]. Additional growth factors were also added to improve cell survival in the serum-free conditions. They included 0.1 μg/ml of L-thyroxine, 10 ng/ml of Epidermal growth factor (Sigma-aldrich) and 0.5 μg/ml of Hydrocortisone (BD biosciences). Cells were plated at a density of 1000 cells/mm2 on the MEAs. The medium was changed 24 hours after plating. Subsequent changing of the medium was performed every third day.

Immunostaining—The full paragraph should be (the missing text is bolded and underlined) - Patterned Cardiomyocytes were immunostained for F-Actin with Rhodamine Phalloidin (Invitrogen, R415), using a protocol provided by the company. Briefly, the cells were washed with PBS and fixed using 3.7% Formaldehyde. The coverslips were extracted with 0.1% Triton X. The staining solution (with 1% Bovine Serum Albumin to prevent background staining) was added at a dilution of 1:40 in PBS and coverslips were incubated for 30 minutes. Imaging was done using confocal microscopy.

Multielectrode extracellular recordings—The cardiac myocytes were cultured on patterned metal MEAs (Planar 10 μm electrodes, 200 μm separation, Multichannel-systems). A 60 channel amplifier (MEA1040, Multichannel-systems) was used to record electrical activity from the spontaneously beating cardiac cells. The same electrodes were also used for stimulation utilizing a stimulus generator (STG 1002, Multichannel systems). The cells were stimulated utilizing 500 mV, 1 ms wide bipolar pulses at 2 Hz. The recording medium

was the same as the plating medium with the pH adjusted to 7.3 using HEPES buffer. After a 30 minute incubation period, APs were detected and recorded using built in functions of the Multichannel System software. For drug experiments, 50 μM 1-Heptanol (Gibco/Invitrogen) was added to the bathing medium and recordings were performed before and 15 minutes after drug administration with additional recordings done at 15 minute intervals. For Sparfloxacin (Sigma-aldrich), 2 μM of the drug was added to the recording medium and recordings were taken in 15 minute intervals before and after drug administration. The data was further analyzed using software written using Matlab and Clampfit (Axon instruments).

RESULTS

Surface modification of the microelectrode arrays

Our laboratory routinely uses SAMs to modify and pattern glass coverslips for cell culture applications[15, 16, 30–32, 42]. However, patterning cardiac myocytes on MEAs presented a challenge for three primary reasons: 1) The complex composition of the MEA surface made the verification of the surface modification step difficult, 2) MEAs are expensive, thus methods needed to be developed that enabled them to be cleaned and refunctionalized for repeated use and 3) patterned cardiac myocytes do not grow optimally on the standard trimethoxysilylpropyldiethylenetriamine (DETA) surfaces[15] nor on clean glass. Thus, a cell resistant background surface was needed which was also resistant to protein adsorption as this would allow fibronectin incubation on the foreground and prevent it's adherence to the background, enabling cardiac myocyte attachment. Thus, poly(ethylene glycol) (PEG) was chosen for the background because of its excellent protein adsorption resistance. X-ray Photoelectron Spectroscopy (XPS) and contact angle measurements were used to analyze the results of the surface modifications and throughout the entire study for quality assurance purposes (See supplementary data).

Cultured neonatal rat cardiomyocytes on patterned surfaces in serum-free medium

The growth and activity of neonatal rat cardiomyocytes on the patterned MEAs was assessed for over 2 weeks. The cells attached to the fibronectin, but not to the PEG background and showed clearly delineated regions (Figure 1). This allowed the beating cells to grow exclusively over specific electrodes. The cells formed monolayers by day 2 and spontaneous contraction and beating activity began on day 4 and was consistent throughout the pattern. Cell survival and activity improved markedly with the addition of L-thyroxine, Epidermal growth factor and Hydrocortisone to the culture medium as indicated in the Methods. In a previous publication cells were shown to maintain spontaneous beating actively for up to 2 months in vitro[15].

