Published in final edited form as:

Biofabrication. 2014 June ; 6(2): 024109. doi:10.1088/1758-5082/6/2/024109.

Controlling the Structural and Functional Anisotropy of Engineered Cardiac Tissues

W Bian^{#1}, C P Jackman^{#2}, and N Bursac^{2,#}

¹Department of Anesthesia and Medicine and Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

²Department of Biomedical Engineering, Duke University, Durham, North Carolina

[#] These authors contributed equally to this work.

Abstract

The ability to control the degree of structural and functional anisotropy in 3D engineered cardiac tissues would have high utility for both *in vitro* studies of cardiac muscle physiology and pathology as well as potential tissue engineering therapies for myocardial infarction. Here, we applied a high aspect ratio soft lithography technique to generate network-like tissue patches seeded with neonatal rat cardiomyocytes. Fabricating longer elliptical pores within the patch networks increased the overall cardiomyocyte and extracellular matrix (ECM) alignment within the patch. Improved uniformity of cell and matrix alignment yielded an increase in anisotropy of action potential propagation and faster longitudinal conduction velocity (LCV). Cardiac tissue patches with a higher degree of cardiomyocyte alignment and electrical anisotropy also demonstrated greater isometric twitch forces. After two weeks of culture, specific measures of electrical and contractile function (LCV = 26.8 ± 0.8 cm/s, specific twitch force = 8.9 ± 1.1 mN/mm² for the longest pores studied) were comparable to those of neonatal rat myocardium. We have thus described methodology for engineering of highly functional 3D engineered cardiac tissues with controllable degree of anisotropy.

1. Introduction

Cell-based therapies hold promise as a new treatment modality for significant loss of functional cardiomyocytes during myocardial infarction[1, 2]. While clinical trials with cell injections in the infarct site have shown modest benefits[3-6], implantation of a pre-assembled, tissue-engineered cardiac patch may support improved retention and survival of transplanted cells and, through direct electromechanical coupling, enable more efficient cardiac repair[7-9]. In designing an ideal engineered cardiac tissue patch, an important consideration is the anisotropic structure and function of native myocardium. Specifically, the alignment of elongated cardiomyocytes and their surrounding extracellular matrix and distribution of cell-cell junctions have profound implications on the electrical and mechanical function of cardiac muscle, yielding anisotropy in both action potential

[#]Corresponding Author: Nenad Bursac, PhD, nbursac@duke.edu, 3000 Science Drive, Hudson Hall, Room 136, Durham, NC 27708, Phone: (919) 660-5510, Fax: (919) 684-4488.

propagation and generation of contractile force[10, 11]. Thus, engineered myocardium should ideally be anisotropic to accurately mimic the structure and function of the native heart. In addition, the ability to control the degree of anisotropy in engineered cardiac tissues would enable *in vitro* modeling of cardiac pathologies with altered anisotropy[12-15] and better matching between donor and host cardiac tissue properties after implantation.

A variety of techniques to align monolayers of cardiac cells have been previously described, including those employing aligned surface topography[16, 17], micropatterning of extracellular matrix (ECM) proteins[16, 18, 19], and unidirectional stretch[20, 21]. In 3D cell cultures, cardiomyocyte alignment has been achieved by use of anisotropic scaffolds fabricated by sucrose leaching[22] or laser microablation[23]. However, these scaffolds were either too rigid to support macroscopic tissue contractions[22], or separated into discrete compartments that prevented continuous electrical communication throughout the entire tissue[23]. Cardiomyocytes can be also aligned by encapsulation within soft, naturally-derived 3D hydrogels shaped as tissue rings[24, 25] or bundles[26, 27]. However, cylindrical geometry and small cross-sectional area of these constructs do not allow systematic variations of cell alignment and structural and functional anisotropy.

Previously, we have developed a technique to control the 3D architecture of engineered skeletal muscle tissues by casting myogenic cells and fibrin-based hydrogels in PDMS tissue molds with staggered hexagonal posts[28, 29]. Resulting muscle tissues contained elliptical pores that directed local cell alignment and facilitated diffusion of nutrients to encapsulated cells. By varying the post dimensions, we controlled the geometry of the pores and degree of skeletal muscle cell alignment, as well as amplitude of generated contractile force[30]. More recently, we have applied similar hydrogel molding techniques to fabricate highly functional tissue patches using mouse and human stem cell-derived cardiomyocytes[31-33]. Building on these results, we set to study how varying the length of the elliptical pores in engineered cardiac tissue patches affects their structural anisotropy, including the global alignment of cardiomyocytes and spatial organization of ECM proteins. Furthermore, by optically mapping the propagation of action potentials and measuring generated contractile forces, we determined how resulting changes in cardiomyocyte alignment affect electrical and mechanical function of engineered cardiac patches.

2. Methods

2.1 Fabrication of tissue molds

The technique for fabricating PDMS tissue molds with staggered hexagonal posts (figure 1) is described elsewhere[28]. Briefly, a layer of SU-8 100 photoresist was coated on a silicon wafer and exposed to UV light through a photomask containing a pattern of hexagonal holes corresponding to different post lengths (PL) of 0.6 mm and 1.2 mm, and otherwise identical dimensions. Exposed wafers were etched with PGMEA solution to remove uncross-linked photoresist and silanized overnight. PDMS solution was then double-cast off the patterned silicon wafers to yield final tissue molds. PDMS molds without posts were used to create non-porous, control tissue constructs.

