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Design of a 3D aligned myocardial tissue construct from biodegradable polyesters

H. Kenar · G. T. Kose · V. Hasirci

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Abstract The heart does not regenerate new functional tissue when myocardium dies following coronary artery occlusion, or if it is defective. Ventricular restoration involves excising the infarct and replacing it with a cardiac patch to restore the heart to a more healthy condition. The goal of this study was to design and develop a clinically applicable myocardial patch to replace myocardial infarcts and improve long-term heart function. A basic design composed of 3D microfibrous mats that house mesenchymal stem cells (MSCs) was developed from human umbilical cord matrix (Wharton's Jelly) cells aligned in parallel to each other mimicking the native myocardium. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), poly(L-D,L-lactic acid) (P(L-D,L)LA) and poly(glycerol sebacate) (PGS) were blended and electrospun into aligned fiber mats with fiber diameter ranging between 1.10 and 1.25 μm. The micron-sized parallel fibers of the polymer blend were effective in cell alignment and cells have penetrated deep within the mat through the fiber interstices, occupying the whole structure; 8–9 cell layers were obtained. Biodegradable macroporous tubings were introduced to serve as nutrient delivery route. It was possible to create a thick myocardial patch with structure similar to the native tissue and with a capability to grow.

H. Kenar · V. Hasirci (✉)
METU, BIOMAT, Department of Biological Sciences, Biotechnology Research Unit, 06531 Ankara, Turkey
e-mail: vhasirci@metu.edu.tr

G. T. Kose
Department of Genetics and Bioengineering, Yeditepe University, 34755, Istanbul, Turkey

1 Introduction

The function of the heart is vital to supply oxygen and nutrients to, and remove waste products from the body via the blood in order to maintain the balance that is necessary to sustain life [1]. Strong muscular contractions in the ventricles pump blood out of the heart and into the circulatory system. The myocardium is the layer of functional beating muscle that consists of fibroblasts and highly oriented cardiomyocytes (muscle fibers) connected end-to-end in the longitudinal direction and side-to-side in the transverse direction in a matrix of collagen [2]. Heart failure, stemming from cardiovascular diseases, is the number one cause of death in the industrialized countries. The heart does not regenerate new functional tissue when myocardium dies following a coronary artery occlusion, or is defective, like in congenital heart defects. Ventricular reconstruction involves removing the infarcted region of the ventricle and reshaping the heart from a spherical shape, to a more efficient normal (elliptical) shape. This can be done using the linear suture technique (direct closure), circular external reorganization or endoventricular circular patch plasty [3]. Direct linear closure is a procedure in which the infarcted section of the ventricle is removed, and the remaining heart tissue is sutured together. However, sometimes there is not enough myocardial tissue to restore the ventricle to the correct dimensions. The Dor procedure, also known as Endoventricular Patch Plasty, has been used since 1984. Infarcted myocardium is removed in this procedure and the heart structure is restored using a patch that is sutured into the opening [3]. The use of a patch ensures the restoration of ventricular volume and prevents further ventricular distortion. Although the currently available prostheses are adequate for restoring ventricular geometry and maintaining ventricular pressure, and

thus may be life saving, they do not actively adapt to the physiological environment and mechanical demands as they represent non-living materials. They have limited durability and are prone to infection, immunologic reactivity, and thrombosis, which often requires repeat operations in the future [4]. When implanted into the immature heart of a child, these materials do not grow with the paediatric patient, which is a disadvantage for the repair of congenital defects.

Efforts to regenerate functional myocardial tissue are pursued through cell grafting by syringe injection directly in the ventricular wall or in the coronary vessels. Most studies support the idea that cell engraftment in animal models of myocardial infarction can improve contractile function [5], but the mechanism behind this functional improvement still needs to be clarified; there has been no convincing demonstration of hematopoietic or myoblastic adult stem cell differentiation into the cardiac phenotype [6–9]. In addition, the efficiency of cell engraftment is very low as more than 90% of the cell suspension injected is lost [9]. Much effort is now directed to the development of tissue engineering strategies using scaffolds to successfully engraft new cells into the myocardium.

An advantage of tissue-engineered patches is our ability to control their various properties, such as scaffold degradation time. Utilization of different combinations of polymers and various scaffold preparation techniques enable researchers to develop patches with varying biomechanical and degradation profiles for numerous applications [4]. Although the micro and nanotechnology used to generate the native architecture of complex tissues are premature yet, tissue engineered constructs with higher complexity are on the way with a hope to get more functional grafts for total elimination of the need for organ transplantation.

The success of bioengineering heart muscle in vitro will largely depend on the use of an appropriate matrix-like biomaterial capable of providing structural support and organization as well as the molecular cues necessary for functional tissue formation [10]. The first materials used for heart tissue engineering were members of the synthetic, degradable, biocompatible poly(lactide) family: polylactic acid (PLA), polyglycolic acid (PGA) and their copolymer polylactic-co-glycolic acid (PLGA) [11]. Subsequently, it was realized that the mechanical properties of the material used had to be adapted to match the elastic properties of the heart tissue as much as possible. Promising results were obtained with collagen, the major constituent of the cardiac ECM-based grafts or 'patches' containing beating cardiomyocytes [12–17]. Radisic et al. applied electrical signals designed to mimic those in the native heart to rat cardiomyoctes seeded onto Ultrafoam collagen sponges using Matrigel, and were successful in inducing synchronous contractions [18]. Poor mechanical properties, the lack of structural stability, and large degree of swelling (approximately 30% immediately following hydration in culture medium [19]), however, may hamper clinical applications of collagen sponges. P4HB is another natural polymer considered for use in cardiovascular tissue engineering due to its suitable elastic property, but it degraded rapidly (in 8 weeks) in vivo [20, 21]. Composites of natural and synthetic polymers were used, too, to obtain cell carriers; for example, sponges based on poly(epsilon-caprolactone-co-L-lactide) reinforced with knitted poly(L-lactide) fabric (PCL-PLLA), gelatin or PGA [9, 22].