Extracellular recordings from patterned cardiomyocytes on the MEAs

The electrical activity of the spontaneously contracting cells patterned on the microelectrodes was extracellularly recorded using the MEA system. Cardiac myocytes formed a morphologically homogenous, aligned, integrated network of cells that communicated through gap junctions and displayed normal tissue electrophysiology. The recorded field potential (FP) signals correlated with the contraction cycle.

In random cultures the direction and speed of the propagation of excitation (action potentials) has been shown to depend on a number of variables, including intercellular resistance across the gap junctions and the depolarizing sodium current[43–45]. For extracellular MEA recordings, previous research had indicated that the excitation wave could be followed through the monolayer as the increasing delay in field potential peak times and could be used to determine the pathway of the excitation as it moved away from the initiation site[46]. However, a direct determination of the CV has been difficult in

unpatterned monolayers, even though the timing of FP generation could be estimated. With patterning of the cells on the electrode array, the exact path of a spontaneous excitation wave can be determined and then, using the path length, conduction velocity can be calculated with a high degree of accuracy. Moreover, because of the pre-determined pathway, there is no need to image the wave propagation, just the measurement of the start and end times of the waves on the patterns as recorded by the microelectrodes in the array. Thus, in later high-throughput applications significantly fewer electrodes would be necessary for these measurements and imaging would not be a necessary requirement for analysis. Figure 2 shows a typical recording and the method for the determination of propagation direction. Figure 2B emphasizes the delay between FPs at different electrodes on the pattern, which enabled the determination of CV with high temporal resolution. The signal propagation was also visualized using an Imaging software routine written in Matlab 6.5[47]. The program converted recordings from the electrodes into a video indicating the field potential movement across an 8 × 8 grid. Snapshots from the video are shown in the supplementary data. This program also verified the origin of the excitation wave and confirmed our CV results obtained using the earlier method (Fig. 2.).

The conduction velocity was calculated to be 0.190 ± 0.025 m/s for spontaneous firing of the patterned cardiomyocytes over eight different MEAs. Previous research has shown that in vitro conduction velocities ranged from 0.12 m/s[48] to 0.242 m/s[49]. In a computer simulation of AP propagation in cardiac fibers in realistic conditions (taking into account extracellular solutions and spaces) the conduction velocity was 0.504 m/s[50]. In the human heart the AP generated by the Sino-atrial node spreads through the atria at a conduction velocity of 0.5 m/sec[51].

The conduction velocity on patterned cardiac monoloyers was also measured with stimulation. In these experiments, the cardiac pattern was stimulated at the two ends of the pattern, over a distance of 0.77 mm with 1 ms wide bipolar pulses with an amplitude of 500 mV at a frequency of 2 to 4 Hz (Fig. 3). The conduction velocity at 2 Hz stimulation was calculated to be 0.315 ± 0.011 m/s (n = 8). Previous research has indicated that rapid electrical stimulation in cultured, neonatal rat cardiomyocytes had an effect on the expression of Connexin 43 leading to changes in conduction properties[52], including an increase in conduction velocity.

This study showed that rapid electrical stimulation caused an increase in conduction velocity compared to the non-stimulated or spontaneous case. These results also indicated that accurate estimation of conduction velocity and propagation path could be determined in both the stimulated and in the spontaneous case. The measurement of the frequency dependence of conduction velocity could help in the understanding of the electrophysiological properties of cultured, neonatal rat cardiac myocytes, and ultimately, the physiology and pharmacology of the heart. The stimulated conduction velocity was also closer to the modeled values in the literature as well as to those generated by the SA node in the heart in vivo.

Electrical stimulation optimization experiments and measurement of refractory period after the AP

The stimulation protocol was optimized by determining a minimal threshold which reliably generated contraction from the cells. The cardiomyocytes were stimulated with 2 Hz, 500 mV, 1 ms wide bidirectional pulses at different electrodes. As seen in Fig. 3, the time-delay between the stimulation artifact and the FP at the different electrodes is in good correlation with the distance between the stimulation and recording electrodes. The excitation wave spread evenly on branched patterns as evidenced by the zero time difference at the end of the branches.

