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Influence of fiber orientation in electrospun polymer scaffolds on viability, adhesion and differentiation of articular chondrocytes[1]

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Abstract. Degradable polymers with a tailorable degradation rate might be promising candidate materials for biomaterial-based cartilage repair. In view of the poor intrinsic healing capability of cartilage, implantation of autologous chondrocytes seeded on a biocompatible slow degrading polymer might be an encouraging approach to improve cartilage repair in the future. This study was undertaken to test if the fiber orientation (random *versus* aligned) of two different degradable polymers and a polymer intended for long term applications could influence primary articular chondrocytes growth and ultrastructure.

A degradable copoly(ether)esterurethane (PDC) was synthesized via co-condensation of poly(*p*-dioxanone)diol and poly(ε-caprolactone)diol using an aliphatic diisocyanate as linker. Poly(*p*-dioxanone) (PPDO) was applied as commercially available degradable polymer, while polyetherimide (PEI) was chosen as biomaterial enabling surface functionalization. The fibrous scaffolds of PDC and PPDO were obtained by electrospinning using 1,1,1,3,3,3 hexafluoro-2-propanol (HFP), while for PEI dimethyl acetamide (DMAc) was applied as solvent. Primary porcine articular chondrocytes were seeded at different cell densities on the fibrous polymer scaffolds and analyzed for viability (fluorescein diacetate/ethidiumbromide staining), for type II collagen synthesis (immunolabelling), ultrastructure and orientation on the fibers (SEM: scanning electron microscopy).

Vital chondrocytes adhered on all electrospun scaffolds irrespective of random and aligned topologies. In addition, the chondrocytes produced the cartilage-specific type II collagen on all tested polymer topologies suggesting their differentiated functions. SEM revealed an almost flattened chondrocytes shape on scaffolds with random fiber orientation: whereby chondrocytes growth remained mainly restricted to the scaffold surface. On aligned fibers the chondrocytes exhibited a more spindle-shaped morphology with rougher cell surfaces but only a minority of the cells aligned according to the fibers. As a next step the reduction of the fiber diameter of electrospun scaffolds should be addressed as an important parameter to mimic cartilage ECM structure.

Keywords: Chondrocytes, electrospinning, scaffold, differentiation, multiblock copolymer

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1. Introduction

Matrix assisted autologous chondrocyte implantation (MACI) describes the employment of a polymeric (preferably degradable) matrix (or scaffold) that serves as an initial supporting structure to achieve mechanical load transmission in a physiological range and enhances chondrogenesis as a novel method to improve the poor intrinsic regeneration capability of articular cartilage [5]. The development of suitable matrices requires understanding of the influence of the chemical, mechanical and structural scaffold properties, whether the scaffold should comprise solely polymeric fibers, on which the cells are directly seeded or polymeric fibers embedded in a viscous component (e.g. a hydrogel) containing the cells [6, 18, 20]. Therefore our group examined in a first step the influence of the surface topography of poly(*p*-dioxanone) (PPDO) and a copoly(ether)esterurethane (PDC) multiblock copolymer containing poly(*p*-dioxanone)- and poly(ε-caprolactone)-segments as film and as electrospun scaffold on chondrocyte viability, adhesion and differentiation [17]. PPDO is a desirable polymer due to its flexibility, moderate degradation rate and lower inflammatory response compared to poly(glycolic acid) and poly(lactide-*co*-glycolide) and which has already been investigated as electrospun scaffold material [14, 19]. PDC is a multifunctional polymer with tailorable degradation rate, adjustable mechanical properties and is capable of a shape-memory effect [9, 10]. Scaffold structures from PDC have been produced by thermally-induced phase separation [12] and electrospinning [8]. The results of our study showed that an electrospun surface morphology beneficially alters chondrocyte growth compared to flat films.

Motivated from these results we wanted to investigate in the present study the influence of the fiber orientation (random *versus* aligned) of electrospun scaffolds on the development of chondrocytes *in vitro*, since chondrocytes are typically embedded in a highly hierarchical surrounding, where the extracellular matrix consists of aligned fiber components withstanding and counterbalancing distinct mechanical load and tension [4]. Furthermore, scaffolds composed of aligned fibers might direct the cells alignment or orientation and ECM deposition as it has been shown for other cell types [7]. The three polymers PPDO, PDC and polyetherimide (PEI) chosen for this study were electrospun in a random and an aligned fashion. While the utilization of PPDO and PDC has already been motivated, PEI was chosen as a polymer intended for long term applications, whose surface can be chemically functionalized, e.g. by attachment of proteins and enzymes [2, 11].