Cardiac myocytes are responsive to the geometry of their environment [23]. It was shown that cardiomyocytes can align parallel to micron range protein lanes and fibers [24, 25]. With these studies it was shown that it is possible to mimic the anisotropic, i.e. aligned, organization of cardiomyocytes on two dimensional scaffolds, however, these constructs still need to be developed into thick tissue grafts to be able to offer a realistic solution for cardiac tissue replacement.

The goal of this study was to develop a clinically applicable myocardial patch to replace myocardial infarctions and improve long-term heart function. A construct composed of 3D microfibrous mats that house mesenchymal stem cells (MSCs) from human umbilical cord matrix (Wharton's Jelly) aligned parallel to each other was prepared mimicking the cell organization in the native myocardium. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), poly(L-D,L-lactic acid) (P(L-D,L)LA) and poly(glycerol sebacate) (PGS) were blended to produce aligned fiber mats with micron-sized fibers through electrospinning. Cell growth and alignment on the mats and penetration within the structure were studied.

2 Methods

2.1 Preparation of aligned microfiber mats through electrospinning

PHBV (MW: 222.2 kDa), containing 5% 3-hydroxyvalerate from Sigma-Aldrich Co. (USA), P(L-D,L)LA (70:30, i.v.: 5.5–6.5 dl/g) from Boehringer-Ingelheim (Germany), and PGS (MW: 9929 Da, polydispersity index: 1.8) were blended using chloroform (Chl) : *N,N*-dimethyl formamide (DMF) (95:5 v/v) as the solvent. PGS was synthesized under N_2 from a mixture of glycerol and sebacic acid (1:1 molar ratio) at 120°C for 24 h and for another 24 h at 120°C without nitrogen. Biodegradable aligned fiber mats of PHBV-P(L-D,L)LA-PGS (PPG) blend were prepared by electrospinning the PHBV:P(L-D,L)LA:PGS 49:49:2 (w/w) from a 5% solution (w/v) in Chl:DMF (10:1). The conditions

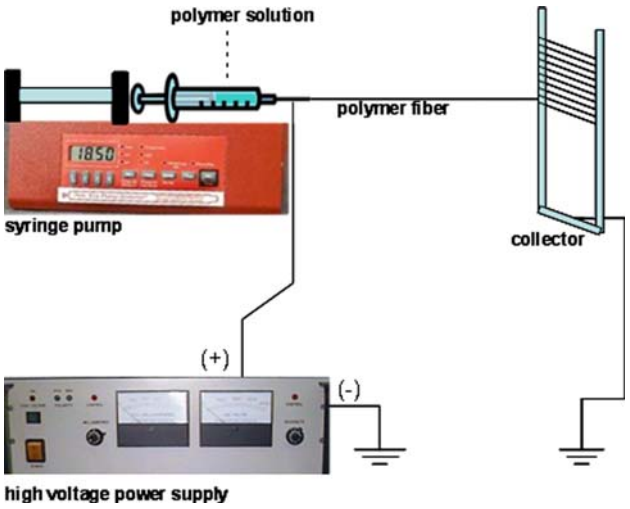

Fig. 1 Electrospinning setup used to obtain aligned microfibrous mats

were: flow rate = 30 μl/min, potential = 18 kV, needle tip to receiver metal frame distance = 24 cm (Fig. 1).

2.2 Preparation of macroporous biodegradable tubings

For preparation of macroporous tubings, a 96:4 (w/w) blend of P(L,DL)LA (70:30) with PGS (MW: 9929 Da, polydispersity index: 1.8) in dioxane was coated on a stainless steel wire and freeze dried. Pore size was calculated from the crosssectional SEM images of the tubings.

2.3 WJ MSC isolation from umbilical cord matrix and its culture

Human Wharton's Jelly MSCs (WJ MSCs) were isolated from umbilical cord matrix. Human umbilical cords were collected from full-term births with informed consent of the mother after either caesarean section or normal delivery and aseptically stored at 4°C in sterile saline until processing. The interval between collection and isolation of WJ MSCs was at most 24 h. To isolate WJ MSCs, the cords were cut into 2- to 4-cm lengths, the vessels were removed and the cords were cut open. The explants were transferred to 6-well plates containing αMEM/Ham F-12 (1:1) with 2% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin and left undisturbed for 2 weeks to allow migration of cells from the explants. WJ MSCs were fed 3 times a week and passaged and cryopreserved.

In order to prove their mesenchymal origin, the cells were subjected to osteogenic and chondrogenic differentiation. The WJ MSCs were cultured in an osteogenic medium consisting of DMEM with 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 10 nM dexamethasone, 50 μg/ml ascorbic acid, and 10 mM β-glycerophosphate for 14 days in an incubator at 37°C. Differentiation to osteoblasts was shown by von Kossa staining, which stains the calcium phosphate mineral deposits in the extracellular matrix to brownish-black upon exposure to UV. For chondrogenic induction, confluent layers of WJ MSCs were supplied with a chondrogenic medium: DMEM with 50 μg/ml ascorbic acid, 1% non-essential amino acids, 5 μg/ml bovine insulin, 10 nM dexamethasone, and 5 ng/ml TGF β1. The cells were grown in this medium for 21 days in an incubator at 37°C and fixed with 4% formaldehyde prior to Alcian Blue staining for extracellular mucosubstances and immunostaining for collagen Type II.