As shown in Fig. 4, paired pulse stimulation protocols could also be used with the defined cardiac patterns. By varying the time-delay between stimulations the absolute/relative refractory period after AP generation could be mapped (Fig. 4). If the stimulation delay between the two electrodes was too short, the second stimulus failed to evoke a second propagating AP. This experiment suggests new possibilities to study phenomena difficult to measure in random (not constrained) monolayers, such as re-entry, the refractory period after an AP and arrhythmia.

Pharmacology and toxin studies

One of the most important applications of this system could be the measurement of the effect of drugs and toxins on cardiac function. Two pharmacological agents, 1-Heptanol and Sparfloxacin, were tested to demonstrate the usability of this system for pharmacological and toxicology studies.

Patterned spontaneously beating cardiomyocytes were exposed to 50 μM 1-Heptanol in the recording medium and the FPs were recorded at 15 minute intervals. A control recording was taken before the toxin application. As shown in Figure 5, 1-Heptanol had a marked effect on the time for propagation of the excitation wave; it significantly increased the value measured before treatment. The increase in the time delay indicates a block and uncoupling of the gap junctions and delays the FP between the two recording electrodes. The CV was calculated to be 0.0066 ± 0.0004 (n = 4), a significant decrease from 0.197 ± 0.014 m/s (n = 4), and this result clearly shows that the effect of a gap junction blocker can be analyzed using this system.

Effect of Sparfloxacin

Sparfloxacin is a fluoroquinone antibiotic and HERG channel antagonist that is known to cause QT prolongation, Tdp and ventricular fibrillation[13, 53]. Application of 2 μM of Sparfloxacin to the recording medium caused a significant change in the spontaneous beating frequency within 25 minutes, with an increased FP length, non-synchronous contractions similar to fibrillation and burst-like beating activity as shown in Figure 6. The beating frequency changed from a stable value of 0.6 Hz-1 Hz to intra-bursts with a higher frequency range of 1 Hz –1.2 Hz. The loss of synchrony between the electrodes measured also indicated a propagation block in the monolayer.

DISCUSSION

Surface modification, photolithographic patterning and patterned cardiac myocyte cultures on microelectrode arrays

In this study, it was shown that the surface of commercial multielectrode arrays could be functionalized with SiPEG self-assembled monolayers. The PEG layer could be ablated and subsequently patterned using photolithographical techniques and that incubation of the patterns with 5 μg/ml fibronectin for 20 minutes did not affect the cell resistive properties of PEG, but significantly improved the attachment of cardiac cells to the glass surface. Surface analysis of the MEAs after modification indicated that both PEG-Silane modification and fibronectin adsorption on the ablated areas of the MEA surface was successful. The patterned commercial electrodes were re-usable 6–10 times without an observable decline in the quality of electrophysiological recordings. Rat neonatal, cardiac myocytes demonstrated normal physiology and formed beating monolayers on the areas designated by the patterns.

Manipulating cells, determining their attachment, growth and differentiation in cultures has became important in applications such as tissue engineering, toxin detection, drug screening and robotics. Unfortunately, there is no generally applicable method to pattern all cell types.

The method developed here using a protein adsorption resistant background, SiPEG, followed by protein adsorption (fibronectin) to the ablated area is a relatively simple, fast and cheap solution for this problem and could be generalized for 'difficult to pattern' cells.

Electrophysiology, toxin and drug effects

The patterned cells formed a confluent, aligned interconnected network of spontaneously contracting cells communicating via gap junctions. The activity of the cells could be measured using the MEA. The extracellular electrodes allowed not only recording, but also stimulation of the patterned cardiac myocyte monolayers. For the measurement of conduction velocity three different methods were used: 1) traditional video-monitoring of the excitation wave in the monolayer, 2) measurement of the speed of the spontaneous excitation waves on the patterns via the MEA and 3) measurement of the propagation of a stimulation-evoked extracellular field potential. All of these methods produced consistent results which were in reasonable agreement with published in vivo data. Conduction velocity increased with rapid electrical stimulation indicating that cardiac cell physiology can be studied using this method. Also, it was seen that the variability of the CV measurments decreased from about 15% as seen with the recordings from spontaneous APs to about 5% with stimulated APs. Patterning of the monolayers made CV measurements simpler, faster and more reliable. In future commercial applications this may be a more cost effective and a higher throughput technique for screening the cardiac side effects of drug candidates as it also eliminates the need for imaging of the propagation waves in the system. Patterning would also drastically reduce the number of required electrodes.

