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Cell adhesion and viability of human endothelial cells on electrospun polymer scaffolds

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Abstract: The usage of electrospun polymer scaffolds is a promising approach for artificial heart valve design. This study aims at the evaluation of biological performance of nanofibrous polymer scaffolds poly(L-lactide) PLLA L210, PLLA L214 and polyamide-6 fabricated by electrospinning via analyzing viability, adhesion and morphology of human umbilical vein endothelial cells (EA.hy926). Nanofibrous surface topography was shown to influence cell phenotype and cell viability according to the observation of diminished cell spreading accompanied with reduced cell viability on nonwovens. Among those, highest biocompatibility was assessed for PLLA L214, although being generally low when compared to the planar control surface. Electrospinning was demonstrated as an innovative technique for the fabrication of advanced biomaterials aiming at guided cellular behavior as well as the design of novel implant platforms. A better understanding of cell–biomaterial interactions is desired to further improve implant development.

Keywords: biocompatibility; cell morphology; electrospinning; endothelial cells; polymeric nanofiber nonwovens; prosthetic heart valve.

1 Introduction

Structural heart diseases are of increasing incidence and research on artificial heart valves has been steadily intensified [1]. In contrast to mechanical or bioprosthetic heart valves, heart valve design using polymeric materials is a promising approach towards the development of advanced cardiovascular prosthetics. Major disadvantages of bioprosthetic heart valves, most commonly being xenografts of porcine aortic valves or calf pericardium, are structural valve deterioration often accompanied with subsequent valve thickening and calcification as well as limited durability [2]. The application of polymeric scaffolds holds great potential to overcome these limitations and thus, is promising for future prosthetic heart valve design seeking e.g. longer durability, enhanced cell compatibility, cell guidance as well as reduced risk of infection and thrombosis and optimized anticoagulant therapy. Therefore innovative techniques, such as electrospinning, will support the development of novel scaffold designs for cardiovascular implants. Electrospinning enables the fabrication of fused fiber biomaterial scaffolds from several natural and synthetic polymers with random or aligned fibers of tunable fiber diameters ranging up to a minima of 5 nm [3], including the range of feature sizes known to facilitate cellular contact guidance [4].

Beyond the physico-mechanical parameters that have to be fulfilled to ensure mechanical integrity and functionality of the implant, biological properties of the biomaterials are of crucial relevance for implant's success. Implants have to ensure biocompatibility as well as additional features such as anti-infectiveness or anti-thrombogenicity, the latter especially demanded for cardiovascular devices. Moreover, implants that hold the capability to induce directed cellular response are highly demanded.

Cell–biomaterial interactions are strongly influenced by both, the surface topography and the chemistry of a biomaterial [4, 5]. The adhesion of cells to a surface triggers a signaling cascade subsequently regulating diverse cell functions, i.e. viability, proliferation and activation of structural and signaling proteins [6]. The knowledge about the complexity of cell physiology in dependence of the characteristics of biomaterials is of elementary clinical relevance regarding the development of optimal implant designs.

This study aims at the investigation of the effect of chemical and topographical characteristics of polymeric nonwovens on adhesion, morphology and viability

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of human endothelial EA.hy926 cells. The resulting evaluation of the biological performance of nanofibrous polymer scaffolds will be of great benefit for the determination of biomaterial's potential as innovative biomaterials for heart valve design finally leading to the development of novel or improved for cardiovascular applications and a better understanding of cell–biomaterial interactions.

2 Material and methods

2.1 Fabrication of polymeric nanofiber nonwovens by electrospinning

Nanofibrous nonwovens were fabricated of polymers poly-(L-lactide) Resomer L210 S (PLLA L210) and poly-(L-lactide) Resomer L214 (PLLA L214) (Evonik Industries AG, Darmstadt, Germany) and polyamide-6 (PA-6) (BASF Ludwigshafen, Germany) by free-surface electrospinning. Solvents used were: chloroform ($CHCl_3$, J.T. Baker, Deventer, Netherlands) for PLLA L210 and $CHCl_3$ added with Tetrahydrofuran (THF) for PLLA L214. Polymer concentrations were 2% (w/w) and 5% (w/w) for PLLA L210 and PLLA L214, respectively. PA-6 was 16% (w/w) in solution of formic acid and acetic acid (1:2). Free-surface electrospinning was performed with Nanospider LAB 200 (ELMARCO, Liberec, Czech Republic) under high voltage at 50–65 kV at ambient temperature and ambient humidity with a distance of 160 mm between the wire electrode and the substrate. The generated polymeric nonwoven mats were dried for 12 h at 40°C using the vacuum oven VO 200 (Memmert GmbH & Co., Schwabach, Germany).