2.4 WJ MSC attachment and proliferation on the aligned microfiber mats

Upon obtaining confluent monolayers, third passage WJ MSCs fed with αMEM/Ham F-12 (1:1) with 2% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin were detached from the flask surface with trypsin, resuspended in αMEM/Ham F-12 (1:1) with 10% FCS, 1% non-essential amino acids and 100 U/ml penicillin and 100 μg/ml streptomycin to 6×10^4 cells/ml, and 3×10^4 cells were seeded on the PPG aligned fiber mats with 1×1 cm^2 area that had collagen type I adsorbed onto them (from 40 μg/ml collagen solution). Cell seeded mats were incubated at 37°C in the CO_2 incubator for 1 and 14 days and cell number on the mats was determined with MTS test at these time points according to previously published procedure [26]. Cell numbers were calculated using a calibration curve that was prepared using the same protocol.

2.5 Analysis of cell alignment and penetration within the mats

The fibrous mats were stained with FITC-labeled Phalloidin from Sigma Aldrich Co. (USA) in order to observe the orientation of cytoskeletal actin filaments, and the cell nuclei were counterstained with propidium iodide (PI). Samples were fixed in formaldehyde (4%) for 30 min at room temperature. The cells were then permeabilized with 0.1% Triton X-100 solution for 5 min at room temperature. Non-specific binding was blocked by incubating at 37°C for 30 min in 1% BSA. The samples were stained with FITC-labeled Phalloidin (1:100 dilution in 0.1% PBS-BSA from stock solution of 0.1 mg/ml FITC-Phalloidin). Finally, the samples were counterstained with PI solution for 10 min at room temperature and washed with PBS. The samples were transferred to microscope slides and observed using a CLSM (Leica DM 2500, Germany) at 488 nm for Phalloidin and 532 nm for PI.

2.6 Assembly of the 3D construct

Electrospun aligned fiber PPG mats were seeded with WJ MSCs, cultured for 14 days and finally rolled around the macroporous biodegradable tubings to obtain the 3D construct. It was designed as such to be able to allow the flow of the cell growth medium through the tubings to provide nutrients for the cells residing within the layers of 3D aligned fiber mats. This arrangement would also allow cell-to-cell communication among the cell layers on the way to get a 3D syncytium.

The electrospun aligned fiber mats (3.5×6 cm^2) were placed in sterile petri plates of 10 cm diameter, sterilized under UV C for 15 min each side, coated with Collagen Type I and then seeded with 6×10^5 WJ MSCs (from 10^5 cells/ml suspension) and cultured for 14 days in αMEM/Ham F-12 (1:1) with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin medium in the CO_2 incubator. The medium was refreshed twice a week. The aligned fiber mats with WJ MSCs grown on them for 14 days were wrapped around the macroporous UV sterilized biodegradable tubings, so that the fibers are parallel to the main axis of the tubings, to form a 3D construct (Fig. 2). It is known that if the distance of a cell to a capillary is more than 200 μm then this cell cannot survive due to oxygen and nutrient diffusion limitations [27]. Keeping this principle in mind, 10 layers of mats, each 20 μm thick, were wrapped around one tubing. Having two tubings enabled us to have a broader construct; one mat (3.5×3 cm^2) was rolled around one tubing and then rolled together with one half rolled mat (3.5×6 cm^2) around another tubing.

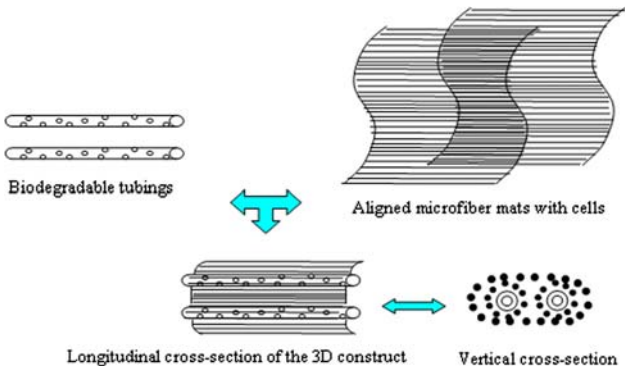

Fig. 2 Scheme of the 3D construct assembly. One unit consisted of two macroporous tubings wrapped in electrospun nanofibrous mats with WJ MSCs grown on them for 14 days

In order to examine with SEM, the constructs were washed with PBS and fixed with 2.5% glutaraldehyde in 0.1 M, pH 7.4 cacodylate buffer for 45 min at room temperature. Prior to SEM analysis, the constructs were washed with distilled H_2O, frozen at −80°C and cross-sections were obtained. The sections were dried at room temperature, and coated with Cd–Au for SEM.

3 Results

3.1 Physical properties of the aligned microfiber mats and macroporous tubings

PPG blend was electrospun to obtain micron-size aligned fiber mats. The experimental parameters were fine tuned until a good Taylor cone and a continuous thin polymer jet were obtained. Conditions were optimized to lead to minimum fiber fusion and bead formation (Fig. 3).

The parallel orientation of the fibers was more pronounced in the initial layers of the mat and this decreased as the mats became thicker. Mats with 12 ± 3 μm thickness and fiber diameters in the range of 1.10–1.25 μm were obtained.