We have also demonstrated that alternating stimulation at different electrodes on the patterns could be used to measure the refractory period after APs, an important parameter for determining potentially fatal pharmacological side effects. This technique could also be a valuable tool for the study of cardiac defects, such as reentrant arrhythmia. Further development of this technology, especially for use with human cells, could significantly affect pharmaceutical drug development enabling high information content cardiac side effect screening.

Two pharmacological agents, 1-Heptanol and Sparfloxacin, were used to demonstrate the effectiveness of this method for drug screening. Both of these compounds are known to have cardiac side effects. 1-Heptanol is a gap junction blocker, whereas Sparfloxacin is an antibiotic that was taken off the market due to cardiac side effects. Our results were in agreement with the literature as 1-Heptanol drastically decreased the CV in the cardiac myocyte monolayers without completely blocking the conduction, whereas Sparfloxacin caused fibrillations which initiated a complete conduction block. These results indicated that specific drug actions can be studied using this system. 1-Heptanol affected the conduction velocity properties, while maintaining the synchronicity of the beating cells. The addition of Sparfloxacin to the cells caused a loss in rhythmicity resulting in a burst-like activity similar to fibrillations that are shown to have occurred in animal and human trials for the drug. These promising initial results support the need for further development of this system as a high-throughput, high-information content functional drug screening platform.

CONCLUSION

A simple and reliable method for patterning cardiac myocytes on multielectrode arrays was developed. This method is compatible with standard silicon manufacturing steps. Patterning of the cardiac myocytes increased the information content of the traditional multielectrode array recordings by enabling measurement of CV in a fast and simple way. Moreover, using paired stimulation, the measurement of the refractory period after the action potentials became possible. Measurement of the effect of drugs with known cardiac effects, was in

agreement with the literature. Further development of this method could result in cheaper, faster, pharmaceutical side-effect screening with higher predictive value. This method could also easily be adapted for use with human cardiac myocytes to eliminate interspecies differences in drug side effect screening and could become an alternative to the existing more complex, expensive and time consuming methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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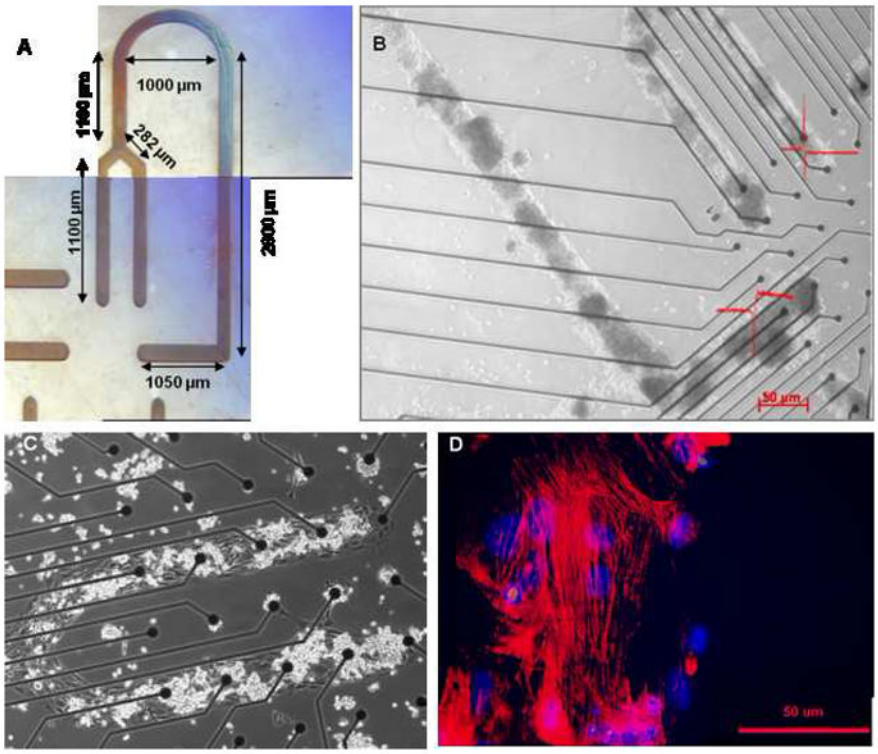