2.2 Isolation of neonatal rat ventricular myocytes

Neonatal rat ventricular myocytes (NRVM) were isolated from 2-3 day old Sprague-Dawley rats using enzymatic digestion with trypsin and collagenase as previously described[34, 35]. A 45 minute pre-plating step was used to remove faster attaching non-myocytes, enriching the proportion of cardiomyocytes in the cell mixture[36].

2.3 Fabrication and culture of engineered tissues

A hydrogel solution containing 2 mg/ml fibrinogen, 10% matrigel, 5×10^6 cells/ml, and 1 U/ml thrombin was prepared as previously described[28]. For large ($14 \times 14 \text{ mm}^2$) tissue patches, 500 μl of hydrogel mixture was pipetted into each mold containing a Velcro frame. For small ($7 \times 7 \text{ mm}^2$) tissue patches, 110 μl of hydrogel was pipetted into each mold containing a nylon frame (Cerex Advanced Fabrics). In all cases, the tissue patch was fully attached to the frame in one direction and by three thin connections in the other direction (figure 1B). Tissue patches were cultured for 2 weeks in DMEM containing 10% horse serum, 2% chick embryo extract, 100 U/ml penicillin G, 1mg/ml aminocaproic acid, and 50 $\mu\text{g}/\text{ml}$ ascorbic acid.

2.4 Immunostaining

Tissues were fixed in 2% paraformaldehyde, permeabilized with 0.5% Triton X, and blocked with 20% chick serum in 1% bovine serum albumin. After blocking, tissues were incubated overnight at 4°C in primary antibodies (mouse monoclonal anti- α -actinin, Sigma; mouse monoclonal anti-vimentin, Sigma; rabbit polyclonal anti-collagen I, Abcam; rabbit polyclonal anti-laminin, Abcam; rabbit polyclonal anti-connexin 43, Invitrogen). Secondary antibodies (Alexa Fluor, Invitrogen), nuclear stain (4',6-diamidino-2-phenylindole, or DAPI), and filamentous actin stain (Alexa Fluor 488 Phalloidin, Invitrogen) were incubated at room temperature for 2 hours. Tissue samples were imaged using a Zeiss 510 laser scanning confocal microscope.

2.5 Quantification of cell alignment

Alignment of phalloidin-labeled cardiomyocytes was analyzed using custom MATLAB software as previously described [18, 29]. After acquiring images, the local fiber angle (direction of cell alignment) in each $50 \times 50 \mu\text{m}$ subregion was calculated by first applying a Sobel edge-detection filter to the image, followed by assigning an intensity gradient vector and corresponding angle to each subregion. The resulting fiber angle map was then compiled over all subregions to produce an angle histogram. The mean angle and standard deviation of the angles were then calculated, with the standard deviation adopted as a quantitative measure of cell alignment (0° - perfect alignment, 45° - random alignment). From the standard deviation of the angles, the cell alignment index was calculated as $1 - \text{StandardDeviation}/45^\circ$ (0 - random alignment, 1 - perfect alignment) [30].

2.6 Optical mapping of action potential propagation

Optical mapping of action potential propagation was performed as previously described [37]. Engineered tissues were stained using voltage-sensitive dye di-4 ANEPPS and mapped in 37°C Tyrode's solution supplemented with 10 μM blebbistatin to reduce motion artifacts.

Tissue samples were stimulated at 2Hz rate by a centrally positioned bipolar point platinum electrode (Goodfellow Metals). Optical signals were recorded at 750 μm spatial and 0.83 ms temporal resolution by a photo diode array (PDA, 504 recording sites), or at 60 μm spatial and 2 ms temporal resolution by a high speed camera (MiCAM ULTIMA-L, SciMedia, 10,000 recording sites). Activation time at each recorded site was calculated as the time of maximum signal upstroke, and action potential duration (APD80) was calculated from activation time to 80% repolarization. Local conduction velocities (CV) were calculated by comparing activation times of neighboring channels. The longitudinal and transverse CVs were defined parallel and perpendicular to the long pore axis, respectively. Stimulus rate was increased in steps of 1Hz, and maximum capture rate was defined as the maximum rate at which a 1:1 stimulus:response pattern was maintained for at least 30 sec.

2.7 Measurement of contractile force

Passive tension and twitch force responses to electrical stimulation were measured isometrically, as previously described [30, 31, 38]. Briefly, engineered tissues were immersed in 37° C Tyrode's solution, and thin connections between patch and two frame sides parallel to long axis of elliptical pores (figure 1B1-3) were detached. These frame sides were then cut to allow tissue elongation and contraction. The two frame sides with fully attached tissue were pinned, one side to the chamber wall and the other to a floating PDMS holder that was connected to a sensitive force transducer mounted on a computer-controlled linear actuator (Thorlabs). Contractions were elicited by two parallel platinum electrodes positioned at the sides of the patch. Length of the patch was set by the actuator and active and passive force amplitudes were recorded at 0% (culture length), 4%, 8%, 12%, 16%, and 20% tissue elongation. For each patch, passive force vs. % elongation data were zeroed at the origin by subtracting the passive force value at 0% elongation, and fit by an exponential relationship with growth constant A, i.e., $y = -1 + \exp(x \cdot A)$. Rise time of a twitch response was calculated as the time between 10% and 90% of peak twitch force amplitude during the onset of contraction, and relaxation time between 90% and 10% of peak twitch force amplitude during relaxation.

