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The influence of polymer scaffolds on cellular behaviour of bone marrow derived human mesenchymal stem cells

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Running Head: The interaction of human mesenchymal stem cells and polymer surfaces

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Abstract

Mesenchymal stem cells (MSCs) are multipotent stem cells that can differentiate into a variety of cell types. Therefore, they are widely explored in regenerative medicine. The interaction of MSCs with biomaterials is of great importance for cell proliferation, differentiation and function, and can be strongly influenced by numerous factors, such as the chemical nature and the mechanical properties of the material surface.

In this study, we investigated the interaction of bone marrow derived human MSCs with different amorphous and transparent polymers namely polystyrene (PS), polycarbonate (PC), poly(ether imide) (PEI), polyetherurethane (PEU) and poly(styrene-*co*-acrylonitrile) (PSAN). To ensure that the MSCs were solely in contact to the testing material we applied polymeric inserts, which were prepared from the aforementioned polymers via injection molding. The explored inserts exhibited a similar wettability with advancing contact angles ranging from 84±7° (PEU) to 99±5° (PS) and a surface roughness of $R_q \leq 0.86$ µm. The micromechanical properties determined by AFM indentation varied from 6±1 GPa (PEU) to 24±5 GPa (PSAN). Cells presented different adhesion rates on the polymer surfaces 24 hours after cell seeding (45±7% (PS), 63±1% (PC), 75±4% (PEI), 69±2% (PEU) and 61±5% (PSAN)). The cells could proliferate on the polymer surfaces, and the fold change of cell number after 16 days of culture reached to 1.93±0.07 (PS), 3.38±0.11 (PC), 3.65±0.04 (PEI), 2.24±0.15 (PEU) and 3.36±0.09 (PSAN). Differences in cell apoptosis could be observed during the culture. After 7 days, the apoptosis of cells on PC, PEI and PSAN decreased to a level comparable to that on standard tissue culture plate (TCP). All of the tested polymers exhibited low cytotoxicity and allowed high cell viability. Compared to cells on TCP, cells on PC and PEI showed similar morphology, distribution as well as F-actin cytoskeleton organization, whereas cells on PSAN were distributed less evenly and cells on PEU were less oriented. Cells were more likely to form clusters on PS. Conclusively, we demonstrated the influence of polymer scaffolds on the cellular behaviour of MSCs, which could be included in the development of novel design concepts based on polymeric biomaterials.

Keywords: mesenchymal stem cells, polymer surface, cell-material interaction, cell adhesion, proliferation, apoptosis

1. Introduction

Human mesenchymal stem cells (hMSCs) are self-renewing cells with multilineage differentiation potential, which makes them an ideal cell source for regenerative medicine [5, 9]. They can be expanded *in vitro* and give rise to various cell lineages, including adipocytes, chondrocytes, myoblasts and osteoblasts [42, 58]. In order to utilize hMSCs to treat debilitating disease, it is crucial to produce hMSCs on a large scale with a well defined condition. Increasing evidence suggested that the interaction of stem cells and their microenvironment, including the chemical and physical properties of the extracellular matrix (ECM) and the presence of the growth factors, plays an important role in regulating cell proliferation, differentiation and self-renewal [32, 39, 40, 43, 50, 60]. However, cultures with recombinant purified human growth factors are very expensive and may suffer a lack of reproducibility from batch to batch variations. Therefore it is of great value to develop a reliable alternative to control the cell fate and proliferation process of hMSCs.

Synthetic polymers are reliable alternatives for the *ex vivo* maintenance, expansion and proliferation of hMSCs, which are inexpensive and relatively easy to fabricate [33, 52]. It has been reported that material parameters especially physicochemical properties strongly influence the interaction of cells and polymers [34]. However, there is still very limited knowledge available how material properties can control stem cell behaviour such as adhesion, morphology, differentiation and functionality. Hence, it is necessary to investigate the cellular behaviour of hMSCs in response to different polymer surfaces.

To address this question, we have prepared polymeric inserts for cell culture plate via injection molding as previously reported [20]. These inserts could serve as a suitable platform to assess solely the interaction of hMSCs and polymers, whereby the influence of other materials such as cell culture dishes or materials utilized for fixation of the testing materials are excluded. The polymeric inserts prepared via this process presented low endotoxin level and did not induce the innate immune reactions, indicating their immuno-compatibility (data not shown). Five different polymeric inserts from polystyrene (PS), polycarbonate (PC), poly(ether imide) (PEI), polyetherurethane (PEU) and poly(styrene-*co*-acrylonitrile) (PSAN) were systematically explored with bone marrow derived hMSCs with respect to cell adhesion, proliferation, morphology and apoptosis.

2. Materials & Methods

The study was performed in accordance with the ethical guidelines of the journal Clinical Hemorheology and Microcirculation [3].

2.1 Polymers

The following five polymers (Fig. 1) were used without any further purification, polystyrene (PS, Type 158K, BASF, Germany), polycarbonate (PC, trade name Makrolon® 2805, Bayer, Germany), poly(ether imide) (PEI, trade name ULTEM® 1000, General Electric, USA), polyetherurethane (PEU, trade name Tecoflex® MG8020, Lubrizol, USA) and poly(styrene-*co*-acrylonitrile) (PSAN, trade name Luran HD-20, BASF, Germany), to prepare the polymeric inserts via injection molding.