2. Material and methods

2.1. Polymers

PPDO was purchased from Boehringer Ingelheim (Resomer X®, Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany) and PEI was obtained from General Electric (Ultem® 1000, General Electric, New York, USA). Both polymers were used as received. The copoly(ether)esterurethane (PDC) is a multiblock copolymer, which was synthesized via co-condensation from identical weight contents of poly(ε-caprolactone)diol (M_n = 2000 g·mol^{-1} Solvay Caprolactones, Warrington, U.K.) and poly(*p*-dioxanone)diol with M_n = 5300 g·mol^{-1} using an aliphatic urethane linker (HDU) as junction unit [8, 10]. The resulting PDC had an average molecular weight M_w of 75000 g·mol^{-1}, as determined by gel permeation chromatography (GPC), and showed two melting transitions in differential scanning calorimetry (DSC) experiments at 37 °C ($T_{m,PCL}$) and 89°C ($T_{m,PPDO}$) indicating a phase-segregated morphology. The chemical structures of the different polymers are shown in Fig. 1.

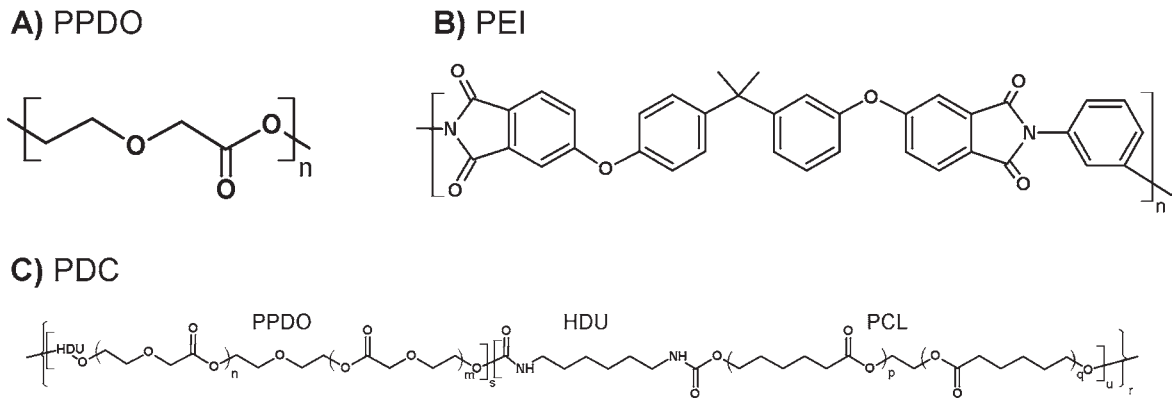

Fig. 1. Chemical structures of the polymers PPDO (A), PEI (B) and PDC (C).

2.2. Production of random or aligned fibrous scaffolds

Both PDC and PPDO were dissolved in 1,1,1,3,3,3 hexafluoro-2-propanol (HFP), which was purchased from ABCR GmbH & CO. KG (Karlsruhe, Germany), and a concentration of 11% (w/v) was chosen. PEI was dissolved in dimethyl acetamide (DMAc) (Merck Schuchardt OHG, Hohenbrunn, Germany) at a concentration of 31% (w/v), while the solution was handled under argon atmosphere to prevent precipitation of PEI at increased humidity levels. The electrospinning setup consisted of a metallic needle at the tip of a syringe containing the polymer solution, which served as the hot electrode, and a rotating mandrel as the collecting electrode. A constant mass flow of $2.5\,mL{\cdot}h^{-1}$ for the PDC and the PEI solution and $4\,mL{\cdot}h^{-1}$ for the PPDO solution was maintained throughout the process, while the electric field strength between the metallic needle and the collecting electrode was $0.6 \pm 0.1\,kV{\cdot}cm^{-1}$ for all solutions. For the production of random fibers (abbreviation: ran) the mandrel was rotated at 1 rotation per minute (rpm), while aligned fibers were obtained with a velocity of 2000 rpm (abbreviation: aln). The porosity (P) of the resulting scaffolds was calculated according to $P = (1 - m_{sc}{\cdot}(\rho{\cdot}V_{sc})^{-1}){\cdot}100$. In this equation m_{sc} and V_{sc} are the weight and the volume of the electrospun scaffold and ρ is the density of the polymer.

2.3. Mechanical characterization

The stress-strain relationships for the different polymers (PDC, PPDO and PEI) and the different topologies (random, aligned) were assessed on a tensile tester (Zwick Z2.5, Zwick, Ulm, Germany) at ambient temperature (AT). Five consecutive measurements were performed for each polymer type and topology with the dimensions $40 \times 10 \times 0.1\,mm^3$. The effective cross-sectional area from the porous scaffold was calculated by multiplying the width w_{sc} with the effective thickness $d_{eff} = m_{sc}{\cdot}(w_{sc}{\cdot}l_{sc}{\cdot}\rho)^{-1}$, where m_{sc}, w_{sc} and l_{sc} are the mass, width and length of the test specimen.

