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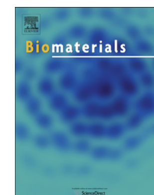

Flexible nanofilms coated with aligned piezoelectric microfibers preserve the contractility of cardiomyocytes

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ABSTRACT

The use of engineered cardiac tissue for high-throughput drug screening/toxicology assessment remains largely unexplored. Here we propose a scaffold that mimics aspects of cardiac extracellular matrix while preserving the contractility of cardiomyocytes. The scaffold is based on a poly(caprolactone) (PCL) nanofilm with magnetic properties (MNF, standing for magnetic nanofilm) coated with a layer of piezoelectric (PIEZO) microfibers of poly(vinylidene fluoride–trifluoroethylene) (MNF+PIEZO). The nanofilm creates a flexible support for cell contraction and the aligned PIEZO microfibers deposited on top of the nanofilm creates conditions for cell alignment and electrical stimulation of the seeded cells. Our results indicate that MNF+PIEZO scaffold promotes rat and human cardiac cell attachment and alignment, maintains the ratio of cell populations overtime, promotes cell-cell communication and metabolic maturation, and preserves cardiomyocyte (CM) contractility for at least 12 days. The engineered cardiac construct showed high toxicity against doxorubicin, a cardiotoxic molecule, and responded to compounds that modulate CM contraction such as epinephrine, propranolol and heptanol.
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1. Introduction

Several scaffolds that mimic the architecture and biophysics of the myocardium's extracellular matrix (ECM) have been proposed in the last decade, with the aim of generating engineered cardiac tissue [1–3]. Most of the cardiac tissue engineering approaches have used preformed scaffolds based on polymers such as poly(glycerol sebacate) [4] and macroporous nanowire electronic scaffolds [5], as well as hydrogels, including alginate [6], fibrin [7], tropoelastin [8] and collagen [9]. These scaffolds have impact in cardiac cell attachment/alignment, electrical signal propagation and cell maturation. Cardiac cells respond to scaffold stiffness and topographical cues by changing their cytoskeleton and morphology [10,11]. Therefore, scaffolds that favor cardiac cell alignment have been shown to improve cell-cell interaction and tissue cohesion [11] as well as action potential duration and transient calcium direction [12]. On the other hand, scaffolds that favor electrical signal propagation between cardiac cells lead to a cardiac tissue construct that has synchronous contraction and shows high levels of proteins involved in muscle contraction and electrical coupling [6,13]. Moreover, external electrical fields applied to scaffolds seeded with cardiac cells originate tissue constructs with higher maturation [14,15]. In this case, the number [14] and organization [15] of mitochondria between myofibrils is higher, however, the cell's metabolic profile has not been evaluated. Overall, although the molecular mechanisms behind the inductive properties of scaffolds

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in cardiac biology are only partially understood, it is expectable that scaffolds that couple mechanical, anisotropy and electric properties may foster the development of a functional cardiac tissue [16].

Engineered cardiac tissue may be a relevant platform for cardiotoxicity and drug screening studies. Cardiotoxicity assessment is of paramount importance in the development of new drugs. Cardiac toxicity has been implicated in 28% of drug withdrawals over the last 30 years [17] and it represents one of the most stringent exclusion criteria in the licensing process [18]. Although the use of CMs in a culture dish or within microfluidic systems [19] has been reported, the use of engineered cardiac tissue for automatized high-throughput drug screening/toxicology assessment remains largely unexplored. One example has been reported which encompasses a miniaturized and automated method based on engineered heart tissue [20]. Unfortunately, more than 20% of the cardiac cells died over a period of 3 days and the variability in the force and frequency between cardiac constructs was relatively high. The development of engineered cardiac tissue for drug screening requires the creation of scaffolds that are easy to produce and miniaturize, flexible, and able to preserve the contractility of CMs, ideally in the absence of an external electrical stimulation since it will facilitate its implementation in any laboratory.

Here we propose a scaffold that mimics some aspects of cardiac ECM (Supplementary Fig. 1A) while preserving for at least 12 days the contractility of fetal rat and human CMs derived from pluripotent stem cells. The scaffold is based on a PCL nanofilm (MNF) coated with PIEZO microfibers of poly(vinylidene fluoride–trifluoroethylene) (PVDF-TrFE). When a mechanical force is applied to a piezoelectric material, a change in the polarization density occurs due to the shifting or rotation of the constitutive dipole crystals. As a result, a transient electric charge is generated. Therefore, PIEZO fibers may act as sinoatrial node cells, which in the native tissue are responsible for cardiac pacemaking [21]. We hypothesize that (i) the MNF provides a flexible support for cell contraction and (ii) the aligned PIEZO fibers deposited on top of the MNF create conditions for cell alignment and electrical stimulation of the seeded cells.