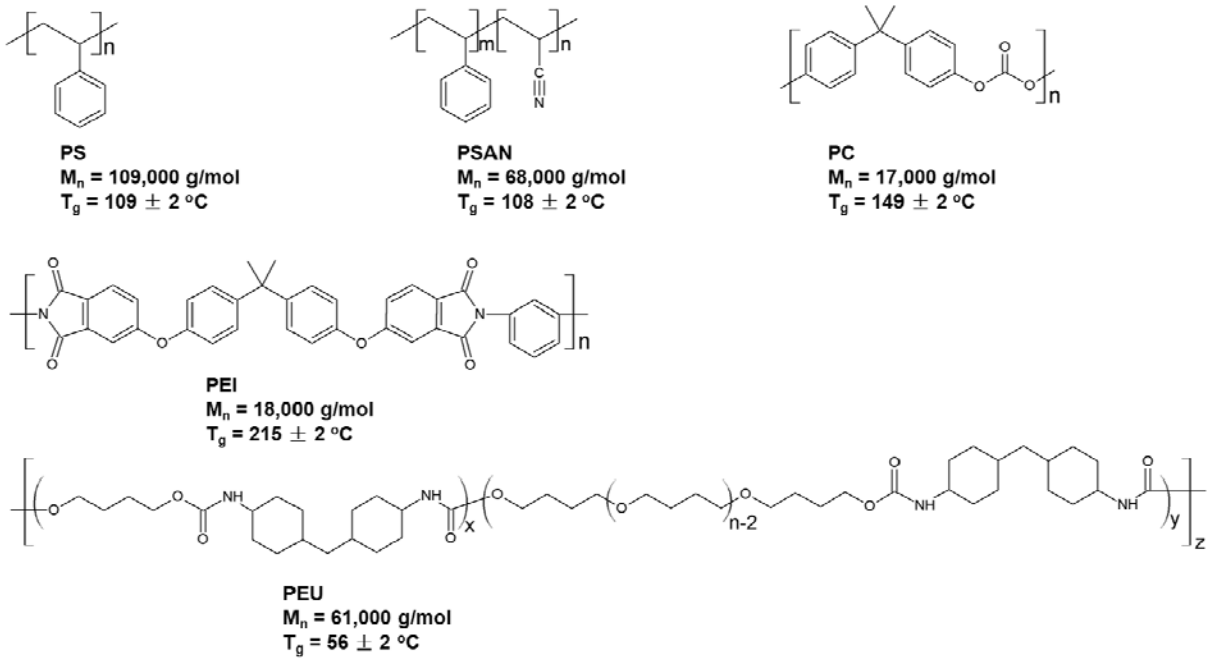

Fig. 1. Chemical structures, average molecular weights (M_n) and glass transition temperatures (T_g) of the investigated polymers.

2.2 Processing of polymeric inserts

For polymer processing an injection molding automat (Alrounder 270U, Arburg Corp., Münsingen, Switzerland) equipped with a custom made mold (Dreuco Formenbau GmbH, Berlin, Germany), allowing the parallel fabrication of 4 inserts with the suitable size to be put into the standard 24-well tissue culture plate (lower inner diameter 10.5 mm, upper inner

diameter 12.4 mm, height 16.8 mm and wall thickness 1 mm) was utilized. The processing parameters for the different polymers were summarized in Table 1.

Table 1. Applied processing parameters

Materials	Temperature of melt – four zone heating [^{o}C]				Temperature of mold [^{o}C]	Injection rate – two-stage [cm^3/s]	Injection pressure – two-stage [bar]	Injection volume [cm^3]
	I	II	III	IV				
PS	35	210	230	230	30	14-20	1200-1800	6.5
PC	35	280	310	320	60	37-40	1500-1800	11
PEI	35	320	350	380	180	35-40	1800-2000	11
PEU	35	150	180	190	20	25-30	1200-1800	6.5
PSAN	35	220	240	260	70	35-40	600-800	10

The sterilization of the inserts was performed prior to the characterizations and biological tests. PS, PC, PEU and PSAN inserts were sterilized by gas sterilization (gas phase: 10% ethylene oxide, 54 ^{o}C, 65% relative humidity, 1.7 bar, 3 hours of gas exposure time and 21 hours of aeration phase). PEI insert was sterilized via steam sterilization (121 ^{o}C, 2.0 bar, 20 minutes) using a Systec Autoclave D-65 (Systec GmbH, Wettenberg, Germany).

For surface characterisation by optical profilometry, contact angle measurements and micromechanical testing the bottom of the insert was detached using a water-cooled histological saw.

2.3 Contact angle measurements

The wettability of the insert bottom was determined by measuring the water contact angles (CA) at room temperature with a Drop Shape Analysis System DSA 100 (KRÜSS, Hamburg, Germany) using the captive bubble method as previously described [20]. For each polymer 10 samples were investigated by 10 measurements on at least two different locations of the insert bottom. The resulting data were averaged to yield the mean value of contact angles and their standard deviation (SD).

2.4 Optical profilometry

Surface profiles of the inner bottom of the inserts were obtained with an optical profilometer type MicoProf 200, equipped with a CWL 300 (Fries Research & Technology GmbH (FRT)) chromatic white-light sensor in according to the method described previously [20]. Each

analysis was performed on an area of 7×7 mm^2 (4000 lines per image, 250 dots per line, 300 Hz). The raw data were corrected for the sample tilt (subtraction of a plane), smoothed by a median filter and modified to the region of interest, removing invalid data.

2.5 Micromechanical characterization

Micromechanical characterization of the insert bottom was conducted at ambient temperature with an atomic force microscope (MFP-3D Bio-AFM, Asylum Research, Santa Barbara, CA, U.S.A) equipped with an indenter (spring constant k = 488.3 N/m) and a diamond Berkovich-tip with a Poisson's ratio of $\nu_{indenter} = 0.2$ and Young's modulus of $E_{indenter} = 865$ GPa [15]. For each sample 36 indents were recorded within a quadratic area of 90×90 μm^2 with an indentation/scan rate of 0.1 Hz, a maximum force of 50 µN and a trigger force of 70 µN. The Young´s modulus was calculated by the Oliver-Pharr model [36] in a range from 20% to 80% at the force-distance curve.

2.6 Human mesenchymal stem cells

For studies involving human tissues we obtained ethical approval of the local ethical committees, Medical Ethics Commission II, Medical Faculty Mannheim, Heidelberg University and Heidelberg University Ethical Board. Bone marrow for research purposes was received according to the approval by the Heidelberg University Ethical Board; approval nos.: 251/2002 and S-076/2007. All samples were taken after written consent using guidelines approved by the Ethic Committee on the Use of Human Subjects at the University of Heidelberg.