2.4. Scanning electron microscopic analysis

The electrospun non-woven scaffolds were analyzed using scanning electron microscopy (SEM) (Zeiss Supra 40 VP, Zeiss, Jena, Germany) to observe the fibrous morphology and to determine the fiber

diameters, the alignment of the fibers as well as the pore sizes. The fiber diameter was calculated by an average of 10 measurements using the ImageJ processing software [1]. Fibers were defined as fully aligned, if the deviation of the fiber angle compared to a reference line was below 5° for all measurements. Pore sizes were determined at 500× magnification by measuring the diameter of a virtual sphere in between embracing fibers, while only fibers of the in-focus plane were utilized for calculation. Pores and in-focus fibers were distinguished by introducing a grey scale threshold. For each material three different SEM images with an area of $50 \times 50\,\mu m^2$ were analyzed, while the minimum pore size was chosen with $5\,\mu m^2$. All samples were sputtered with platinum/palladium (80 : 20) under vacuum and the SEM pictures were taken at a voltage of 3 keV and at 300×, 500× and 2000× magnification.

For the investigation of the chondrocyte ultrastructure and its interaction with the fibrous polymer matrix the cells were fixed with 4% glutaraldehyde and dehydrated in an ascending alcohol series after a cultivation time of 36 or 72 hours. During the fixation process the temperature was kept at 4°C and the samples were rinsed three times in 0.1 M cacodylate buffer (arsenate buffer, pH 7.2) before and after fixation.

2.5. Sterilization of the samples and biological evaluation of polymer scaffolds by determining endotoxin content and cytotoxicity testing

Electrospun scaffolds were clamped in circular shaped support structures (Minusheet®, Minucells and Minutissue Vertriebs GmbH, Bad Abbach, Germany) with a diameter of 14 mm. Subsequently, the scaffolds were sterilized by gas sterilization with 600 $mg \cdot mL^{-1}$ ethylene oxide for 3 hours at 50–80% humidity and thereupon left for 3 days at 35–45°C to induce desorption. The endotoxin content was analyzed by a Limulus Amebocyte Lysate assay (QCL-1000, Lonza, Basel, Switzerland) and the cytotoxicity of the specimen was assessed in direct contact using L929 cells for cell viability (MTS assay, Promega, Madison, WI, USA) and cell integrity (LDH-assay, Roche, Mannheim, Germany). The cell morphology was investigated according to the USP 23-NF18 [3] by transmission light microscopy in phase contrast mode.

2.6. Chondrocytes isolation

Porcine cartilage chips were explanted from the knee joints of domestic pigs (3–6 month old hybrid pigs), whereby the cartilage from the femoral grove, condyles and patella back site was pooled. Porcine cartilage was subsequently minced into 1 mm slices and enzymatically digested with 0.4% pronase ($7\,U \cdot mg^{-1}$, Roche, Basel, Switzerland) diluted in Ham's F-12/Dulbecco's modified Eagle's (DMEM) medium 1 : 1 (Biochrom AG, Berlin, Germany) for 1 hour at 37°C and subsequently digested with 0.2% (w/v) collagenase ($\geq 0.1\,U \cdot mg^{-1}$, SERVA Electrophoresis GmbH, Heidelberg, Germany) diluted in growth medium (Ham's F-12/DMEM 1 : 1 containing 0.5% (v/v) fetal calf serum (FCS) [Biochrom AG], $25\,\mu g \cdot mL^{-1}$ ascorbic acid [Sigma-Aldrich, St. Louis, MO, USA], $50\,IU \cdot mL^{-1}$ streptomycin, $50\,IU \cdot mL^{-1}$ penicillin, $0.5\,\mu g \cdot mL^{-1}$ partricin, essential amino acids, 2 mM L-glutamine [all: Biochrom AG]) for 16 hours at 37°C. Isolated chondrocytes were resuspended in growth medium supplemented with 10% FCS and seeded at 2.8×10^4 $cells \cdot cm^{-2}$ in culture flasks.

2.7. Chondrocytes seeding onto scaffolds

PDC, PPDO and PEI scaffolds were placed in the wells of a 6 well plate. Scaffolds equilibrated in chondrocyte growth medium containing 10% (v/v) FCS for at least two days. 2×10^5 primary chondrocytes

(passages 1–3) were suspended in 50–70 μL growth medium before directly pipetted onto the scaffolds and let to adhere for 30 minutes at 37°C in the incubator. Later, 2 mL culture medium was added to each well.

