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Plasma-functionalized electrospun matrix for biograft development and cardiac function stabilization

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ABSTRACT

Cardiac tissue engineering approaches can deliver large numbers of cells to the damaged myocardium and have thus increasingly been considered as a possible curative treatment to counteract the high prevalence of progressive heart failure after myocardial infarction (MI). Optimal scaffold architecture and mechanical and chemical properties, as well as immune- and bio-compatibility, need to be addressed. We demonstrated that radio-frequency plasma surface functionalized electrospun poly (ε-caprolactone) (PCL) fibres provide a suitable matrix for bone-marrow-derived mesenchymal stem cell (MSC) cardiac implantation. Using a rat model of chronic MI, we showed that MSC-seeded plasma-coated PCL grafts stabilized cardiac function and attenuated dilatation. Significant relative decreases of 13% of the ejection fraction (EF) and 15% of the fractional shortening (FS) were observed in sham treated animals; respective decreases of 20% and 25% were measured 4 weeks after acellular patch implantation, whereas a steadied function was observed 4 weeks after MSC-patch implantation (relative decreases of 6% for both EF and FS).

1. Introduction

Despite significant improvements in medical and surgical interventions, cardiovascular diseases remain the major cause of death in industrialized countries. Consequently, the urgent need for alternative therapies has been addressed, including strategies focusing on the possible reverse remodelling of the cardiac structure and recovery of the contractile function [1]. During the past decade, myocardial stem cell injection has provided encouraging clinical outcomes and might become an accepted therapy [2]. However, the ischemic myocardium represents a hostile environment and successful clinical implementation of cell therapy is thus hampered by several challenges, including poor survival of injected cells and their limited retention and engraftment [3–5].

The use of biomaterials to engineer exogenous micro-tissues for cell delivery may be an attractive strategy to promote reproducible prolonged cell survival and retention [6,7]. Polymeric matrices aim at providing a favorable environment for ex vivo cell adhesion and growth, and for facilitated implantation onto the ischemic myocardium [4,8–13].

However, the pertinence of acellular patches for cardiac function improvement has been demonstrated [14,15]. Epicardial implantation of acellular patches after myocardial injury resulted in left ventricular (LV) function improvement and limited progression of LV dilation. Therefore, the relevance of cells implanted together with a scaffold remains an open question. The systematic comparison of therapeutic efficacy of the scaffold alone or with cells is of paramount importance to provide specific answers for any new patch.

Nevertheless, the choice of material, the consequential role of cell–matrix interactions and the vascularization of the biograft are major aspects to consider. Recent studies have highlighted the suitability of synthetic electrospun-based scaffolds that can be fine-tuned, with respect to their biocompatibility and their architectural, mechanical and chemical properties, in order to provide an optimal microenvironment for cell growth [16,17]. Electrospun fibres have been successfully used for the engineering of soft tissues [18] or cardiac valves [19]. Their application in myocardial tissue engineering is, however, still in its infancy. In vitro

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evaluations underpinned the suitability of electrospun membranes for cardiomyocyte guidance and differentiation as well as maintenance of their contractile function [20–22]. The fibrous, anisotropic architecture of electrospun scaffolds compared to solid substrates proved superior for cardiomyocyte maturation and, in particular, cell infiltration and multi-layered tissue formation [23–25].

Poly(ε-caprolactone) (PCL), a material approved by the US Food and Drug Administration, holds great potential for successful implementation due to its good immune-tolerance, high availability and practicable processability by electrospinning [26,27]. Previous studies highlighted the versatility of electrospun PCL to create synchronously beating multi-layered cardiac constructs [28,29]. However, in vivo evaluations of the efficiency of cell-seeded electrospun-based matrices applied onto infarcted myocardium are still lacking.

Due to the strongly hydrophobic nature of electrospun PCL matrices, an enhanced biological interface, holding particular importance in cell–matrix interactions, must be generated by surface functionalization. Several methods, among them blending of synthetic polymers with ECM proteins, are known to induce enhanced cell adhesion [30,31]. With respect to clinical application, naturally occurring proteins raise concerns due to their xenogenic origin, potential contamination and low availability at high costs [32]. To this end, the substitution of proteins or peptides by functional groups generated in wet-chemical or plasma-based processes presents a versatile advantage. Adapted process parameters during plasma polymerization allow for tissue specific functionalities and the formation of stable surface coatings [16,33,34].

In the current study, we aimed at developing a new plasma-coated, parallel-oriented electrospun PCL substrate that structurally and chemically provides adhesion sites for cellular attachment, is immune-tolerant and guarantees scaling up possibilities for translational research. The designed cardiac patch, acellular vs. seeded with mesenchymal stem cells (MSCs), was evaluated in a rodent model of chronic myocardial infarction (MI). We provide evidence that the designed matrices provide a safe and efficient substrate for MSC growth and delivery thereof to the ischemic heart. We furthermore report that this treatment induces improved performance of infarcted hearts with attenuation of LV dilatation and stabilized cardiac function.