The hMSCs from bone marrow (BM) were isolated and prepared as previously described in detail [16, 23]. The hMSCs were cultured in stem cell medium (MSCGM™, Lonza, Walkersville, MD, USA) at 37 °C in a humidified atmosphere containing 5% CO_2. Cell passaging was performed at a ratio of 1:3 when cells reached ~90% confluence. For trypsinization, cells were washed two times with pre-warmed phosphate buffered saline (PBS) and incubated with 0.25% trypsin/0.53mM EDTA solution for 4 minutes at 37 °C to detach cells from flask bottom. Next, fresh medium was added to stop trypsinization reaction. Then, cell suspension was collected and cells were spun down by centrifugation at 300 g for 10 minutes. After removing the supernatant, cell pellet was resuspended with 1 ml fresh medium to carry out subculturing or cell counting. Cells were characterized by flow cytometry and the multipotency was tested using standard protocols as previously described [16, 59].

2.7 Cell adhesion assay

To assess the early cell attachment, cells were seeded at the density of 0.50×10^4 cells/cm^2 of insert bottom. After 24 hours of incubation, the medium was carefully aspirated to remove the nonadherent cells. The relative cell number was determined using Cell Counting Kit-8 (CCK-8, Dojindo Molecular Technologies, Munich, Germany). In brief, 200 µl fresh medium was added into each inserts, followed by adding 20 µl CCK-8 solution. After 2 hours of incubation at 37 ^{o}C, 110 µl medium/CCK-8 mixture was transferred from each insert into transparent 96-well plate, and the absorbance of the mixture was measured at a wavelength 450 nm and a reference wavelength 600 nm using a microplate reader (Infinite 200 PRO®, Tecan Group Ltd., Männedorf, Switzerland). The cell number was calculated via a standard curve, which was made by measuring a series of samples with known cell number. The experiment was performed in triplicates and the cell adhesion rate was defined as the percentage of adherent cell number out of seeded cell number. The tissue culture plate (TCP) was involved as a positive control.

2.8 Cell proliferation assay

To study the influence of polymeric inserts on cell proliferation, one of the most important parameters is to evaluate the compatibility of biomaterials. We measured the cell proliferation rate in the polymeric inserts up to more than 2 weeks. Cells were seeded at the density of 0.50×10^4 cells/cm^2 of insert bottom. The cell culture medium was regularly changed and the cell number at various time points was measured using CCK-8 as described above. The experiment was performed in triplicates and the TCP was involved as a positive control.

2.9 Apoptosis assay

Spontaneous apoptosis test was conducted to study the apoptotic rate of cells cultured in polymeric inserts. Cells were seeded in day 0 at the density of 0.50×10^4 cells/cm^2 of insert bottom. The caspase-3/7 activation, which is a key biomarker of apoptosis, was measured in day 1, 4 and 7 respectively using an apoptosis kit (Caspase-Glo®, Promega, Madison, WI, USA). In brief, the old culture medium was replaced by new medium (120 µl/per insert), followed by adding Caspase-Glo® 3/7 reagent (100 µl/per insert) and shaking orbitally at 300 rpm for 30 seconds. After incubating the mixture at room temperature for 90 minutes, 200 µl liquid in each insert was transferred into 96-well opaque (white) tissue culture plate and the luminescence intensity was measured using a microplate reader (Infinite 200 PRO®, Tecan

Group Ltd., Männedorf, Switzerland). The experiment was performed in triplicates and the TCP was involved as a positive control. The result was given as relative light units (RLU) normalized against cell number, which was measured by CCK-8.

2.10 Fluorescent staining

Live/dead staining was conducted to identify the death of cells cultured in the polymeric inserts. Cells were seeded at the density of 0.25×10^4 cells/cm^2 of insert bottom. After 28 days of culture, cells were stained by fluorescein diacetate (FDA, 25μg/ml) and propidium iodide (PI, 2μg/ml) for 5 minutes. Then, cells were washed with phosphate buffered saline (PBS) and observed using a confocal laser scanning microscope (LSM 510 META, Carl Zeiss, Jena, Germany). For fibrous actin (F-actin) cytoskeleton staining, cells were seeded at the density of 0.30×10^4 cells/cm^2 of insert bottom. After 14 days of culture, cells were stained using rhodamine conjugated phalloidin (Invitrogen, Paisley, UK) following the given protocol and the cell nuclei were counterstained by 4',6-diamidino-2-phenylindole (DAPI). Then, cells were observed using a confocal laser scanning microscope (LSM 510, Carl Zeiss, Jena, Germany).

2.11 Statistics

The values for all samples were expressed as mean ± standard deviation (SD). Statistical analysis was performed using Independent-samples t test, and a significance level (Sig.) $<$ 0.05 was considered to be statistically significant.

3. Results

3.1 Characterization of polymeric inserts

In order to evaluate the wettability or hydrophilicity of the tested polymers, which is known as an important parameter for cell attachment, spreading as well as proliferation, the water contact angle of the polymer surface was measured (Fig. 2A). PC and PEU exhibited the lowest values of advancing contact angles (PC: 85 ± 8^o and PEU: 84 ± 7^o), which can be attributed to the polar urethane or carbonate groups. Whereas, for PS a higher advancing angle of 99 ± 5^o was obtained.

In order to determine the surface roughness of the polymeric inserts, all samples were analysed by optical profilometry (Fig. 2B). While the obtained values for the root mean square roughness R_q of PS, PC, PEI and PSAN were in the range from 0.12 to 0.34 µm ($R_{q,PS}$ = 0.12±0.04; $R_{q,PC}$ = 0.34±0.13; $R_{q,PEI}$ = 0.23±0.07; $R_{q,PSAN}$ = 0.26±0.07), significantly higher values were found for PEU with $R_{q,PEU}$ = 0.86±0.07, which can be attributed to the relatively low and broad mixed glass transition of PEU in the range from 20 ^{o}C to 90 ^{o}C, which allows relaxation of the polymer chains at ambient temperature [11].

The micromechanical property of the cell culture substrate is another important parameter, which can influence stem cells cultured on it. The elastic properties of the samples measured by AFM indentation method indicated an increase of the Young's modulus in the order of PEU, PEI, PC, PS and PSAN (Fig. 2B). Compared to PS (16±4 GPa), PEU showed a lower value of Young's modulus (6±1 GPa), whereas PSAN showed a higher value (24±5 GPa). Both PEI (12±1 GPa) and PC (13±2 GPa) presented relatively comparable values of Young's modulus to PS.