Figure 1.
Patterned neonatal cardiomyocytes. A: Pattern design and dimensions. B,C: Phase contrast pictures on day 12 of different regions of the pattern indicated in A. Field potentials measured at individual electrodes are marked in red in B. Electrode distance: 200 µm, D: Immunostaining with Rhodamine phalloidin for F-actin indicating cardiac myofibrils (note the visible striations). Nuclei are stained with DAPI (blue).

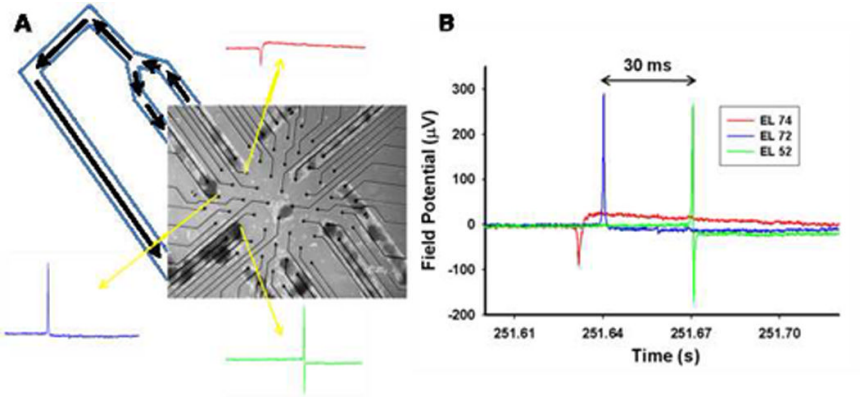

Figure 2.

Action potential (AP) conduction in patterned cardiac myocyte monolayers. A: phase contrast picture of cardiac myocyte patterns on the top of the substrate embedded electrodes (electrode distance is 200 μm) with a sketch of the conduction pathway on the completed pattern and with the recorded extracellular signals on the given electrodes. B: Overlay of the recorded traces showing the temporal relationship of the signals. A spontaneous AP was generated in the cardiac myocyte monolayer in the right fork of the pattern, close to electrode 74. This AP spread through the pattern reaching electrode 74 first, electrode 72 second and with a much longer delay (due to the longer path) electrode 52 last. Conduction velocity (CV) was determined based on the time delay in the signal between electrode 74 and 52 and the known pattern geometry. The calculated CV in this experiment was 0.190 m/s.

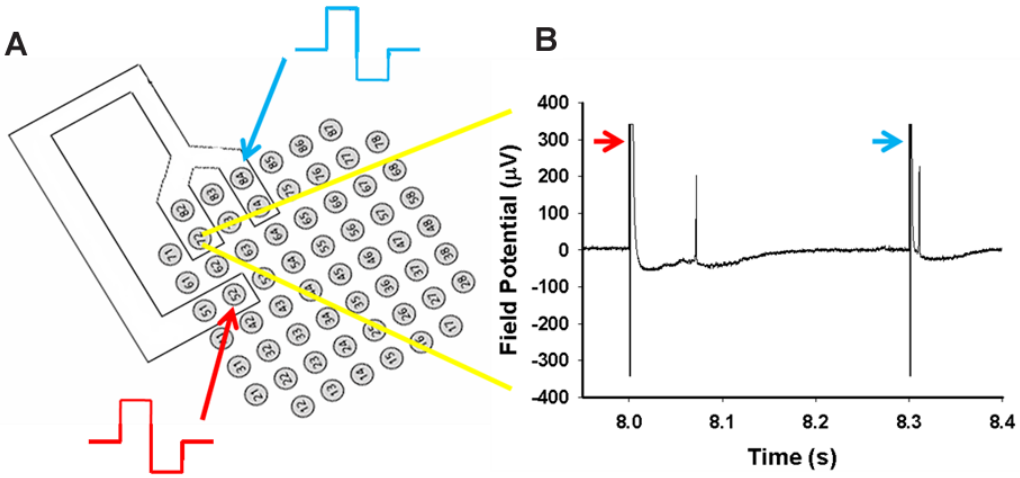

Figure 3.