Macroporous biodegradable tubings were obtained using a 96:4 w/w blend of P(L,DL)LA with PGS in dioxane. Macroporous tubings with pore size of 21.29 ± 7.11 μm were obtained by freeze drying the polymer solution coated on a stainless steel wire. The porosity was continuous from

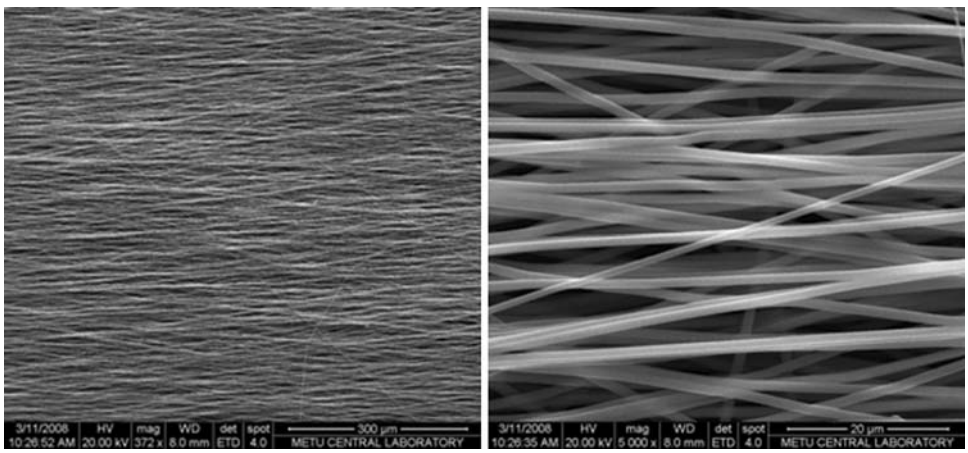

Fig. 3 SEM of electrospun PHBV-P(L-D,L)LA-PGS mats

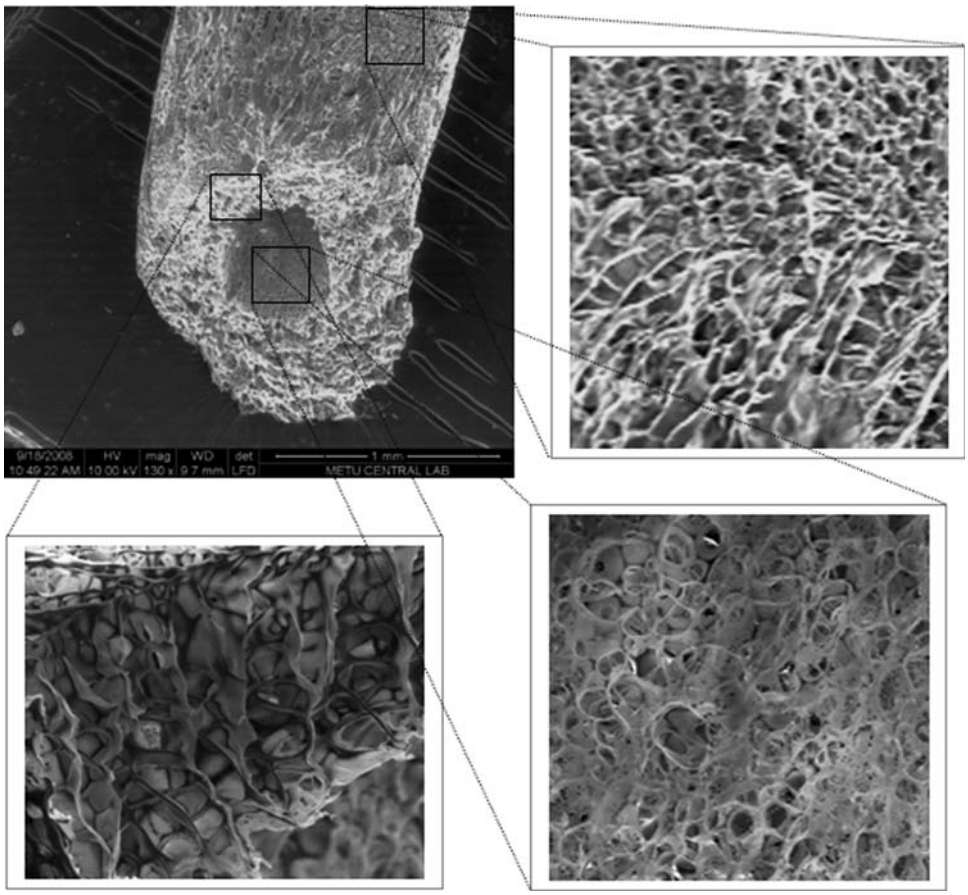

Fig. 4 SEM of the macroporous tubing of P(L,DL)LA and PGS. Insets show close ups (clockwise from the top): outer surface, inner surface, and cross section of the tube

the lumen to the outer surface of the tubings; a property necessary for adequate fluid flow from inside to outside (Fig. 4). The cross-section of the tubing wall examined with SEM revealed the interconnectivity of the porous structure.

3.2 Mesenchymal properties of WJ cells

The cells isolated from human Wharton's Jelly were characterized in terms of their capability to differentiate into cells of mesodermal lineage (e.g. osteoblasts, chondrocytes), to prove their mesenchymal origin. Undifferentiated and WJ MSCs cultured under osteogenic conditions were stained with von Kossa stain, in order to detect the mineral deposition (Fig. 5). It was shown that the WJ cells were able to differentiate into osteoblasts since they deposited Ca–P minerals on the TCPS surface when induced by the osteogenic factors (dexamethasone, ascorbic acid, and β-glycerophosphate). Upon chondrogenic induction a change to a more circular cell morphology was