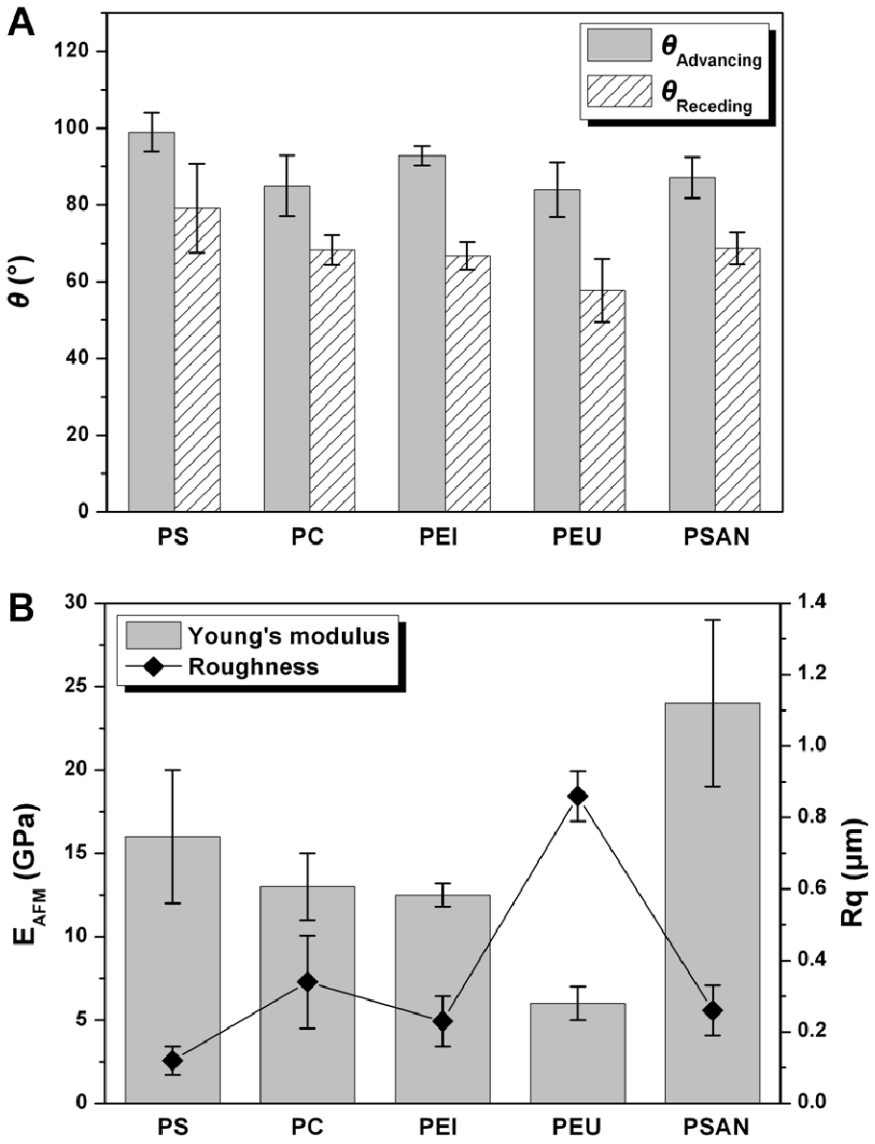

Fig. 2. Contact angle θ (A), Young´s modulus E_{AFM} and root mean square roughness R_q (B) of the insert bottom.

3.2 Cell adhesion

The early stage cell adhesion rate was measured 24 hours after cell seeding (Fig. 3A). Compared to PS, on which 45±7% cells were adherent, the other four polymers showed significantly higher cell adhesion rate: 63±1% (PC), 75±4% (PEI), 69±2% (PEU) and 61±5% (PSAN). Among these polymers, PEI exhibited highest cell adhesion rate. As expected, the commercial TCP allowed almost the attachment of all of the seeded cells, showing a significantly higher cell adhesion rate (104±4%) than the tested polymers. The morphology of adherent cells was observed by phase-contrast microscopy (Fig. 3B-D). The cells on different

polymer surfaces showed typical MSCs' spindle-shape, and no obvious difference on morphology was observed.