Measurement of conduction velocity in patterned cardiac myocyte monolayers with electrical stimulation. A: Electrodes 52 and 84 were stimulated alternatively with a time delay of 300 ms and the response was recorded at electrode 72. B: E72 exhibited both long and short time delays in FP generation based on the distance the AP had to travel from the stimulation site.

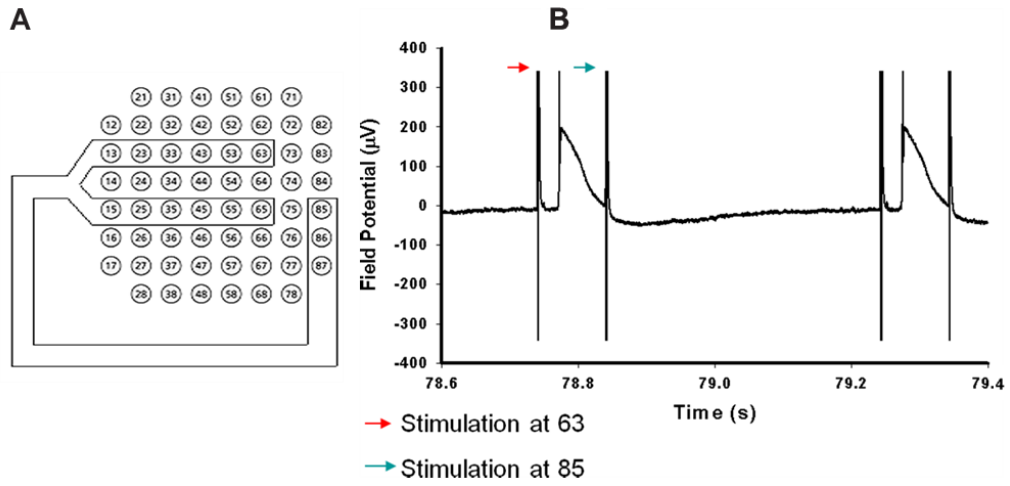

Figure 4.

Measurement of refractory period after cardiac action potential. Electrodes 63 and 85 (see pattern geometries on the left) were stimulated alternatively according to the following protocol: E63, 100 ms delay, E85, 400 ms delay, E63 again. Right panel shows recording at electrode 87. Stimulating E85 100 ms after the stimulation of E63 failed to evoke an AP because this delay was too short, cells were stimulated in their refractory period. By chance, cell electrode coupling on electrode E85 was especially strong in this experiment, thus the extracellular recording approximately replicated the shape of the intracellular AP illustrating the relationship of the AP and the stimulation. When the stimulation delay between E63 and E85 was 250 ms, both stimulations evoked an action potential at electrode 87.