2.8 Statistics

Data are expressed as mean $\pm$ standard error (SE), and differences among groups were compared by one-way analysis of variance (ANOVA) with Tukey's *post hoc* test, with statistical significance defined as $p < 0.05$.

3. Results

3.1 Engineered cardiac tissue patches with controllable pore size

Upon casting the cardiomyocyte-hydrogel suspension into the PDMS tissue molds, the size of the initial tissue pores was determined by dimensions of the hexagonal PDMS posts. With culture time, the process of cell-mediated gel compaction resulted in gradual increase in the pore size (figure 1A) while the tissue patches remained firmly attached to the surrounding frames and were easily removed after two weeks of culture for subsequent histological and functional analysis (figure 1B). The final size of the pores was controlled by varying the length of the hexagonal PDMS posts. Specifically, pore length remained unchanged and

approximately equal to the PDMS post length (0.604 ± 0.015 mm for PL = 0.6 mm, and 1.257 ± 0.006 mm for PL = 1.2 mm), while longer posts yielded wider pores (0.350 ± 0.011 mm for PL = 0.6 mm, and 0.631 ± 0.013 mm for PL = 1.2 mm, figure 1C). The length-to-width ratio of the resulting elliptical pores was significantly higher for PL = 1.2 mm vs. PL = 0.6 mm (1.997 ± 0.043 vs. 1.732 ± 0.043 , figure 1D). Increasing the porosity of the cardiac patches resulted in significantly thicker tissues (55 ± 2 μ m in non-porous controls, 76 ± 2 μ m for PL = 0.6 mm, and 87 ± 3 μ m for PL = 1.2 mm, figure 1E)

3.2 Alignment of cardiomyocytes

Cardiac myocytes in the tissue patches spread and attained standard cylindrical shape. Whereas myocytes in the non-porous controls oriented in random directions (figure 2:A1, B1), the presence of hexagonal posts in the tissue mold served to locally guide the cell alignment. Specifically, myocytes within the resulting tissue bundles between staggered pores oriented in the direction of the bundle's axis (figure 2:A2-3, B2-3)[29, 30]. Analysis of standard deviation of cell alignment angles (figure 2C) showed that the use of longer posts yielded better co-alignment of cells within network bundles (figure 2D). Furthermore, analysis of global (average) cell alignment within the tissue patch was performed by quantifying cell orientation within repeatable rectangular subunits representative of overall tissue structure (figure 2C)[29, 30]. It was found that the global cell alignment was statistically increased in PL=1.2 mm (0.39 ± 0.03) but not PL = 0.6 mm (0.18 ± 0.05) patches compared to non-porous controls (0.22 ± 0.02 , figure 2E).

3.3 Spatial distribution of ECM

To determine whether changes of cell alignment in patches with different PL affected the spatial distribution of ECM, we stained for ECM proteins most abundantly found in the native myocardium. In both nonporous and porous tissue patches, collagen I was detected at highest density at the outer tissue surface, which likely resulted from the preferential location of ECM-depositing fibroblasts at the tissue periphery (figure 3A-B). Higher magnification images revealed that introduction of pores and changes in myocyte orientation also affected the spatial organization of ECM within the patch, with interstitially deposited collagen I appearing to orient parallel with surrounding cardiomyocytes (figure 3C-D). Compared to collagen I, laminin distribution appeared more concentrated at the cardiomyocyte boundaries, forming a sheath-like structure around individual myocytes (figure 3E-F).

3.4 Anisotropy in action potential propagation

Since myocyte alignment is one of the main determinants for anisotropic action potential propagation in native myocardium, we applied optical mapping of transmembrane voltage to investigate whether changes in cardiomyocyte alignment in patches with different PL alter their degree of conduction velocity anisotropy. Application of point electrical stimulus to the centers of all tissue patches yielded uniform and continuous action potential propagation with elliptically shaped wavefront (figure 4A). The longitudinal conduction velocity (LCV) in patches with PL = 1.2 mm (26.8 ± 0.8 cm/s) was significantly higher than those measured in PL = 0.6 mm (20.3 ± 0.7 cm/s) and nonporous control (17.8 ± 1.5 cm/s) patches (figure 4B). No significant difference was observed in the transverse conduction velocity between

the 3 groups. Interestingly, we also observed significant differences between the LCV and TCV in the nonporous controls (figure 4B). Conduction velocity anisotropy ratio was found to be significantly higher in PL = 1.2 mm group (1.72 ± 0.07) relative to the other two groups (1.30 ± 0.05 for PL = 0.6 mm and 1.33 ± 0.10 for non-porous control, figure 4C). No significant difference was observed in the action potential duration (APD) or maximum capture rate (MCR) among the three groups (figure 4D-E). Furthermore, as characteristic of the native neonatal myocardium, uniform membrane distribution of connexin-43 gap junctions was found in cardiomyocytes from all tissue patches regardless of the degree of cell alignment (figure 4F).