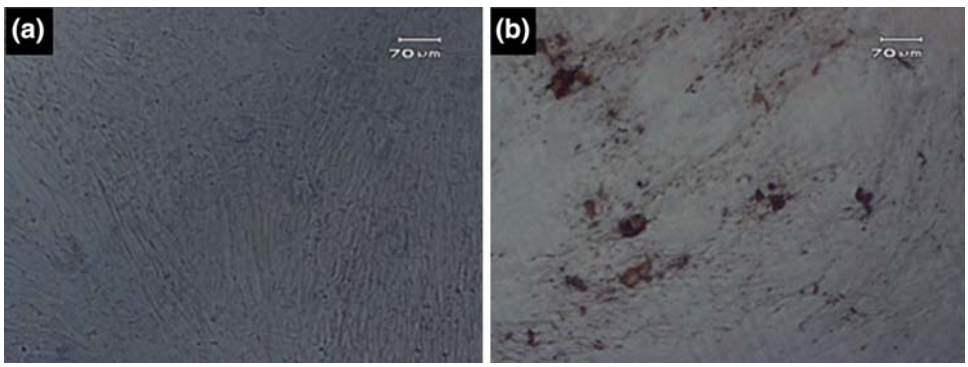

Fig. 5 Light micrographs of WJ MSCs. **a** undifferentiated, **b** differentiated into bone cells. von Kossa staining. (×100)

observed, and the increase in collagen Type II and mucosubstance production was more distinct (data not shown).

3.3 Cell attachment and proliferation on the aligned microfiber mats

Collagen Type I coated aligned fiber mats of PPG blends (1×1 cm^2) were seeded with WJ MSCs and then incubated for up to 14 days at 37°C in the CO_2 incubator, to study the suitability of the property of mats coated with collagen for cell attachment and proliferation. WJ MSCs were seeded on TCPS to serve as positive control.

To determine cell attachment the cell numbers were determined with MTS assay 24 h after cell seeding. Number of cells attached on the TCPS was found to be almost twice that of the cells on the PPG mats (18813 ± 815 vs. 9410 ± 1207). However, the rate of cell proliferation within 14 days was better with the PPG mats. A reasonable explanation for this behavior could be that cells can penetrate easily within the mat through the gaps between the aligned fibers, and find more space to adhere to and occupy. At the end of 14 days almost the same cell number was attained on both surfaces: 115154 ± 5874 on Coll/PPG and 111599 ± 2496 on TCPS.

3.4 Cell alignment and penetration within the mats

The ultimate aim in this study was to produce an artificial myocardial patch with a cellular organization mimicking the native tissue with macroporous tubings in between to provide nutrition to the cells. The WJ MSCs were first seeded on collagen Type I coated aligned fiber mats of PPG (3.5×6 cm^2) and cultured for 14 days to obtain cell sheets where cells aligned parallel to each other and were in contact, as in the native tissue. Confocal micrographs of these cell sheets were obtained to study the cell distribution, alignment and penetration within the mats (Fig. 6). The micron-sized electrospun, parallel fibers of the polymer blend were effective in cell alignment (Fig. 6a) and were soft enough to be retracted by the cells; this softness of the cell carrier designed for use in cardiac tissue engineering is of utmost importance because it makes it possible for cardiomyocytes to contract. Especially in the cross section of the mat it is seen that cells have penetrated deep within the mat through the fiber interstices, occupying the

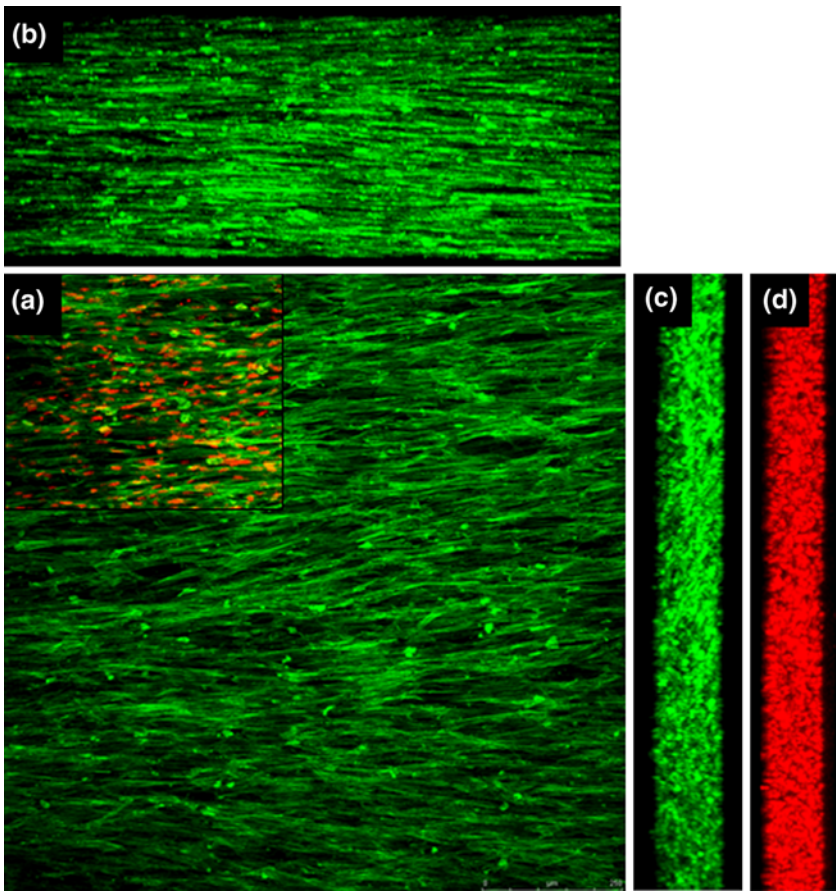

Fig. 6 Confocal micrographs of WJ MSCs grown on aligned fiber PPG mats. **a** top view, filamentous actin, **b** cross sectional view, filamentous actin alignment (X ~60°), **c** cross sectional view, filamentous actin (Y 90°), **d** cross sectional view, cell nuclei (Y 90°). Filamentous actin stained with FITC-Phalloidin (green), cell nuclei stained with PI (red). Time: 14 days. (Color figure online)

full thickness of the mat, and at least 8–9 cell layers could be formed within the mat (Fig. 6d).