2.8. Vitality testing

To test the viability of the chondrocytes cultured on the polymers, constructs were washed with PBS, and incubated with fluorescein diacetate (FDA) (3 $\mu g \cdot mL^{-1}$ dissolved in acetone [stock solution], Sigma-Aldrich and further diluted 1 : 1000 in PBS [working solution]) for 15 minutes at 37°C. Then, they were rinsed three times with PBS before being counterstained with ethidium bromide solution (1 μL dissolved in 1 mL PBS) for 1 minute in the dark at ambient temperature (AT). Photos of the green and red fluorescence of the cells were taken using fluorescence microscopy (Axioskop 40, Zeiss, Jena, Germany) and using a XC30 camera (Olympus Soft Imaging Solution GmbH, Muenster, Germany)

2.9. Type II collagen immunolabelling

Seeded scaffolds were fixed in 4% (v/v) paraformaldehyde for 15 minutes. For immunolabelling, samples were rinsed in Tris buffered saline (TBS: 0.05 M Tris, 0.015 M NaCl, pH 7.6) before being blocked with protease-free donkey serum (5% (v/v) diluted in TBS) for 30 minutes at AT. Then, constructs were rinsed and incubated with the polyclonal rabbit anti-type II antibody (both 27.5 $\mu g \cdot mL^{-1}$, Acris Antibodies, Herford, Germany) in a humidifier chamber overnight at 4°C. Constructs were subsequently washed with TBS before incubated with donkey-anti-rabbit-Alexa-Fluor®488 (10 $mg \cdot mL^{-1}$, Invitrogen, Carlsbad, CA, USA) and donkey anti-mouse Cy3 [Invitrogen] secondary antibody for 30 minutes at AT. Negative controls included omitting the primary antibody during the staining procedure. Cell nuclei were counterstained using 4′,6-diamidino-2-phenylindole (DAPI) (0.1 $\mu g \cdot mL^{-1}$, Roche, Basel, Switzerland). Finally, seeded scaffolds were rinsed several times with TBS and examined using fluorescence microscopy (Axioskop 40, Carl Zeiss, Jena, Germany). Images were taken using a XC30 camera (Olympus Soft Imaging Solution GmbH, Muenster, Germany).

The manuscript has been written in accordance with the ethical guidelines of Clinical Hemorheology and Microcirculation [14].

2.10. Statistics

All data were expressed as mean value ± standard deviation.

3. Results

3.1. Mechanical and morphological characterization of random or aligned scaffolds

Non-woven scaffolds with a deposit thickness of 80 ± 20 μm could be obtained for all polymers. The porosity for the random as well as for the aligned scaffolds was around $80 \pm 10\%$ and the fiber diameter was in the range of 2 ± 1 μm for the PDC and PEI scaffolds and around 3.5 ± 1 μm for PPDO scaffolds. The morphologies of the randomly oriented and aligned fibers of the different polymers have been visualized by SEM and are shown in Fig. 2. The degree of alignment, where full alignment was

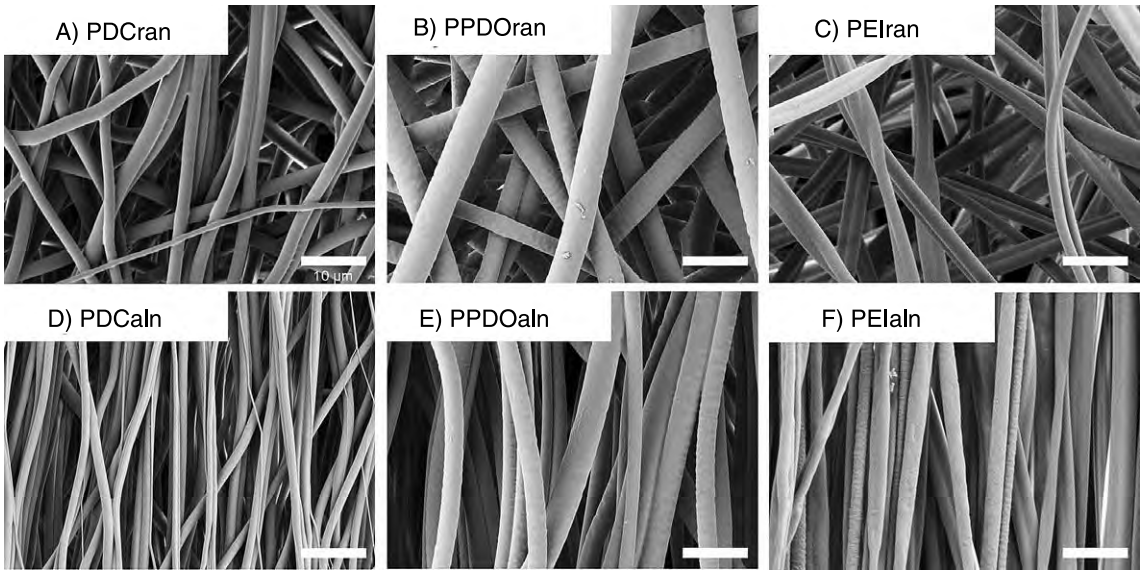

Fig. 2. SEM analysis of the different electrospun materials PDC (A and D), PPDO (B and E) and PEI (C and F) with different fiber orientations (random, aligned). Scale bars indicate 10 μm.

defined as a deviation less than 5° of the fibers from a reference line, was above 80% for PPDOaln and PDCaln, while for PEIaln a degree of alignment of around 90% was achieved. Furthermore, PPDOaln fibers tended to form a wavy structure, probably due to residual strain within the fibers after detaching from the collector. The pore diameter of the randomly oriented scaffolds was found to be in the range from 6 to 7 μm, whereas the pore diameter of aligned scaffolds was slightly lower around 5 μm.