3.5 Passive and active force generation

We further tested whether the more uniform cell alignment in cardiac tissue patches altered their passive mechanical properties and contractile force generation in response to electrical stimulation. Increase in tissue length yielded expected increase in active force generation in all three patch groups with active forces reaching maximum values near 10% strain (figure 5A). Tissue patches with PL = 1.2 mm exhibited significantly higher maximum twitch forces (2.39 ± 0.25 mN) relative to PL = 0.6 mm (1.61 ± 0.19 mN) and nonporous (1.66 ± 0.13 mN) groups (figure 5B). When normalized for the corresponding cross-sectional areas, porosities, and alignments [26, 28], specific contractile forces in PL = 1.2 mm, PL = 0.6 mm, and non-porous group amounted to 8.9 ± 1.1 , 4.7 ± 0.6 , and 5.2 ± 0.6 mN/mm², respectively. Furthermore, all patches showed increase in passive tension and stiffness with increase in tissue length (figure 5C), with the PL = 1.2 mm group exhibiting a trend (NS, $p=0.09$) towards fastest exponential growth (figure 5D). We also analyzed the kinetics of contraction and relaxation, but found no significant difference in contraction or relaxation time between the 3 groups. Contraction time remained relatively constant at different levels of strain (figure 5E), whereas relaxation was significantly slowed with increase in tissue length (linear regression $R^2 = 0.971, 0.965, 0.978$ for control, PL = 0.6 mm, and PL = 1.2 mm patch, respectively, $p < 0.05$ for all groups when testing for non-zero slope, figure 5F).

4. Discussion

In this study, we describe a methodology to fabricate 3D cardiac tissue patches with varying degree of structural and functional anisotropy. Specifically, changes in global cardiomyocyte and ECM orientation and tissue-level electrical and mechanical properties of cardiac network patches were controlled by altering the dimensions of microfabricated network pores. We found: (1) increased local and global alignment of cardiomyocytes with increased length/width ratio of the pores, (2) alteration in orientation of ECM proteins consistent with changes in orientation of cardiomyocytes, (3) increased anisotropy of electrical function in tissue patches with longer pore sizes, as measured by propagation velocity of action potentials, and (4) augmented contractile force generation in patches with higher structural and electrical anisotropy.

Described fabrication methodology enables the control of structural and functional anisotropy of 3D cardiac tissue patches by simple tuning of a single geometrical parameter (the length of hexagonal posts) within the microfabricated PDMS molds. The presence of

elliptical pores resulting from gel compaction around the PDMS posts generates higher tissue surface area and number of free tissue boundaries in the patch, thereby increasing the magnitude and altering the spatial distribution of local tissue strains, which in turn promote cell alignment throughout the patch. Increasing the post length both lengthens and widens the tissue pores while simultaneously guiding the average alignment of cardiomyocytes and surrounding cell matrix along the direction of the pores' long axis. The resulting increase in cardiomyocyte unidirectional alignment is based on the changes in tissue network topology and does not require application of external biophysical stimuli (e.g. stretch, electrical current); rather it stems from an increase in passive tension within the longer and thinner tissue bundles formed between the ends of the longer network pores. In addition to enhanced global cell alignment, this methodology offers potential for control of local 3D alignment of cardiomyocytes by altering the direction of individual PDMS posts.

One unexpected finding of this study was that the uniformity of global cell alignment (figure 2E), conduction velocity anisotropy (figure 4C), and contractile force amplitude (figure 5B) did not statistically differ between PL = 0.6 mm and non-porous patches, suggesting that there is a minimum required post length needed to induce tissue-level structural and functional anisotropy in the network patch. The small degree of anisotropy observed in non-porous tissue patches (figure 4B-C) likely resulted from the use of three thin side connections that served to attach the patch to the nylon frame on two opposite sides (figure 1B-1). These side connections allowed efficient separation of patch from the frame during force tests, but also resulted in slight tissue compaction away from the frame and cardiomyocyte alignment along the free tissue boundaries[35] (not shown), yielding increase in global tissue anisotropy. While the 0.6 mm PL significantly increased cell alignment between the network pores (figure 2D), these localized changes in tissue architecture were not sufficient to significantly affect global structural and functional properties of the patch. Decreasing transverse spacing between hexagonal posts could be one method to significantly increase structural and functional anisotropy in PL = 0.6 mm patches.