3.5 Analysis of the 3D construct

To assemble the 3D construct, two aligned fiber mats on which WJ MSCs were grown for 14 days were wrapped around two biodegradable tubings, so that the fibers and tubings are parallel to each other (Fig. 7). The tubings also served as a supporting component in obtaining closely packed fiber sheets (Fig. 7c).

Ten layers of mat could be wrapped around one tubing, and the final construct, with the 2 tubings, was 3.5 mm wide, 2 mm tall and 3.5 cm long. The dimensions of the construct could be arranged by changing the number of mat layers. SEM of the sections of the 3D construct showed that the mat thickness was 20 μm and the interlayer distance ca. 20 μm. So in this construct, 10 layers of mat with 20 μm mat thickness and a 20 μm spacing among layers around one tubing constituted a 400 μm thick structure with the cells in the mid layers being not further than 200 μm to a nutrition source. Cells were found to spread among the fibers of the mat layers, but were not as easy to discern in SEM images as was in the confocal microscopy images.

4 Discussion

Cardiac muscle contracts like a syncytium owing to the multicellular assembly of myofibers oriented parallel to each other, connected end-to-end via intercalated disks in the longitudinal direction and side-to-side in the transverse direction. It is this cellular arrangement that integrates individual contraction into a pumping action and indeed generating myofiber disarrays is known to cause arrhythmia. It was proposed in this study that it is possible to obtain in vitro a 3D cell organization similar to that of cardiomyocytes in the native myocardium by aligning the cells on microfibrous mats and subsequent stacking of these cell sheets.

The very limited ability of adult cardiomyocytes to proliferate has triggered an intense search for progenitor cells that can replace damaged myocardium. It is clear that natural cardiomyocyte regeneration—including differentiation of progenitors residing in the myocardium or the recruitment of stem cells from outside (e.g., from endothelial cells or a niche in the bone marrow)—is insufficient to overcome cardiomyocyte death in the acutely or chronically damaged heart [28]. Whole bone marrow mononuclear cell transplants are already under clinical investigation, but the mechanism of improvement in cardiac function is not clear [29, 30]. Mesenchymal stem cells with a capability to differentiate into cells of mesodermal lineage (osteoblasts, chondrocytes, adipocytes) can be derived from Wharton's Jelly of the human umbilical cord in very high numbers [31, 32]. Previous studies with Wharton's Jelly MSCs indicate the possibility of their being more primitive relative to bone marrow MSCs [31, 33]. They were investigated for their potential to differentiate into cardiomyocytes by treating with 5-azacytidine or maintaining in cardiomyocyte-conditioned medium and it was found that both treatments resulted in expression of the cardiomyocyte markers N-cadherin, cardiac troponin I, connexin 43, α-actinin, and desmin [33]. The therapeutic potential of human umbilical cord derived stem (UCDS) cells in a rat myocardial infarction model was investigated by Wu et al., and statistically significant improvement of cardiac function was observed [34]. Some of the UCDS cells expressed cardiac troponin-T, von Willebrand factor, and smooth muscle actin, indicating regeneration of damaged myocardium by cardiomyocytic, endothelial, and smooth muscle differentiation. The capillary and arteriole

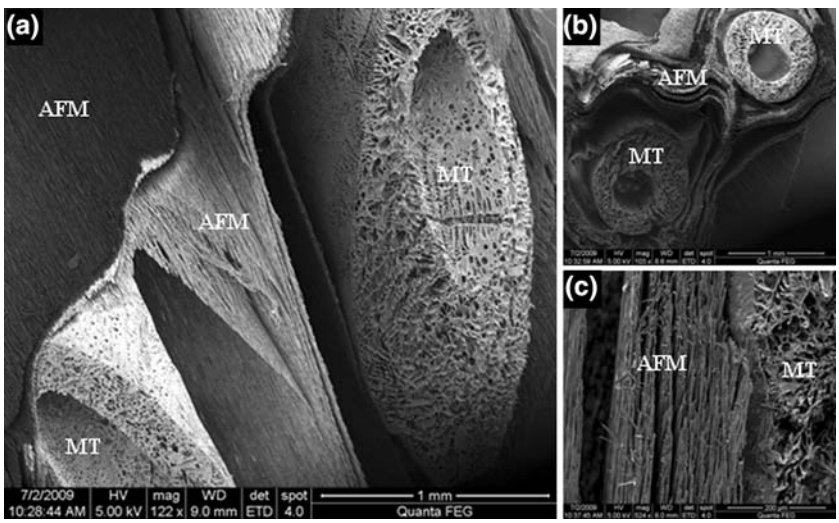

Fig. 7 SEM of the sections from the 3D construct. **a** Diagonally sectioned macroporous tubings wrapped in aligned fiber mats **b** cross section of the 3D construct, **c** longitudinal cross section of the 3D construct showing the mat-tubing interface. MT: Macroporous tubing; AFM: Aligned fiber mats

density were also markedly increased. WJ MSCs have been used in generation of living tissue engineered arteries [35] and heart valves [36, 37]. Valve leaflets showed mature layered tissue formation and extracellular matrix production comparable with that of native tissues. Therefore, if differentiation pathways of WJ MSCs could be elucidated, they can be used in overcoming the lack of living autologous replacements. Human WJ MSCs were used in this study as the cellular component of the 3D myocardial construct considering their potential to improve cardiac function either through cytokine production or differentiation to cardiac cells.