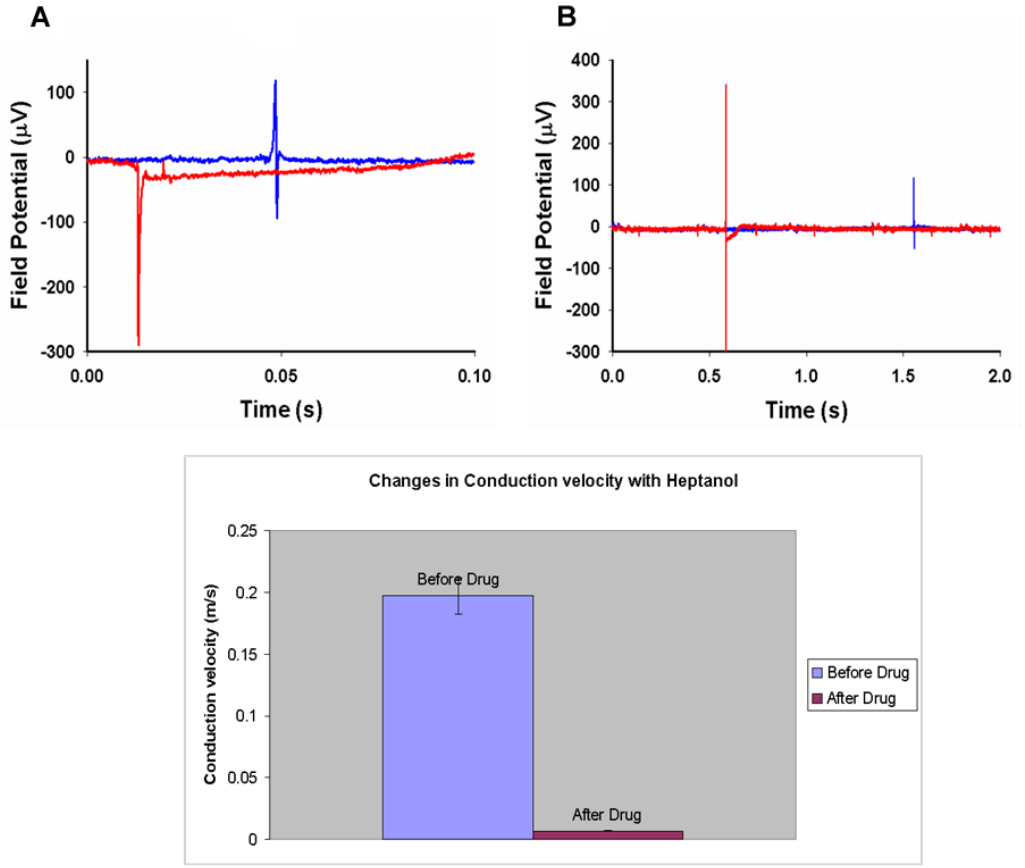

Figure 5.

Effect of the gap junction blocker 1-Heptanol. A: Propagation of excitation between the two end points in the pattern (Electrodes 84 and 52) before addition of the drug. The CV was measured to be 0.197 m/sec B: Propagation of excitation after the drug. The CV was calculated as 0.0066 m/sec. The drug effect on the CV measured over 4 MEAs.

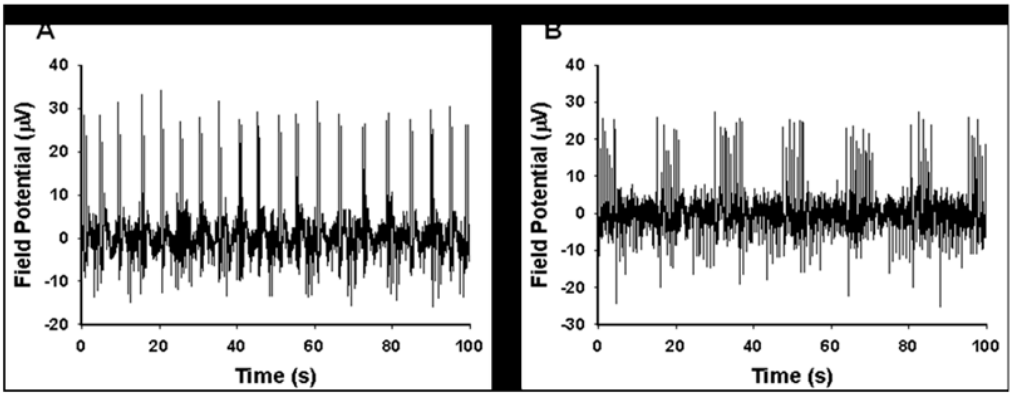

Figure 6.
Effect of the HERG channel antagonist Sparfloxacin. A: Field potential recordings before addition of Sparfloxacin indicated stable beating frequencies and synchronous activity B: Field potential recording after the addition of 2 μM Sparfloxacin showed burst like activity with higher intra-burst frequencies and no synchrony between electrodes.