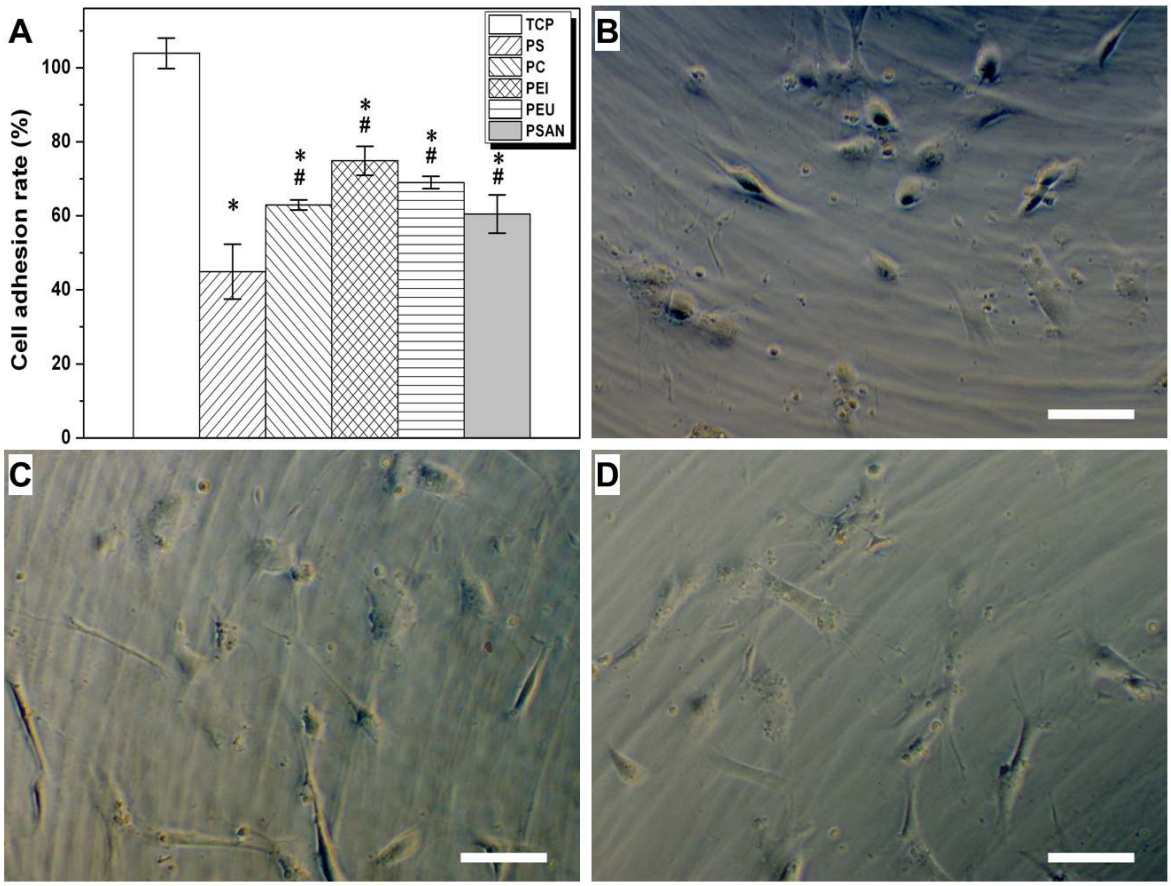

Fig. 3. Adhesion of hMSCs on different polymer surfaces 24 hours after cell seeding. The adhesion rate which was defined as the percentage of adherent cell number out of seeded cell number (A) and the representative images of cells on PS (B), PC (C) and PSAN (D). (n = 3, # Sig < 0.05 compared to PS, * Sig < 0.05 compared to TCP, Bar = 100 μm)

3.3 Cell proliferation

The cells could proliferate in all of the tested polymeric inserts (Fig. 4). Cells cultured on PS had a significantly lower proliferation rate than cells cultured on the other four polymers. PEI exhibited the best compatibility for cell proliferation, which was at the comparable level to TCP from day 7. PC and PSAN also allowed high cell proliferation rate, which was comparable to TCP after 13 days of culture. Interestingly, cells on PEU had a fast proliferation at the beginning, and the cell number was higher than that on other polymers in day 4. However, after that, the cell number increased slowly and was exceeded by the number

of cells on PC, PEI and PSAN from day 10. Compared to the number of seeded cells in day 0, the fold change of the cell number in day 16 was 3.67±0.07 (TCP), 1.93±0.07 (PS), 3.38±0.11 (PC), 3.65±0.04 (PEI), 2.24±0.15 (PEU) and 3.36±0.09 (PSAN), respectively.

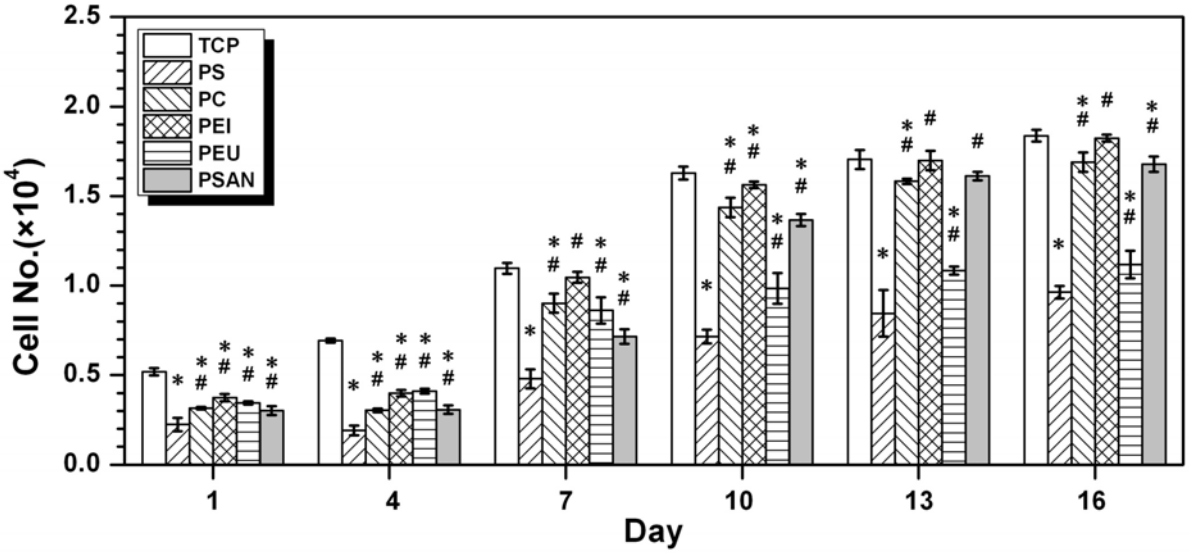

Fig. 4. Proliferation of hMSCs cultured in TCP and polymeric inserts. The cell number was determined using CCK-8. (n = 3, # Sig < 0.05 compared to PS, * Sig < 0.05 compared to TCP)

3.4 Apoptosis

In order to study the influence of different polymers on cell apoptosis, the apoptotic level was evaluated (Fig. 5). Cells on polymers except PEU exhibited decreasing tendency of apoptosis. The apoptosis level of cells on PS was the highest in day 1 and day 4, and decreased to a lower level in day 7, which was similar with that of cells on PC, PEI and PSAN. The apoptosis level of cells growing on PEU was relatively constant, which had a lower apoptosis value in day 1 and a higher apoptosis value in day 7 compared to other polymers. The lowest apoptosis levels were detected on TCP at all of the testing time points. However, in day 7, the apoptosis level of cells on PC, PEI and PSAN decreased to the comparable level to that of cells on TCP.