On the other hand, increasing PL to 1.2 mm yielded significant increase in the longitudinal velocity of propagation to 26.8 ± 0.8 cm/s, which is comparable to values measured in the ventricles of neonatal (21.8 cm/s)[35] and 10-day-old (27 cm/s) rats[40]. Simultaneously, longitudinal-to-transverse velocity anisotropy ratio was increased to ~ 1.7 , a value similar to those of neonatal rat and dog myocardium (1.7-2.1)[41, 42]. While longer elliptical pores would be expected to further increase uniformity of global cell alignment, longitudinal conduction velocity, and electrical anisotropy, reaching the adult heart properties would require significant increase in cardiomyocyte size, acquisition of rod cell shape, and changes in gap junctional distribution. Specifically, while we have observed robust cardiomyocyte coupling via Cx43⁺ gap junctions (figure 4F), these gap junctions showed random membrane distribution characteristic of neonatal cardiomyocytes, instead of being localized at end-to-end junctions seen in adult cells[43].

Interestingly, despite the presence of multiple pores, we observed no significant conduction slowing or the formation of sustained reentrant arrhythmias in tissue network patches. We partly attribute this finding to the elliptical shape of the pores that presents gradual (rather than sharp) tissue expansions in front of a propagating wave as it exits the regions between

the pores. This reduces mismatch between local current supply and downstream current sink[44], preventing the occurrence of arrhythmogenic conduction block remote from the pacing site. Furthermore, the perimeter of elliptical pores (< 3 mm) is significantly shorter than the wavelength (the product of CV and APD) of the propagating waves (~ 15 mm at the maximum sustainable excitation rate), which would prevent stable anchoring of a reentrant wave around the tissue pore.

Increase in post length in this study also augmented force generating capacity of tissue network patches, consistent with our previous findings for similarly fabricated skeletal muscle tissues [30]. Qualitatively, the shape of measured active and passive force-length curves as well as observed slowing of relaxation at increased strains was consistent with the measurements in native myocardium [45, 46]. The average contractile force for patches with $PL = 1.2$ mm was 2.39 ± 0.25 mN, corresponding to a specific force value of 8.9 ± 1.1 mN/mm². While this is considerably lower than values measured in adult rat ventricular muscle ($40\text{--}58$ mN/mm²)[47–49], it is comparable to forces measured in ventricular muscle preparations from neonatal rats ($7.8\text{--}8.7$ mN/mm²)[48, 49]. Interestingly, the significant increase in specific force found in $PL = 1.2$ mm group suggests that 3D cell alignment and/or changes in passive tissue properties may contribute to enhanced functional maturation of neonatal cardiomyocytes, consistent with previous studies by others[25, 50, 51], and our findings in engineered skeletal muscle networks[30]. Understanding the underlying mechanisms of these results will be a subject of our future studies.

5. Conclusion

In this study, we have shown that increasing the length of microfabricated pores in 3D cardiac tissue network patches improves the local and global uniformity of cardiomyocyte alignment and distribution of critical ECM components including collagen I and laminin. Most importantly, these changes in cell and matrix alignment were accompanied with increase in tissue-level functionality resulting in rapid impulse propagation and robust force generation, comparable to those of native neonatal myocardium. The described method to control structural and functional anisotropy of engineered cardiac tissues is expected to enable systematic *in vitro* studies of structural heart disease and to promote the design of safer and more efficient cardiac cell therapies.

Acknowledgments

The authors thank A. Krol for NRVM isolations, N. Badie for data analysis software, and J. Kim for assistance with optical mapping. This work was supported by National Heart, Lung, and Blood Institute of the NIH under Award Numbers R01HL104326 to N.B. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

References

- [1]. Donndorf P, Strauer BE, Haverich A, Steinhoff G. Stem cell therapy for the treatment of acute myocardial infarction and chronic ischemic heart disease. *Current Pharmaceutical Biotechnology*. 2013; 14:12–19. [PubMed: 23092255]
- [2]. Anversa P, Kajstura J, Rota M, Leri A. Regenerating new heart with stem cells. *Journal of Clinical Investigation*. 2013; 123:62–70. [PubMed: 23281411]