2.2 Cell culture

Human umbilical endothelial cells EA.hy926 (ATCC) were cultured in DMEM (GIBCO) supplemented with 10% fetal calf serum (FCS, PAN Biotech, Aidenbach, Germany) at 37°C and 5% CO_2 under humidified atmosphere. For the experiments, cells were seeded at a density of 1.5×10^4 cells/cm^2 onto the polymeric scaffolds and incubated for 48 h.

2.3 Cell viability assay

Cell viability was determined by using the CellQuanti-Blue™ assay (BioAssaySystems, Hayward, CA, USA) according to the manufacturer's instructions. Briefly, cell viability was assessed via measuring the metabolic activity by the reduction of the substrate resazurin to resorufin by cellular reductases. Cells were grown on the polymeric surfaces for 48 h and incubated with CellQuanti-Blue™ as 10% of the culture medium volume for 2 h. The resulting fluorescence of resorufin was measured at an emission wavelength of 590 nm with an excitation wavelength of 544 nm using a microplate reader (FLUOstar OPTIMA, BMG Labtech, Offenburg, Germany). Four independent biological replications were performed. Data was normalized to cells grown on plane polystyrene control surface (NC).

2.4 Cell morphology analysis by scanning electron microscopy

Cell morphology of human endothelial EA.hy926 cells was analyzed by scanning electron microscopy. After 48 h cultivation period on the polymeric surfaces, cells were fixed with 25% glutaraldehyde and 0.2 M sodium cacodylate in PBS for 30 min. Samples were then washed with sodium phosphate buffer, dehydrated in a graded series of ethanol (50%, 75%, 90% and 100%) and dried with CO_2 in a critical point dryer (CPD 7501, Quorum Technologies Ltd., Laughton, Lewes, East Sussex, UK). Samples were sputter-coated with gold by Agar Sputter Coater (Canemco Inc., Quebec, Canada) and image acquisition was performed with the scanning electron microscope Quanta™ FEG 250 (FEI Company, Hillsboro, OR, USA).

2.5 Statistical analysis

Data were reported as mean value + standard deviation and were analyzed by one-way ANOVA carried out with GraphPad© Prism 5 software (La Jolla, CA, USA). Statistical significance was assumed at $p < 0.05$.

3 Results

3.1 Cell adhesion and morphology

Cell morphology analysis by scanning electron microscopy revealed phenotypic differences of human endothelial EA.hy926 cells grown on polymeric nanofiber nonwovens compared to those that were grown on the plane control surface (Figure 1A–D). On all polymeric nanofiber nonwovens, comprising PLLA L210, PLLA L214

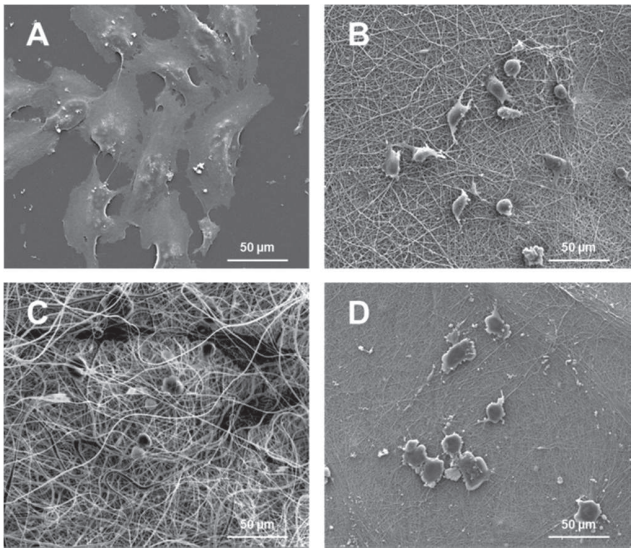

Figure 1: SEM images of human endothelial EA.hy926 cells grown on polymeric nanofiber nonwovens PA-6 (B), PLLA L210 (C) and PLLA L214 (D) and planar control surface (NC) (A) for 48 h.

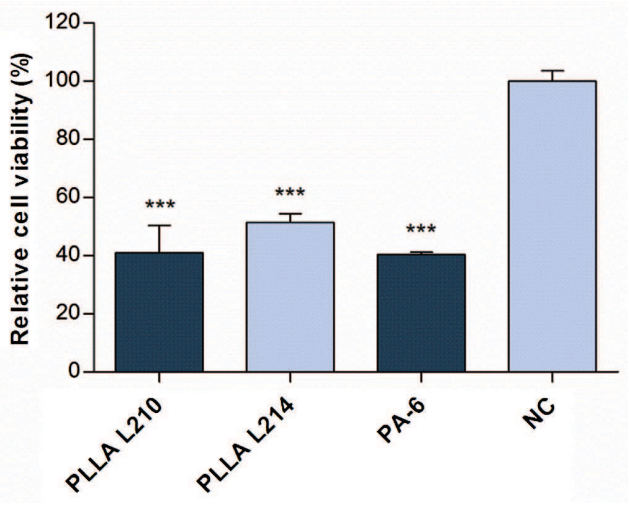

Figure 2: Relative cell viability of human endothelial EA.hy926 cells after 48 h cultivation on polymeric nanofiber nonwovens PLLA L210, PLLA L214 and PA-6 compared to the planar control surface (NC) ($n = 4$, mean + SD, ***$p < 0.001$).