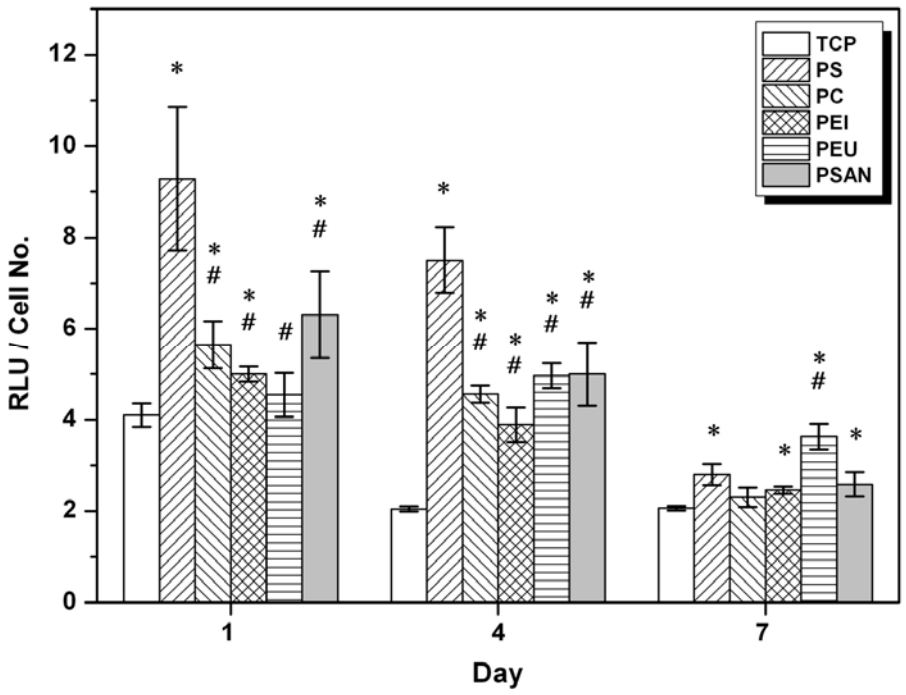

Fig. 5. Apoptotic level of hMSCs cultured in TCP and polymeric inserts was measured at different time points and expressed as relative light units (RLU) normalized against cell number. (n = 3, # Sig < 0.05 compared to PS, * Sig < 0.05 compared to TCP)

3.5 Live/dead staining

FDA/PI staining was conducted after 28 days of cell culture to evaluate the death rate of cells growing on the polymers (Fig. 6). For all of the tested polymers, few dead cells were observed, indicating the low cytotoxicity of the polymers.

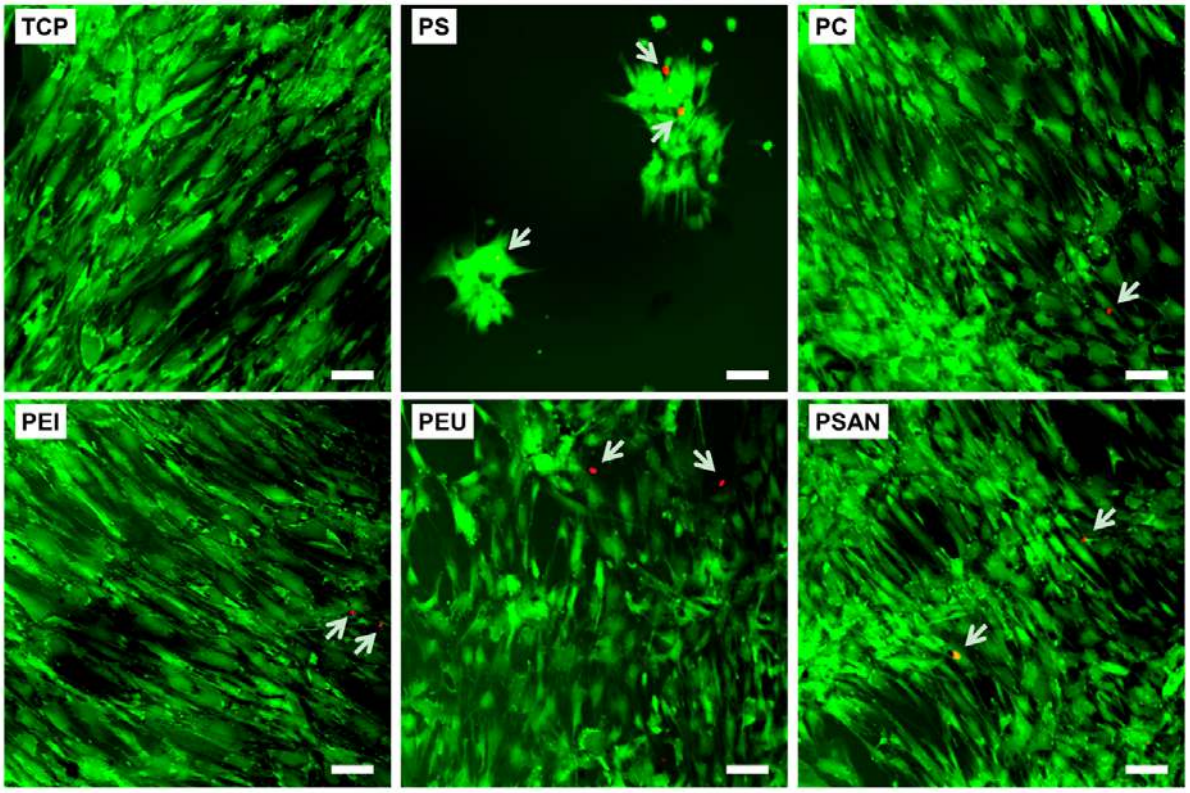

Fig. 6. Fluorescent images of hMSCs with FDA/PI staining. Cells cultured in TCP and polymeric inserts for 28 days were stained with FDA (green) and PI (red). Arrows indicate the dead cells stained by PI. (Bar = 100 μm)

3.6 Cytoskeleton staining

F-actin was stained with phalloidin after 14 days of cell culture to further evaluate the cell morphology and the organization of cytoskeleton (Fig. 7). Cells growing on PS could not spread and distribute evenly, but could only form some cell clusters. Cells growing on PC and PEI showed oriented morphology, even distribution and extensive stress fibers, which were similar like cells on TCP. Cells growing on PSAN were distributed less evenly than cells on TCP, and exhibited highly extensive and oriented stress fibers. Cells growing on PEU were less oriented than cells on TCP, and exhibited more random stress fibers.