- [3]. Delewi R, Andriessen A, Tijssen JGP, Zijlstra F, Piek JJ, Hirsch A. Impact of intracoronary cell therapy on left ventricular function in the setting of acute myocardial infarction: A meta-analysis of randomised controlled clinical trials. *Heart*. 2013; 99:225–232. [PubMed: 22875736]
- [4]. King A. Stem cells. Could it be time to abandon BMCs? *Nature Reviews - Cardiology*. 2013; 10:8.
- [5]. Marbán E, Malliaras K. Mixed results for bone marrow–derived cell therapy for ischemic heart disease. *Journal of the American Medical Association*. 2012; 308:2405–2406. [PubMed: 23117584]
- [6]. Bartunek J, et al. Cardiopoietic stem cell therapy in heart failure: The C-CURE (cardiopoietic stem cell therapy in heart failure) multicenter randomized trial with lineage-specified biologics. *Journal of the American College of Cardiology*. 2013; 61:2329–2338. [PubMed: 23583246]
- [7]. Bursac N. Cardiac tissue engineering using stem cells. *IEEE Engineering in Medicine and Biology Magazine*. 2009; 28:80–89. [PubMed: 19353830]
- [8]. Buikema J, Van Der Meer P, Sluijter JPG, Domian JJ. Engineering myocardial tissue: The convergence of stem cells biology and tissue engineering technology. *Stem Cells*. 2013
- [9]. Liao B, Zhang D, Bursac N. Functional cardiac tissue engineering. *Regenerative Medicine*. 2012; 7:187–206. [PubMed: 22397609]
- [10]. Spach MS, Heidlage JF, Dolber PC, Barr RC. Changes in anisotropic conduction caused by remodeling cell size and the cellular distribution of gap junctions and Na(+) channels. *Journal of Electrocardiology*. 2001; 34(Suppl):69–76. [PubMed: 11781939]
- [11]. Saffitz JE, Kanter HL, Green KG, Tolley TK, Beyer EC. Tissue-specific determinants of anisotropic conduction velocity in canine atrial and ventricular myocardium. *Circulation Research*. 1994; 74:1065–1070. [PubMed: 8187276]
- [12]. Carey PA, Turner M, Fry CH, Sheridan DJ. Reduced anisotropy of action potential conduction in left ventricular hypertrophy. *Journal of Cardiovascular Electrophysiology*. 2001; 12:830–835. [PubMed: 11469438]
- [13]. Jongsma HJ, Wilders R. Gap junctions in cardiovascular disease. *Circulation Research*. 2000; 86:1193–1197. [PubMed: 10864907]
- [14]. Spach MS, Boineau JP. Microfibrosis produces electrical load variations due to loss of side-to-side cell connections: A major mechanism of structural heart disease arrhythmias. *PACE-Pacing and Clinical Electrophysiology*. 1997; 20:397–413.
- [15]. Gupta KB, Ratcliffe MB, Fallert MA, Edmunds LH, Bogen DK. Changes in passive mechanical stiffness of myocardial tissue with aneurysm formation. *Circulation*. 1994; 89:2315–2326. [PubMed: 8181158]
- [16]. Bursac N, Parker KK, Iravanian S, Tung L. Cardiomyocyte cultures with controlled macroscopic anisotropy - a model for functional electrophysiological studies of cardiac muscle. *Circulation Research*. 2002; 91:E45–E54. [PubMed: 12480825]
- [17]. Bien H, Yin LH, Entcheva E. Cardiac cell networks on elastic microgrooved scaffolds. *IEEE Engineering in Medicine and Biology Magazine*. 2003; 22:108–112. [PubMed: 14699943]
- [18]. Badie N, Bursac N. Novel micropatterned cardiac cell cultures with realistic ventricular microstructure. *Biophysical Journal*. 2009; 96:3873–3885. [PubMed: 19413993]
- [19]. Badie N, Satterwhite L, Bursac N. A method to replicate the microstructure of heart tissue in vitro using DTMRI-based cell micropatterning. *Annals of Biomedical Engineering*. 2009; 37:2510–2521. [PubMed: 19806455]
- [20]. Terracio L, Miller B, Borg TK. Effects of cyclic mechanical stimulation of the cellular-components of the heart – in vitro. *In Vitro Cellular & Developmental Biology*. 1988; 24:53–58. [PubMed: 3276657]
- [21]. Zhuang JP, Yamada KA, Saffitz JE, Kleber AG. Pulsatile stretch remodels cell-to-cell communication in cultured myocytes. *Circulation Research*. 2000; 87:316–322. [PubMed: 10948066]
- [22]. Bursac N, Loo YH, Leong K, Tung L. Novel anisotropic engineered cardiac tissues: Studies of electrical propagation. *Biochemical and Biophysical Research Communications*. 2007; 361:847–853. [PubMed: 17689494]
- [23]. Engelmayr GC, Cheng M, Bettinger CJ, Borenstein JT, Langer R, Freed LE. Accordion-like honeycombs for tissue engineering of cardiac anisotropy. *Nature Materials*. 2008; 7:1003–1010.