and PA-6, only moderate spreading of EA.hy926 cells could be observed (Figure 1B–D). In particular, cells that were grown on electrospun polymer scaffolds mainly exhibited spherical phenotypes. Especially on PLLA L210 nonwovens, EA.hy926 cells were shown to circum-grow the scaffold nanofibers (Figure 1C). In contrast, on the plane control surface cells were well attached to the surface while demonstrating a flattened phenotype (Figure 1A).

3.2 Cell viability

Cell viability of human endothelial EA.hy926 cells grown on polymeric nanofiber nonwovens PLLA L210, PLLA L214 and PA-6 for 48 h was significantly reduced ($p < 0.001$) when compared to the control surface (NC) (Figure 2). Among the electrospun polymer scaffolds, relative viability of human endothelial EA.hy926 cells was highest on PLLA L214 (51.43 $\pm$ 2.97%), followed by PLLA L210 (40.97 $\pm$ 9.73%), while being lowest on PA-6 (40.40 $\pm$ 0.70%). Thus, cell viability was nearly similar among the nanofibrous scaffolds PLLA L210 and PA-6. However, observed differences in cell viability between the three polymeric nonwovens were not significant.

4 Discussion

Nanofibrous polymer scaffolds were successfully processed by electrospinning technology which makes them applicable for a variety of biomedical applications, e.g. heart valve prosthetics or novel drug-eluting stent platforms.

The biological evaluation of electrospun polymeric nonwovens by assessing cellular behavior of human endothelial EA.hy926 cells showed altered cell physiology in response to the physico-chemical properties of the nanofibrous polymer scaffolds. Human endothelial EA.hy926 cells grown on PLLA L210-, PLLA L214- and PA-6-nonwovens showed lower cell adhesion and reduced spreading in comparison to cells grown on the plane control surface. This might probably be a result of the distinct nanofibrous topography of the polymeric scaffolds since Badami et al. [7] already reported smaller projected areas of osteoblastic cells grown on, i.e. PLLA-fibers than for cells grown on planar surfaces but similarly a higher cell aspect ratio on the fibers. Thus, topographical factors designed into biomaterial scaffolds can regulate cellular functions like cell adhesion and spreading. Moreover, in the present study, it seems most likely that surface chemistry might be subordinated to surface topography because endothelial EA.hy926 cells exhibited similar phenotypes on all polymeric nonwovens, regardless of their distinct chemical composition, i.e. PLLA vs. PA-6. Regarding the differentiation between the relative influence of topographical and chemical effects, Cousin [8] showed that changes in the morphology of fibroblasts could be predominantly attributed to the surface topography of nanoparticulated coatings, irrespective of the surface chemistry, which is in consistence with the results of the present study.

Similarly, also cell viability of human endothelial EA.hy926 cells was not significantly different among the polymeric nonwovens, which additionally supports the superordinate effect of surface topography. Moreover, results obtained from cell viability study were consistent with those from the cell morphology analysis since altered cell morphology, expressed by lower spreading, was accompanied with altered cell function, expressed by lower cell viability. Similar cell architecture–cell function dependencies are already described in other studies [6].

In order to improve biocompatibility and biological integrity of the nanofibrous scaffolds, surface modifications, by either chemical or biological surface activation, might be of additional benefit. Among them, plasma-functionalization or the immobilization of biological molecules have already been described as promising methods to optimize biomaterials towards a better cell compatibility and implant integrity. Rudolph et al. [9] showed that NH_3-plasma treatment of different polymer surfaces enhanced cell viability as well as cell adhesion of human endothelial cells. In addition, also biofunctionalization of biomaterial surfaces, e.g. with vascular endothelial growth factor (VEGF) can improve biocompatibility of implant surfaces [10].

5 Conclusion

Nanofibrous scaffolds of PLLA L210, PLLA L214 and PA-6 were successfully fabricated by electrospinning enabling its usage as appropriate matrices for endothelialization whereat surface topography-dependent influences on cell physiology were detected. Together with the finely tunable fabrication properties of the electrospinning technique, polymer nonvovens are promising for directing cellular response and several cardiovascular applications. Systematic modulation of biomaterial's physico-chemical properties will support the elucidation of key cell–biomaterial interactions to further improve implant technology.

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