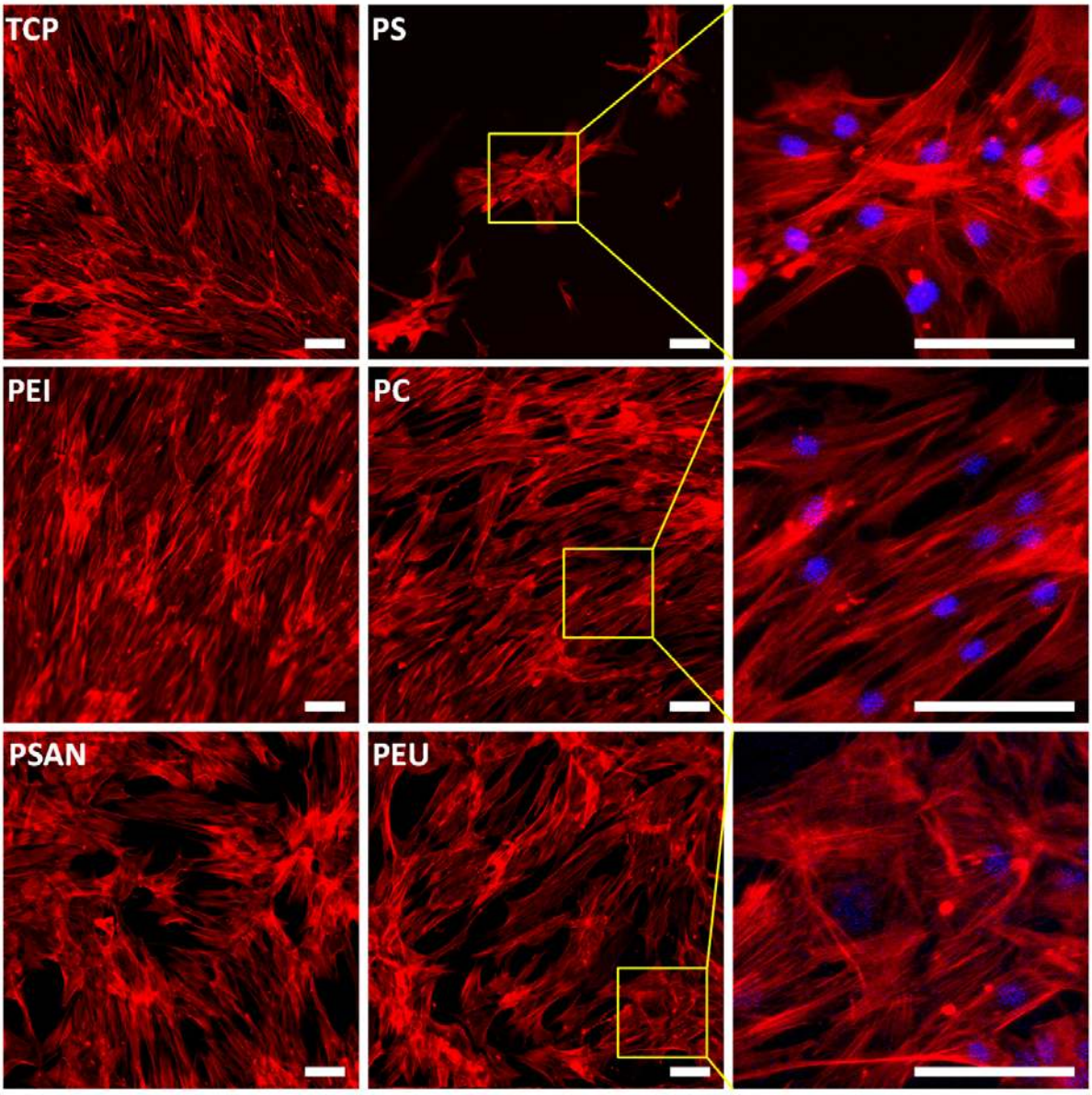

Fig. 7. Confocal images of phalloidin-stained hMSCs cultured in TCP and polymeric inserts. Cells were cultured for 14 days followed by F-actin staining (red) with phalloidin. For higher-magnification pictures, the cell nuclei were identified (blue), which were counterstained with DAPI. (Bar = 100 µm)

4. Discussion

The maintenance, proliferation and fate of stem cells could be regulated and controlled by the physiochemical cues in their microenvironment [6, 19, 62]. The progress in biomaterial and stem cell research has opened the door of the exciting field of regenerative medicine [7, 14, 17, 22, 28, 41, 61]. The potential of biomaterials to influence the stem cell behaviour is being intensively explored and has attracted significant scientific attention. We developed a

polymeric insert system for cell culture plates which allows us to use combinatorial assessment to evaluate the interaction of stem cells and polymer surfaces in a parallel manner. Large amounts of information on cell viability, apoptosis and morphology can be acquired simultaneously by using this system. Here we demonstrated that it is feasible to maintain primary hMSCs culture on the polymer surfaces for a considerably long period. The influence of different polymers on hMSCs behaviour is compared. We are able to show that other than PS based traditional culture material different polymers can promote survival and maintenance of hMSCs.

Because hMSCs are multipotent and can be expanded in culture, there has been much attention in their clinical potential for tissue repair and gene therapy [10, 38, 46, 47]. The cells are expanded to a relevant scale before they are applied clinically. Their cellular property should be stringently examined by the quality inspecting on varied parameters [23, 57]. The to date established method to culture and expand hMSCs is to use tissue culture flasks, which are commonly made from modified polystyrene. Although hMSCs usually show good growth behaviour in these tissue culture flasks, polystyrene has several intrinsic disadvantages to be used as cell culture material including its poor chemical endurance, high fragility and relative chemical inertness to be modified with functional ligands. Most importantly, pure polystyrene has poor biocompatibility for cell culture, which makes it necessary to be chemically or physically modified before its application for culturing adherent cells [4, 25, 35, 37]. Hence, there is a great need to develop an alternative surface for hMSCs expansion, screening and assessment. In this study we examined the cell compatibility and suitability of five polymers with hMSCs: PS as a conventional cell culture plastic ware material was purposely selected as our reference; PSAN, a copolymer of styrene and acrylonitrile, which has a higher Young's modulus and a lower contact angle than PS was included; PC, a very durable material, has been demonstrated to have a good biocompatibility [27, 44]; PEI which can be easily chemically surface modified and further functionalized with biomolecules [2, 53-55] and PEU as biocompatible elastic thermoplastic material with a low Young's modulus exhibiting hydrophilic wetting properties were involved for investigation [26]. To validate the data commercially available TCPs served as our positive control. In order to explore the material and stem cell interaction directly and avoid the influence of other factors, all of the tested polymer surfaces were not modified or treated (e.g. polished) after processing via injection moulding. It has been demonstrated that different surface roughness could affect the cell function [30]. Here, we attempted to remove this variable from our test system and retained a similar smooth surface topography of the tested polymers on a sub-micron scale. It should

also be noted that the micromechanical property of the polymer insert bottom was measured by atomic force microscopy in the dry state at ambient temperature, which might be altered when exposed to aqueous solution at body temperature especially in case of PEU, where the onset of the T_g is around body temperature [26]. All the finding needs to be further confirmed with the micromechanical property in aqueous solution in the future.