One important finding of this study is that electrospun, parallel microfibers of PPG are effective in aligning the cells and are soft enough for retraction by the cells; softness of the cell carrier used in cardiac tissue engineering is of utmost importance to allow cardiomyocyte contraction. Biodegradable polyesters like P(L-D,L)LA and PHBV are biocompatible polymers widely used as materials for scaffold preparation in tissue engineering applications. However, their hydrophobicity and rigidity restrict their use in this field. It is necessary to have polymers with higher flexibility for use in the engineering of soft tissues like heart. PGS, being a hydrophilic and low molecular weight polymer could serve as a plasticizer, and was blended with these polymers to augment their hydrophilicity and softness, in order to make them suitable for cell attachment and contraction. Knowing that addition of PGS will decrease the mechanical properties, and to not change the properties extensively, PGS incorporation was kept limited. Having a low molecular weight, PGS is expected to leave the structure in short time when implanted in vivo, thus making scaffold degradation faster owing to the increase in surface area of the polymer exposed to extracellular fluids.

The aligned cell sheets in the fibrous mats were successfully stacked around the biodegradable macroporous tubings to obtain a 3D construct that can be perfused with growth media from its central portion. Five layers of electrospun mats of poly(epsilon-caprolactone) with random, unaligned fiber orientation that were seeded with cardiomyocytes from neonatal rats were successfully overlayed by Ishii et al. to form a 3D graft where individual layers adhered intimately, and morphologic and electrical communication between the layers was established [38]. By stacking up the aligned cell sheets in our study, it was possible to decrease the distance between layers to 20 μm, a distance which can be spanned by the cells and an interlayer cell-to-cell contact is feasible.

The 3D construct had a relatively loose structure, which may be turned into an advantage. The low ischemia tolerance of thick, terminally differentiated cardiac muscle samples is expected to impede their application in cardiac regeneration in vivo [39]. Eventually, loose but electrically and functionally interconnected cardiac myocyte networks with a low degree of differentiation may have a better chance to survive in vitro and after implantation in vivo.

For a clinically relevant effect, the diameter of the patch should be close to in vivo physiological muscle diameter which is ca. 6 mm. In vitro formation of primitive capillary structures may be supportive but may not be efficient in providing immediate blood circulation to such large engineered tissue grafts in vivo [40]. After obtaining an endothelial cell layer in its lumen and ensuring a proper inner diameter and mechanical strength, the macrovasculature that is incorporated into the 3D myocardial construct in this study may serve as a vasculature to enable surgical anastomoses to the coronary arteries, to provide sufficient blood flow to the region. For treatment of small size myocardial infarctions, the patch can replace the dead tissue by insertion and can be stitched to the viable heart muscle [41]; a seamless electrical and structural integration of the biological graft into the recipient heart is necessary for functional engraftment. Alternatively, the patch can be stitched on top of the ischemic region, i.e. on the ventricular wall [42, 43]. Another way of stabilizing the construct at the target site could be through use of fibrin glue and a biodegradable ventricular restraint [43] could be provided until the full mechanical strength of the cardiac tissue is matched.

To conclude, a novel method was developed in this study to obtain a thick myocardial patch with a structure similar to that of the native tissue and capability to grow and without a need for complex electrical stimulations to align the cells in 3D. This represents an important step towards obtaining a thick autologous myocardial patch for ventricular restorations.

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References

1. Vander AJ, Sherman JH, Luciano DS. Human physiology. 6th ed. New York: McGraw-Hill Inc; 1994.
2. Parker KK, Ingber DE. Extracellular matrix, mechanotransduction and structural hierarchies in heart tissue engineering. Phil Trans R Soc B. 2007;362:1267–79.
3. Dor V, Sabatier M, Montiglio F, Civaia F, DiDonato M. Endoventricular patch reconstruction of ischemic failing ventricle: a single center with 20 years experience, advantages of magnetic resonance imaging assessment. Heart Fail Rev. 2004;9(4):269–86.
4. Mirensky TL, Breuer CK. The development of tissue-engineered grafts for reconstructive cardiothoracic surgical applications. Pediatr Res. 2008;63(5):559–68.
5. Passier R, Mummery C. Origin and use of embryonic and adult stem cells in differentiation and tissue repair. Cardiovasc Res. 2003;58:324–35.