- [24]. Zimmermann W-H, et al. Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. *Nature Medicine*. 2006; 12:452–458.
- [25]. Black LD 3rd, Meyers JD, Weinbaum JS, Shvelidze YA, Tranquillo RT. Cell-induced alignment augments twitch force in fibrin gel-based engineered myocardium via gap junction modification. *Tissue Engineering Part A*. 2009; 15:3099–3108. [PubMed: 19338433]
- [26]. Hansen A, Eder A, Bonstrup M, Flato M, Mewe M, Schaaf S, Aksehirlioglu B, Schworer A, Uebeler J, Eschenhagen T. Development of a drug screening platform based on engineered heart tissue. *Circulation Research*. 2010; 107:35–44. [PubMed: 20448218]
- [27]. Tulloch NL, Muskheli V, Razumova MV, Korte FS, Regnier M, Hauch KD, Pabon L, Reinecke H, Murry CE. Growth of engineered human myocardium with mechanical loading and vascular coculture. *Circulation Research*. 2011; 109:47–59. [PubMed: 21597009]
- [28]. Bian W, Liao B, Badie N, Bursac N. Mesoscopic hydrogel molding to control the 3d geometry of bioartificial muscle tissues. *Nature Protocols*. 2009; 4:1522–1534.
- [29]. Bian W, Bursac N. Engineered skeletal muscle tissue networks with controllable architecture. *Biomaterials*. 2009; 30:1401–1412. [PubMed: 19070360]
- [30]. Bian WN, Juhas M, Pfeiler TW, Bursac N. Local tissue geometry determines contractile force generation of engineered muscle networks. *Tissue Engineering Part A*. 2012; 18:957–967. [PubMed: 22115339]
- [31]. Liao B, Christoforou N, Leong KW, Bursac N. Pluripotent stem cell-derived cardiac tissue patch with advanced structure and function. *Biomaterials*. 2011; 32:9180–9187. [PubMed: 21906802]
- [32]. Zhang D, Shadrin IY, Lam J, Xian H-Q, Snodgrass HR, Bursac N. Tissue-engineered cardiac patch for advanced functional maturation of human esc-derived cardiomyocytes. *Biomaterials*. 2013; 34:5813–5820. [PubMed: 23642535]
- [33]. Christoforou N, Liao B, Chakraborty S, Chellapan M, Bursac N, Leong KW. Induced pluripotent stem cell-derived cardiac progenitors differentiate to cardiomyocytes and form biosynthetic tissues. *PLOS ONE*. 2013; 8:e65963. [PubMed: 23785459]
- [34]. Toraason M, Luken ME, Breitenstein M, Krueger JA, Biagini RE. Comparative toxicity of allylamine and acrolein in cultured myocytes and fibroblasts from neonatal rat-heart. *Toxicology*. 1989; 56:107–117. [PubMed: 2728003]
- [35]. Bursac N, Papadaki M, Cohen RJ, Schoen FJ, Eisenberg SR, Carrier R, Vunjak-Novakovic G, Freed LE. Cardiac muscle tissue engineering: Toward an in vitro model for electrophysiological studies. *American Journal of Physiology - Heart and Circulatory Physiology*. 1999; 277:H433–H444.
- [36]. Naito H, Melnychenko I, Didié M, Schneiderbanger K, Schubert P, Rosenkranz S, Eschenhagen T, Zimmermann W-H. Optimizing engineered heart tissue for therapeutic applications as surrogate heart muscle. *Circulation*. 2006; 114:I72–I78. [PubMed: 16820649]
- [37]. Mcspadden LC, Kirkton RD, Bursac N. Electrotonic loading of anisotropic cardiac monolayers by unexcitable cells depends on connexin type and expression level. *American Journal of Physiology - Cell Physiology*. 2009; 297:C339–C351. [PubMed: 19494239]
- [38]. Hinds S, Bian W, Dennis RG, Bursac N. The role of extracellular matrix composition in structure and function of bioengineered skeletal muscle. *Biomaterials*. 2011; 32:3575–3583. [PubMed: 21324402]
- [39]. Costa KD, Lee EJ, Holmes JW. Creating alignment and anisotropy in engineered heart tissue: Role of boundary conditions in a model three-dimensional culture system. *Tissue Engineering*. 2003; 9:567–577. [PubMed: 13678436]
- [40]. Sun LS, Legato MJ, Rosen TS, Steinberg SF, Rosen MR. Sympathetic innervation modulates ventricular impulse propagation and repolarization in the immature rat-heart. *Cardiovascular Research*. 1993; 27:459–463. [PubMed: 8490947]
- [41]. Litchenberg WH, Norman LW, Holwell AK, Martin KL, Hewett KW, Gourdie RG. The rate and anisotropy of impulse propagation in the postnatal terminal crest are correlated with remodeling of cx43 gap junction pattern. *Cardiovascular Research*. 2000; 45:379–387. [PubMed: 10728358]
- [42]. Den Haan AD, Veldkamp MW, Bakker D, Boink GJJ, Janssen RB, De Bakker JMT, Tan HL. Organ explant culture of neonatal rat ventricles: A new model to study gene and cell therapy. *PLOS ONE*. 2013; 8:e59290. [PubMed: 23516623]

- [43]. Van Kempen MJA, Fromaget C, Gros D, Moorman AFM, Lamers WH. Spatial distribution of connexin 43 the major cardiac gap junction protein in the developing and adult rat heart. *Circulation Research*. 1991; 68:1638–1651. [PubMed: 1645233]
- [44]. Kleber AG, Rudy Y. Basic mechanisms of cardiac impulse propagation and associated arrhythmias. *Physiology Reviews*. 2004; 84:431–488.
- [45]. Julian FJ, Sollins MR. Sarcomere length-tension relations in living rat papillary-muscle. *Circulation Research*. 1975; 37:299–308. [PubMed: 1157219]
- [46]. Janssen PML, Hunter WC. Force, not sarcomere-length, correlates with prolongation of isosarcometric contraction. *American Journal of Physiology-Heart and Circulatory Physiology*. 1995; 269:H676–H685.
- [47]. Hasenfuss G, Mulieri L, Blanchard E, Holubarsch C, Leavitt B, Ittleman F, Alpert N. Energetics of isometric force development in control and volume-overload human myocardium. Comparison with animal species. *Circulation research*. 1991; 68:836–846.
- [48]. Solaro RJ, Lee JA, Kentish JC, Allen DG. Effects of acidosis on ventricular muscle from adult and neonatal rats. *Circulation Research*. 1988; 63:779–787. [PubMed: 3168178]
- [49]. Moreno-Gonzalez A, Korte FS, Dai J, Chen K, Ho B, Reinecke H, Murry CE, Regnier M. Cell therapy enhances function of remote non-infarcted myocardium. *Journal of Molecular and Cellular Cardiology*. 2009; 47:603–613. [PubMed: 19683533]
- [50]. Chung C-Y, Bien H, Entcheva E. The role of cardiac tissue alignment in modulating electrical function. *Journal of Cardiovascular Electrophysiology*. 2007; 18:1323–1329. [PubMed: 17916158]
- [51]. Pong T, Adams WJ, Bray M-A, Feinberg AW, Sheehy SP, Werdich AA, Parker KK. Hierarchical architecture influences calcium dynamics in engineered cardiac muscle. *Experimental Biology and Medicine*. 2011; 236:366–373. [PubMed: 21330361]