The mechanical properties of the scaffolds, which were obtained at AT, are summarized in Table 1 with respect to the different polymer types, the fiber orientation within the scaffolds and the loading direction of the tensile tests. PEI fibers exhibited the highest Young's moduli E from all polymers with 540 MPa for random fiber orientation, while also being the most brittle scaffold material. PPDOran scaffolds offer an E at about a half of that and PDCran scaffolds even a decade lower than PEIran, consequently both PPDOran and PDCran comprise a larger elongation at break ε_b. The effect of alignment increased E and decreased ε_b (except for PPDO fibers) in the direction of the fiber (loading direction: 0°), while perpendicular to the fiber direction E was decreased and ε_b increased (loading direction: 90°) resulting in scaffolds with anisotropic mechanical properties. For PEIaln scaffolds the increase of E, when loaded in the direction of the fibers, was less pronounced with 40% (compared to PEIran) than for PPDOaln and PDCaln scaffolds with about 100% (compared to PPDOran and PDCran). Nevertheless, E was reduced for all polymers over 90% when loaded perpendicular to the fiber direction as compared to random scaffolds. Likewise the ε_b of aligned PEIaln and PDCaln when loaded in the fiber direction was reduced to about 50% of PEIran and PDCran scaffolds, while increased ε_b-values could be observed for all polymer types perpendicular to the fiber direction. Stretching of PPDOaln scaffolds along the direction of the fiber resulted in a sharp increase of the stress indicating a high E; however, above the Yield point necking of the scaffold could be observed which resulted in a high ε_b of PPDOaln in the fiber direction. As compared to phase-segregated multiblock copolymers (PDC) or glassy, amorphous polymers (PEI), semi-crystalline PPDO molecules slip off more easily which might explain the high ε_b of PPDOaln in the fiber direction.

Table 1
Mechanical properties of the scaffolds depending on the fiber orientation

Sample	Loading direction [°]	E[1][MPa]	ε_b[2][%]
PDCran[3]	–	50 ± 10	210 ± 20
PDCaln[4]	0[5]	110 ± 20	100 ± 10
PDCaln	90[6]	4 ± 1	290 ± 20
PPDOran	–	230 ± 20	130 ± 10
PPDOaln	0	470 ± 30	160 ± 20
PPDOaln	90	9 ± 1	240 ± 30
PEIran	–	540 ± 140	40 ± 30
PEIaln	0	770 ± 120	20 ± 1
PEIaln	90	12 ± 2	240 ± 20

[1]E: Young's modulus; obtained by tensile tests at AT (AT: ambient temperature).
[2]ε_b: elongation at break; obtained by tensile tests at AT.
[3]ran: randomly oriented fibers.
[4]aln: aligned fibers.
[5]aligned 0°: Scaffolds composed of aligned fibers were loaded in the direction of the fibers.
[6]aligned 90°: Scaffolds composed of aligned fibers were loaded perpendicular to the direction of the fibers

3.2. Chondrocytes growth on the scaffolds

Seeding experiments revealed that a seeding density of 150000 cells·cm^{-2} was appropriate. Most of the chondrocytes growing on all scaffolds remained viable during the 6 days of culture. Cell viability did not differ between random and aligned scaffold topologies (Fig. 3).

3.3. Type II collagen expression of the chondrocytes on the scaffolds

In addition, the chondrocytes produced the cartilage-specific type II collagen (Fig. 4) on all tested topologies and polymers suggesting differentiated functions. There was no difference in staining intensity in chondrocytes grown on random or aligned fibers. Type II collagen could be detected intra- and extracellularly in the pericellular area.

3.4. Ultramorphology of the chondrocytes on different topologies and polymers

Chondrocytes adherence on PEI-fibers was higher compared with PPDO and PDC independent of the fiber orientation (Fig. 5A–F). As visualized in Fig. 5G–I SEM revealed a more flattened cell shape of the chondrocytes adhering on scaffolds with random fiber orientation: chondrocytes could be detected mostly at the scaffold surface. In contrast, on scaffolds with aligned fibers the chondrocytes exhibited a spindle-shaped morphology and a rough cell surface, as depicted in Fig. 5J–L. Many of them could be found in the spaces between the parallel running fibers. These chondrocytes adhering in the deeper layers of the scaffolds were often aligned as visible at higher magnification. However, only a part of the chondrocytes colonizing the scaffold surface aligned along the fiber direction (see Fig. 5D–F). Furthermore,