6. Dengler TJ, Katus HA. Stem cell therapy for the infarcted heart ('cellular cardiomyoplasty'). Herz. 2002;27:598–610.
7. Murry CE, Soonpaa MH, Reinecke H, et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature. 2004;428:664–8.
8. Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature. 2004;428:668–73.
9. Zammaretti P, Jaconi M. Cardiac tissue engineering: regeneration of the wounded heart. Curr Opin Biotechnol. 2004;15:430–4.
10. Fedak PWM, Weisel RD, Verma S, Mickle DAG, Li RK. Restoration and regeneration of failing myocardium with cell transplantation and tissue engineering. Semin Thorac Cardiovasc Surg. 2003;15(3):277–86.
11. Zund G, Breuer CK, Shinoka T, et al. The in vitro construction of a tissue engineered bioprosthetic heart valve. Eur J Cardiothorac Surg. 1997;11:493–7.
12. Akhyari P, Fedak PW, Weisel RD, et al. Mechanical stretch regimen enhances the formation of bioengineered autologous cardiac muscle grafts. Circulation. 2002;106:I137–42.
13. Kofidis T, Akhyari P, Wachsmann B, et al. A novel bioartificial myocardial tissue and its prospective use in cardiac surgery. Eur J Cardiothorac Surg. 2002;22(2):238–43.
14. Kofidis T, Lenz A, Boublik J, et al. Bioartificial grafts for transmural myocardial restoration: a new cardiovascular tissue culture concept. Eur J Cardiothorac Surg. 2003;24:906–11.
15. Zimmermann WH, Schneiderbanger K, Schubert P, et al. Tissue engineering of a differentiated cardiac muscle construct. Circ Res. 2002;90:223–30.
16. Zimmermann WH, Eschenhagen T. Cardiac tissue engineering for replacement therapy. Heart Fail Rev. 2003;8:259–69.
17. Zimmermann WH, Melnychenko I, Eschenhagen T. Engineered heart tissue for regeneration of diseased hearts. Biomaterials. 2004;25:1639–47.
18. Radisic M, Park H, Shing H, et al. Functional assembly of engineered myocardium by electrical stimulation of cardiac myocytes cultured on scaffolds. PNAS. 2004;101(52):18129–34.
19. Radisic M, Euloth M, Yang L, Langer R, Freed LE, Vunjak-Novakovic G. High density seeding of myocyte cells for tissue engineering. Biotechnol Bioeng. 2003;82:403–14.
20. Sodian R, Hoerstrup SP, Sperling JS, et al. Evaluation of biodegradable three-dimensional matrices for tissue engineering of heart valves. ASAIO J. 2000;46(1):107–10.
21. Hoerstrup SP, Sodian R, Daebritz S, et al. Functional living Trileaflet heart valves grown in vitro. Circulation. 2000;102(suppl III):III 44–49.
22. Park H, Radisic M, Lim JO, Chang BH, Vunjak-Novakovic G. A novel composite scaffold for cardiac tissue engineering. In Vitro Cell Dev Biol Anim. 2005;41(7):188–96.
23. Evans HJ, Sweet JK, Price RL, Yost M, Goodwin RL. Novel 3D culture system for study of cardiac myocyte development. AJP-Heart Circ Physiol. 2003;285:570–8.
24. McDevitt TC, Woodhouse KA, Hauschka SD, Murry CE, Stayton PS. Spatially organized layers of cardiomyocytes on biodegradable polyurethane films for myocardial repair. J Biomed Mater Res A. 2003;66(3):586–95.
25. Zong X, Bien H, Chung CY, et al. Electrospun fine-textured scaffolds for heart tissue constructs. Biomaterials. 2005;26:5330–8.
26. Kose GT, Korkusuz F, Korkusuz P, Purali N, Ozkul A, Hasirci V. Bone generation on PHBV matrices: an in vitro study. Biomaterials. 2003;24(27):4999–5007.
27. Morritt AN, Bortolotto SK, Dilley RJ, et al. Cardiac tissue engineering in an in vivo vascularized chamber. Circulation. 2007;115:353–60.
28. Nakamura T, Schneider MD. The way to a human's heart is through the stomach: visceral endoderm-like cells drive human embryonic stem cells to a cardiac fate. Circulation. 2003;107:2638–9.
29. Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation. 2002;106:1913–8.
30. Perin EC, Dohmann HF, Borojevic R, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. Circulation. 2003;107:2294–302.
31. Weiss ML, Medicetty S, Bledsoe AR, et al. Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. Stem Cells. 2006;24:781–92.
32. Karahuseyinoglu S, Cinar O, Kilic E, et al. Biology of stem cells in human umbilical cord stroma: in situ and in vitro surveys. Stem Cells. 2007;25:319–31.
33. Wang HS, Hung SC, Peng ST, et al. Mesenchymal stem cells in the Wharton's Jelly of the human umbilical cord. Stem Cells. 2004;22(7):1330–7.
34. Wu KH, Zhou B, Yu CT, et al. Therapeutic potential of human umbilical cord derived stem cells in a rat myocardial infarction model. Ann Thorac Surg. 2007;83(4):1491–8.
35. Hoerstrup SP, Kadner A, Breymann C, et al. Living autologous pulmonary artery conduits tissue engineered from human umbilical cord cells. Ann Thorac Surg. 2002;74:46–52.
36. Schmidt D, Mol A, Neuenschwander S, et al. Living patches engineered from human umbilical cord derived fibroblasts and endothelial progenitor cells. Eur J Cardiothorac Surg. 2005;27(5):795–800.
37. Schmidt D, Mol A, Neuenschwander S, et al. Engineering of biologically active living heart valve leaflets using human umbilical cord-derived progenitor cells. Tissue Eng. 2006;12(11):3223–32.
38. Ishii O, Shin M, Sueda T, Vacanti JP. In vitro tissue engineering of a cardiac graft using a degradable scaffold with an extracellular matrix-like topography. J Thorac Cardiovasc Surg. 2005;130(5):1358–63.
39. Zimmermann WH, Didie M, Döker S, et al. Heart muscle engineering: an update on cardiac muscle replacement therapy. Cardiovasc Res. 2006;71(3):419–29.
40. Zimmermann WH, Cesnjevar R. Cardiac tissue engineering: implications for pediatric heart. Surgery Pediatr Cardiol. 2009;30:716–23.
41. Wei HJ, Chen CH, Lee WY, et al. Bioengineered cardiac patch constructed from multilayered mesenchymal stem cells for myocardial repair. Biomaterials. 2008;29:3547–56.
42. Lesman A, Habib M, Caspi O, Gepstein A, Arbel G, Levenberg S, et al. Transplantation of a tissue-engineered human vascularized cardiac muscle. Tissue Eng Part A. 2009. doi:10.1089/ten.TEA.2009.0130.
43. Christman KL, Lee RJ. Biomaterials for the treatment of myocardial infarction. J Am Coll Cardiol. 2006;48:907–13.