2. Materials and methods

2.1. Preparation and characterization of MNF and MNF+PIEZO scaffolds

2.1.1. Preparation of MNF and MNF+PIEZO scaffolds

MNFs were prepared by the combined use of a sacrificial layer and spin coating [22]. Initially, a solution of PVA (1% w/v, in water; 1 mL; Mw = 25.000, 88% hydrolysed; Polysciences, Inc) was added to the surface of a silicon wafer (400 μm thick, 2 × 2.5 cm; Primewafers) and spin-coated (Spincoat G3P-8, Pi-Kem) at 4000 rpm for 20 s. Then a suspension of superparamagnetic magnetite/maghemite nanoparticles (10 mg/mL; EMG1300; diameter of 10 nm; FerroTec Co., USA) suspended in a PCL solution (20 mg/mL in chloroform; Mw = 80.000, Aldrich) was added to the wafer and spin-coated according to the previous spinning parameters. For MNF experimental group, the polymer-coated wafer was immersed in water, the PVA sacrificial layer was dissolved, releasing a freely suspended insoluble nanofilm. For MNF+PIEZO experimental group, the polymer-coated silicon wafers were fixed to a homemade rotating collector for the deposition of aligned fibers (rotator motor: Heidolph RZR2020; rotative collector was made as an aluminium circular frame with the following measurements: 100 mm of diameter, 18 mm of thickness). The fibers were generated from a solution of poly(vinylidene fluoride–trifluoroethylene) (70:30 w; Solvay) dissolved in methylethylketone (Labor Spirit) according to a methodology described elsewhere [23]. The parameters used were: 10–12 kV voltage, 20% (w/v) polymer solution concentration, 12 cm tip-to-collector distance, 2 mL/h injection rate, 40–50% relative humidity at room temperature and 2.000 rpm collector's rotation speed. The selected collection time was 4 min.

2.1.2. Scaffold characterization: atomic force microscopy (AFM) analyses

Imaging was performed with a Bruker Innova Scanning Probe Microscope (Bruker) in dry state, operating in tapping mode, with oxide-sharpened silicon probes (RTESPA-CP, Veeco Instruments Inc.) at a resonant frequency of 300 kHz. The sample was scanned across the edge of the scratch over a 50 μm × 50 μm area, recording 128 × 128 samples. The resulting scan data were elaborated using the Gwyddion SPM analysis tool (http://gwyddion.net). Scan data were leveled with the facet level tool to remove sample tilt, and then the film thickness was evaluated as the difference between the average heights of a region of interest (ROI) selected on the nanofilm surface and the average height of the ROI on the silicon wafer.

2.1.3. Scaffold characterization: mechanical tests

Mechanical properties were evaluated by measuring their strain in response to an applied unidirectional stress. An INSTRON 4464 Mechanical Testing System was used, equipped with a ±10 N load cell. Traction tests were performed on ten samples for each sample typology. The nanofilms were detached from the substrate, by immersing them in water, then gently fished up and allocated between two aluminium clamps. All specimens were pulled at a constant speed of 5 mm/min, until reaching sample failure. Data were recorded at a frequency of 100 Hz. The stress was calculated as the ratio between the load and the cross-section area of a tensile specimen, while the strain was calculated as the ratio between its extension and its initial length. The Young's modulus for each tested sample was extracted from its stress/strain curve.

2.1.4. Scaffold characterization: scanning electron microscopy (SEM) analyses

A Phenom Pro tabletop SEM was used for analyses. At least 3 images were taken per each sample. Fiber diameter was determined by ImageJ software on SEM images. Fiber anisotropy was determined by a protocol described elsewhere [24] on SEM images. In this case, the orientation and anisotropy of fibrillar structures in SEM images was calculated using the image analysis software ImageJ, where computation on the basis of the gradient of pixel intensity level is performed over a region of interest in the image.

2.1.5. Scaffold characterization: assessment of impedance and the direct piezoelectric effect

Scaffold impedance spectra were analyzed by using an impedentiometer (Agilent) in the 40 Hz–20 kHz frequency range. The capacity of piezoelectric microfibers to generate charge (direct piezoelectric effect) was determined using a custom designed flexible gold electrodes connected to a mix signal oscilloscope (InfiiVision, Agilent Technologies). Deformation of the PIEZO microfibers was performed on the surface of two Delrin cylinders having two different curvatures: curvature I – 84 mm cylinder diameter; curvature II – 105 mm cylinder diameter. A stack of PIEZO microfibers was prepared (mass of the PVDF-TFRE fiber layer stack = 4.4 mg), and a flexible polyethylene thin-film was used as non-piezoelectric control condition (mass of control thin-film = 7.8 mg). The samples were placed in contact with the flexible gold electrodes. Next, this setup was fixed on cylinders and manually pressed repeatedly against the cylinder's surface for a short period of time. Variations of charge generation were registered during this period. At least 10 replicate measurements were performed per cylinder. The total voltage generated during each period of stimulation was calculated and normalized by the mass of

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