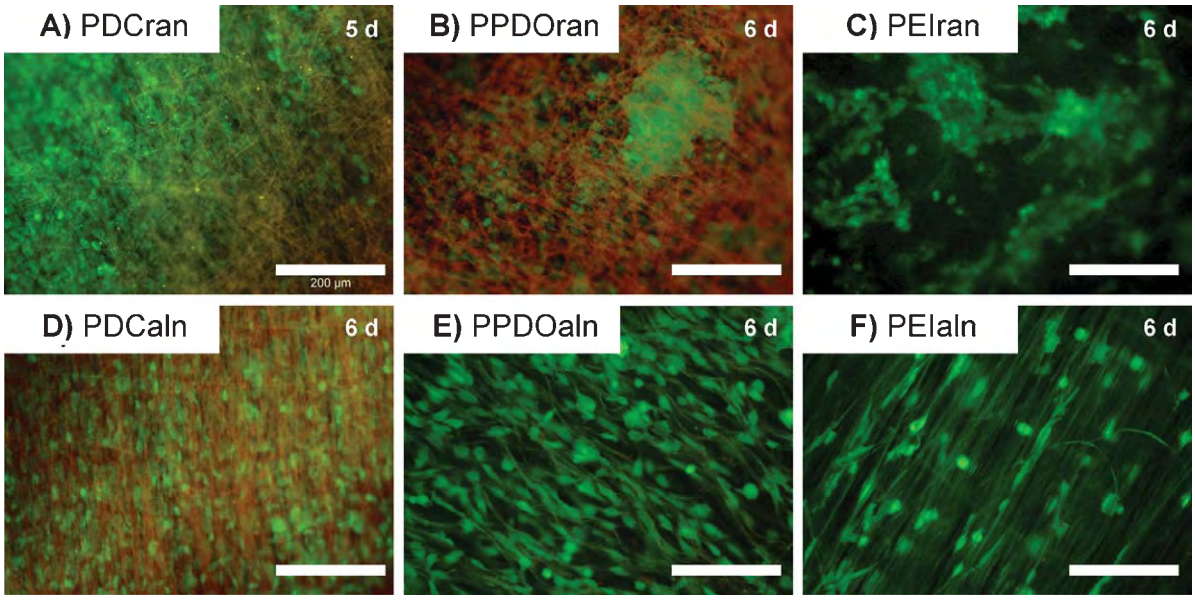

Fig. 3. Vitality testing of articular chondrocytes seeded on PDC (A and D), PPDO (B and E) and PEI (C and F) polymer scaffolds with random or aligned fiber orientation. Scaffolds were adapted in culture medium before seeded with chondrocytes for 5-6 days. Cell seeding density: 150000 cells·cm^{-2}. Green: vital cells, red: dead cells or autofluorescence of the scaffolds. Scale bars indicate 200 µm.