2. Materials and methods

2.1. Substrate production and characterization

Fibrous substrates were produced and characterized as previously established in our group [16]. In short, a 15% w/v PCL (Sigma–Aldrich, Buchs, Switzerland) spinning solution was prepared by dissolving PCL in a mixture of chloroform/methanol (9:1) (Sigma–Aldrich, Buchs, Switzerland). Solutions were processed by electrospinning at a flow rate of 30 μl min^{-1}, with an applied field strength of 1 kV cm^{-1}, and collected on a rotating drum (~1000 rpm).

Electrospun patches were functionalized using a radio-frequency (RF) plasma process. An oxygen functional hydrocarbon layer (a-C:H:O) was deposited according to previously established protocols [35]. Briefly, 4 standard cubic centimetre per minute (sccm) ethene (C_2H_4), 24 sccm carbon dioxide (CO_2) and 50 sccm argon (Ar) were introduced into a symmetric reactor chamber. All gases were of 99.9 vol.% purity and provided by Carbagas, Gümligen, Switzerland. RF power input was set to 34 W at a chamber pressure of 0.1 mbar and plasma exposure time of 15 min. Surface functionalization was characterized by X-ray photoelectron spectroscopy (XPS) analysis as previously reported [16].

Wettability was assessed by static contact angle measurements (Krüss GmbH, Hamburg, Germany). A drop of 10 μl distilled water was deposited on the fibrous substrates. Measurements were repeated three times.

Substrate morphology and fibre diameter (based on 100 individual measurements, Image J, free download under http://rsb-web.nih.gov/ij/) were characterized by scanning electron microscopy (SEM). Prior to imaging, substrates were gold-sputtered (Polaron Equipment, SEM coating Unit E5100, Kontron AG, Switzerland; 5 mA, 1 mbar, 7 min) and recorded on a Hitachi S-4800 (Hitachi High-Technologies, US, Illinois, USA) at an accelerating voltage of 2 kV and 10 μA current flow.

Elasticity (E modulus) of the patches and yield strength were assessed by stress–strain measurements on an MTS 858 Mini Bionix (Bionix systems, MTS Eden Prairie, USA) equipped with a 25 N load cell and a displacement rate of 0.15 mm s^{-1}. Substrates were pre-incubated in culture medium or seeded with MSCs, respectively, prior to measurements.

2.2. In vitro evaluation

2.2.1. MSC isolation and characterization

All experiments were carried out with MSCs obtained from rat bone marrow. Briefly, the bone marrow from the femur and tibia of 5 male Lewis rats (200 g) was flushed with sterile phosphate buffered saline (PBS; Spitalpharmazie, Inselspital Bern, Switzerland), centrifuged and resuspended in red blood cell lysis buffer (Gentra Systems Inc.; Minneapolis, USA). After 10 min incubation, samples were centrifuged, resuspended in fresh medium and seeded in culture flasks. MSCs were selected upon adherence and medium was removed after 2 days. The medium was then transferred into a new flask in order to further collect adherent cells.

Cell culture was performed under standard conditions on tissue treated polystyrene (TCPS; TPP Omnilab, Mettmenstetten, Switzerland and BD, Biosciences, San José, USA), at 37 °C and 5% CO_2 in a humidified incubator (Thermo Forma, Model 371, Thermo Fisher Scientific, Waltham, USA). MSCs were expanded in growth medium (Dulbecco's modified Eagle Medium, Gibco, Invitrogen, Carlsbad, USA) supplemented with 20% fetal bovine serum (FBS; PAA clone, Connectorate AG, Dietikon, Switzerland) and 1.25% penicillin/streptomycin solution (P/S; 100 μg ml^{-1}, Gibco, Invitrogen, Carlsbad, USA). MSCs were maintained in culture and expanded for 1 week. Medium was changed every second day. Accutase was used to detach the cells prior to culture on substrates for 1 week.

MSCs were characterized by fluorescence activated cell sorting (FACS; FACScan, BD, Biosciences, San José, USA) using PE-labelled anti CD90, FITC-labelled anti CD45 and PE-labelled anti CD31 (all BD, Biosciences, San José, USA). Cells were $CD90^{(+)}$ (34.3%), $CD45^{(-)}$ (0%), $CD31^{(-)}$ (0%), $CD29^{(+)}$ (25.7%), $CD44^{(+)}$ (42.9%), $Sca1^{(-)}$ (0%) and $cKit^{(-)}$ (0%). MSCs retained their capacity to differentiate into adipocytes and cardiac troponin $C^{(+)}$ cells when cultured in appropriate differentiation media.

2.2.2. Culture on the substrates

Cell seeding on the substrates was accomplished according to previously established methods from our group [36]. Briefly, bottoms of 12-well culture dishes were coated with silicone (Sylgard-184 two component silicone, Sylgard, Dow Corning Corporation, Midland, USA). Patches of 15 × 10 × 0.1 mm were fixed by stainless steel minutiae insect pins (EntoSPHINX, Pardubice, Czech Republic) on the silicone coating and sterilized under UV light overnight. MSCs were seeded at a density of 2×10^6 cells per patch. A 200 μl cell suspension was deposited at the surface of the patch and cells were allowed to adhere for 3 h prior to medium addition. Substrates were cultured for 7 days in growth medium prior to implantation.

Cell viability was assessed 24 h post-seeding and at the day of implantation with an MTT assay (3-(4,5-dimethylthiazol-2-yl)-

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