Figure 1.

Structural properties of engineered cardiac tissue patch networks. (A1-A3) Representative light microscopy images of two-week old non-porous patches (A1) and tissue networks fabricated with indicated post lengths (PL) of 0.6mm (A2) and 1.2mm (A3). (B1-B3) Corresponding cardiac tissue patches shown removed from PDMS molds. The patches were cultured anchored within nylon frames. (C-E) Morphometric parameters of two-week old patches including average length and width of patch pores (C), pore length-to-width (L/W) ratio (D) and patch thicknesses (E). n = 4-9 patches per group. #significant difference between two denoted groups. *significant difference from other two groups.

Figure 2.

Cell alignment in engineered cardiac tissue patches. (A1-A3) Representative filamentous actin (F-act) stainings of control (non-porous) tissue patches (A1) and tissue networks with PL = 0.6 mm (A2) and PL = 1.2 mm (A3). (B1-B3) Corresponding close-up confocal stack images with side and front projections showing cellular organization within the bundle regions marked by yellow boxes in A. DAPI labels cell nuclei. (C) Representative map of fiber alignment in a rectangular sub-unit of a PL = 1.2 mm patch, with blue lines indicating fiber angles within each 50 μ m tissue region. (D-E) Degrees of cell alignment within bundle regions (D) and entire tissue patch (global alignment, E). n = 6-12 patches per group.

*significant difference from other two groups.

Figure 3.

Distribution of cells and extracellular matrix in engineered cardiac tissue patches. (A1-A2) Representative macroscopic distribution of vimentin (Vim)⁺ fibroblasts and F-actin (F-act)⁺ cardiomyocytes in a control non-porous patch (A1) and bundle segment of a PL = 1.2 mm network patch (A2). Cross-sectional projections on the top show cellular distribution throughout the patch thickness. (B1-B2) Macroscopic distribution of collagen I (Col 1) in tissue patches corresponds to that of fibroblasts. (C-D) Microscopic distribution of collagen I (C1-C2) and F-act⁺ cardiomyocytes (D1,D2) in tissue patches. (E-F) Microscopic distribution of laminin (Lam, E1-E2) and F-act⁺ cardiomyocytes (F1,F2) in tissue patches. DAPI labels cell nuclei.

Figure 4.

Electrical properties of engineered cardiac tissue patches. (A1-A3) Representative activation maps of action potential propagation in control (A1), PL = 0.6 mm (A2), and PL = 1.2 mm (A3) tissue patches. Pulse signs denote position of stimulus electrode. Isochrone lines are labeled in ms. Arrows show directions of measurement for longitudinal and transverse conduction velocity (LCV and TCV, respectively). Insets in (A2) and (A3) show representative activation maps (isochrone lines spaced 5 ms) recorded by a CMOS camera at higher spatial resolution, distinguishing the acellular pores from the surrounding tissue area. (B-D) Summary data for conduction velocity (CV, B), velocity anisotropy ratio (AR= LCV/TCV, C), and action potential duration (APD, D) in cardiac tissue patches stimulated at 2

Hz. (E) Maximum capture rate (MCR) defined as fastest pacing rate able to elicit a 1:1 response from engineered cardiac tissue. n = 9-13 patches per group. #significant difference between two denoted groups. *significant difference from other two corresponding groups. (F1-F3) Expression of connexin 43 (Cx43)⁺ gap junctions in sarcomeric α -actinin (SAA)⁺ cardiomyocytes in control patches (F1) and bundles of PL = 0.6 mm (F2) and PL = 1.2 mm (F3) tissue networks.

Figure 5.

Contractile function of engineered cardiac tissue patches. (A) Average force-length relationships showing isometric twitch force amplitudes recorded during 1 Hz electrical stimulation at different tissue elongations (strains), with 0% elongation corresponding to culture length. (B) Maximum isometric twitch forces at optimal tissue elongation. (C) Passive tension in tissue patches as a function of tissue elongation. (D) Growth constants A of an exponential rise fit ($y = -I + \exp(x \cdot A)$) of data points shown in (C). (E-F) Kinetics of contractile force generation in tissue patches quantified via Rise time (from 10% to 90% of peak twitch force during onset of contraction, E) and Decay time (from 90% to 10% of peak twitch force during relaxation, F). $n = 6-7$ patches per group. *significant difference from other two groups.