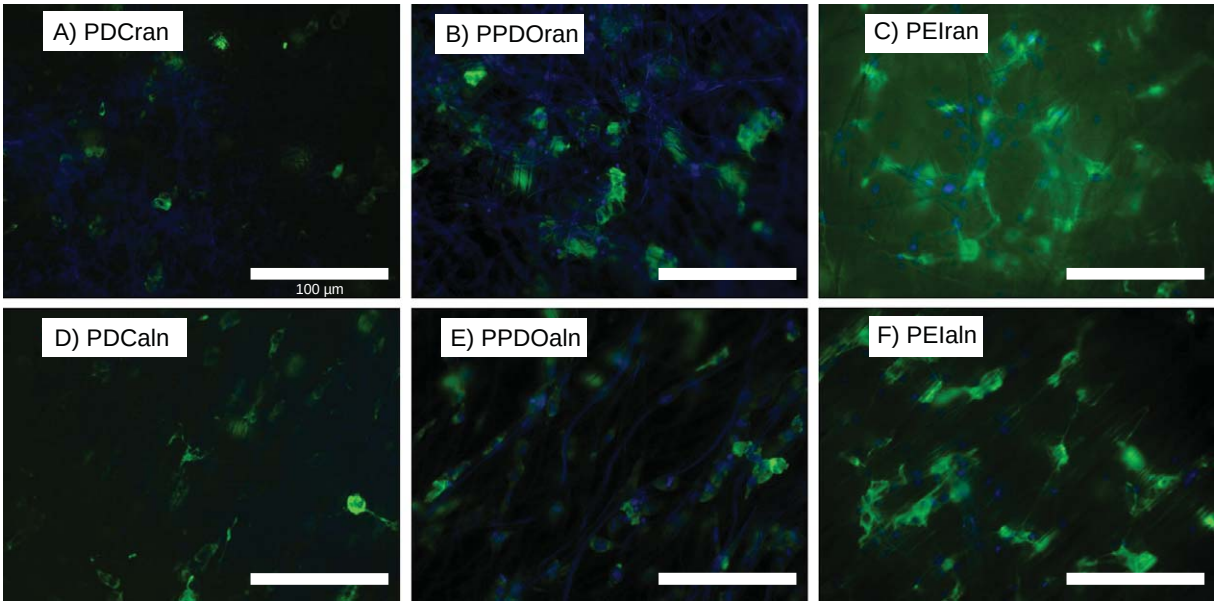

Fig. 4. Type II collagen (green) expression of articular chondrocytes cultured on PDC (A and D), PPDO (B and E) and PEI (C and F) polymer scaffolds. Scaffolds were pre-incubated in culture medium before seeded with chondrocytes for 72 hours. Cells were immunolabeled using an anti-collagen type II antibody and Alexa-488 coupled secondary antibodies (green). Cell seeding density: 150000 cells·cm^{-2}. The cell nuclei were counterstained (blue) using DAPI. Scale bars indicate 100 µm.

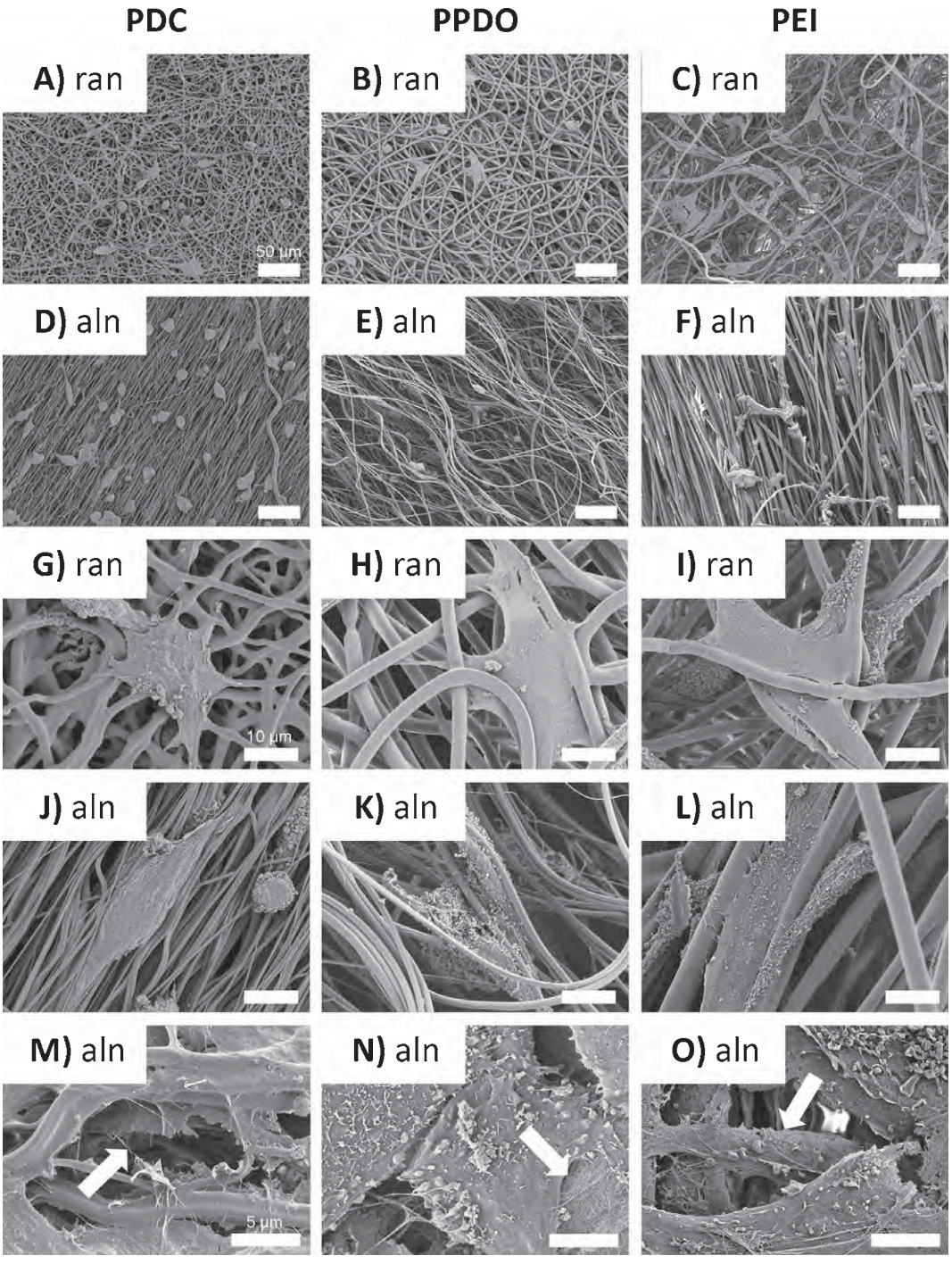

Fig. 5. SEM analysis of articular chondrocytes seeded on random and aligned PDC (A, D, G, J and M), PPDO (B, E, H, K and N) and PEI (C, F, I, L and O) polymer scaffolds. Scaffolds were pre-incubated in culture medium before articular chondrocytes were added and cultivated for 36 (A–L) or 72 hours (M–O). Cell seeding density: 150000 cells·cm^{-2}. Scale bars for A) to F) indicate 50 µm, for G) to L) 10 µm and for M) to O) 5 µm.

chondrocytes produced an ECM on all polymers at 3 days of culture, as indicated by the white arrows in Fig. 5M–O. Chondrocytes developed, except for PDC, a rough surface on aligned fibers with multiple nodular cell surface processes at this time.

4. Discussion

Three polymers with different functions (degradability, shape-memory capability, functionalizable surface) were investigated as fibrous scaffolds for their effects on chondrocytes to assess in future studies their potential to enhance the healing of chondral defects. The focus was placed on the influence of the fiber orientation (random *versus* aligned) on chondrocyte growth. For all polymers the adhesion of chondrocytes could be shown. The majority of the chondrocytes growing on all scaffolds were viable for the whole investigation time (6 days) as indicated by live dead stainings. Accordingly, only moderate cytotoxic effects of the polymers on L929 cells could be detected. The expression of the cartilage-specific ECM protein type II collagen found on all scaffolds and topologies suggests the maintenance of chondrocytes differentiated functions. We could demonstrate previously the expression of both type II collagen and the non-specific type I collagen in PPDOran and PDCran scaffolds as well as PPDO and PDC films [17]. The type II collagen immunolabelling did not reveal major differences in type II collagen deposition dependent on the polymer. To detect discrete differences in chondrocytes synthetic capacity on different scaffold topologies a more sensitive method should be applied in future.

The parallel fiber alignment of PPDOaln and PEIaln scaffolds allowed the chondrocytes to grow not only at the scaffold surface but also between the fibers (Fig. 5E and F), while for random scaffolds only PEIran revealed some chondrocytes surrounding deeper fibers (Fig. 5C). In contrast, the chondrocytes remained mostly at the surface of the PPDOran and PDCran scaffolds (Fig. 5A and B). The crossing points, which fix the fiber arrangement in the scaffolds consisting of random fibers might prevent chondrocytes migration into deeper scaffold layers. In face of the small pore sizes of around 5–7 μm the adhering chondrocytes, which are markedly larger, have to force themselves between the fibers.

The ultrastructure of chondrocytes on the scaffolds revealed a more smooth and flattened shape on the PDCran and PPDOran scaffolds compared with the aligned fiber scaffolds (Fig. 5G, H, J and K). Small microvilli- and knob-like cell surface processes could be detected on PEIaln and PPDOaln scaffolds at 36 hours but also still at 3 days of culture indicating a synthetic cell phenotype as it has been shown previously [16]. Although, at 36 hours obviously no major ECM deposition could be detected by SEM around the cells (see Fig. 5G–I), already three days after seeding on the scaffolds type II collagen was evident (Fig. 4A–F) and the presence of a significant amount of ECM could be confirmed by SEM (Fig. 5M–O). Other studies have shown that seeding of fibrochondrocytes isolated from the annulus fibrosus of the intervertebral disc on aligned PCL scaffolds resulted in a cell orientation along the fibers, while also cell number and the ECM production were increased as compared to random PCL scaffolds [7]. Furthermore, aligned fibers seem to promote an organized ECM deposition along the fibers, which has shown to increase the mechanical properties of cartilage cells seeded on aligned scaffolds compared to random scaffolds over time [13]. Regarding solely the polymers, Figs. 4 and 5 suggests that a higher number of chondrocytes have been attached to PEI scaffolds (regardless of the fiber orientation) compared to the PDC and PPDO scaffolds, which might be due to a significantly different surface chemistry (aromatic structures in the polymer backbone) as displayed in Fig. 1. The diameter of the fibers in the scaffold used for this study remained in the micrometer range. Therefore, in future experiments the diameter should be reduced to mimic natural ECM fibers, since collagen fibers have a diameter of around 0.3 μm. It has been shown

previously that a small fiber diameter of a chitosan scaffold can promote type II collagen expression in chondrocytes [15].

5. Conclusion

In summary, it was possible to show that aligned fibers promoted a more chondrocytic, spindle-like, cell morphology compared to random fibers, where the chondrocytes remained flat. Furthermore, the cells began to infiltrate the aligned scaffolds, which did not happen for PDCran and PPDOran scaffolds. The highest adhesion could be achieved with PEI scaffolds regardless of their fiber orientation. Future studies will evaluate smaller fiber diameter in the sub-micrometer range that more closely mimic the ECM structure.

Acknowledgments

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