Cell attachment was investigated on the characterized polymeric materials. We found that hMSCs were able to attach to different untreated polymer surfaces. However, a vastly different adhesion rate of hMSCs was found on different surfaces already after 24 hours of cell culture time. The early cell matrix interaction such as adhesion and spreading is mediated via integrin or focal adhesion expression of hMSCs in general [12, 13, 49]. It has been shown that different substrates could affect integrin expression and cell behaviour of hMSCs [48]. The ratio of integrin subunit expression may also regulate the cell differentiation [24]. Therefore, we may speculate that the different polymer surfaces may influence not only hMSCs attachment but also cell fate by modulating the expression of integrin. There was excellent cell growth behaviour on PEI surface. The cell number of hMSCs on the untreated PEI surface was significantly higher than that on polystyrene, and comparable with that in TCP. This finding is in accordance with the literature showing cell compatibility of PEI with different cell lines [20]. It has been reported that the protein adsorbed on the polymer surface influenced the cell attachment and subsequently affected the cellular behaviour after cell attachment [18, 51]. However, the mechanism of protein adsorption on polymers is of complexity, which depended on both chemical and physical properties as well as the topography of the polymer surface [1, 21, 45]. For our study, we speculated that the protein adsorption might be one important factor to regulate stem cell behaviour. Proteins in the cell culture medium might first bind to polymer surfaces and then influence the adhesion and proliferation of stem cells. Proteins adsorbed on hydrophilic surfaces should typically support cell adhesion, whereas for hydrophobic surfaces such as polyethylene terephthalate cell adhesion was not favourable because of the inaccessibility of the proteins´ adhesion sites (e.g. fibronection or vitronectin) when adsorbed to the surface [56]. Further studies will be conducted in the future to clarify this hypothesis. Nevertheless, for the first time we showed PEI presented a very good cell compatibility with primary hMSCs, which suggests PEI may be an alternative material for hMSCs expansion.

Apoptosis is the programmed cell death, by which the cell undergoes intentional suicide [29]. This process is distinguished from necrosis and involved in a unique gene expression pattern

[31]. To monitor the effects of different polymer surfaces on the induction of apoptosis of hMSCs, we examined the expression level of caspase-3/7, which is a reliable biomarker of early apoptosis. This assay is based on a highly sensitive luminescent assay, which measures caspase-3/7 activities in culture of hMSCs. We found that the apoptotic rate of hMSCs on the PS surface after 1 day of cell culture time was considerable higher than that on all the other surfaces. This is a spontaneous apoptosis in the absence of any known stimuli. The mechanism of such apoptotic induction remains unknown and is currently a topic under investigation. Nevertheless, it was observed that the apoptotic rate decreased within the observation period in a time-dependent manner. The cells exhibited the highest cell adhesion rate and the lowest apoptotic rate on PEI surface. This result may be attributed to the differences in early cell attachment on the different polymer surfaces, since apoptosis may be a way to remove the nonattached hMSCs with little or no inflammatory consequence [8]. We also analysed the influence of apoptosis on cell proliferation. The apoptosis level induced by PC, PEI and PSAN decreased to a low level after 7 days of cell culture time, which was comparable to TCP. Accordingly, cells growing on these three polymers presented high cell numbers, which were comparable to those obtained on TCP after 16 days of culture. In contrast, the apoptosis level induced by PEU was lower after 1 day of cell culture time and was higher after 7 days compared to PC, PEI and PSAN. As a result, the cell numbers on PEU were higher in the earlier culture stage (day 4) and showed a gently increasing tendency after 10 days of cell culture time. Additionally, a live/dead staining was performed to evaluate the death rate of cells growing on the polymer surfaces. After 28 days of cell culture, we did not find high death rates of cells on all tested polymers, indicating that these polymers have considerably low toxicity to hMSCs.

In order to gain a deeper understanding of the influence of different polymer surfaces on MSCs, morphological characterization of cells was evaluated by light microscopy and confocal laser microscopy. The morphological change may be the early indicator of MSCs differentiation [6]. The hMSCs at early culture stage (24 hours) display typical homogenous spindle-shaped morphology on TCP as well as the other investigated polymer surfaces. However, with the increase of culture time, their morphology was altered in response to the differences of polymer surfaces. After 14 days of culture time, hMSCs growing on PEU displayed flat and round polygonal morphology and exhibited more random stress fibers, whereas cells growing on other polymers maintained a fibroblast like morphology as well as extensive and oriented stress fibers. Morphologic differences might be related with the different macromolecular orientation of the polymer chains on the surface originating from

the fast cooling in a highly oriented manner during injection moulding process. Among the investigated polymers, PEU has a low T_g of 56 °C with a T_g onset around body temperature, which allow relaxation of the previously oriented macromolecular PEU chains. As a result, the polymer chains of PEU at the insert surface might be less oriented compared to those of the other polymer inserts with higher T_g. We hypothesized that these macromolecular cues might influence the organization of cytoskeleton fibers and therefore can affect the cell morphology. Furthermore, the morphological change might be also partially attributed to the difference of the chemical environment of the polymer surfaces. Further systematic studies focusing on the influence of physical and chemical properties of the polymers on the cellular behaviour will be the basis for a knowledge-based approach to design polymers which can modulate hMSCs growth and differentiation in a technical way.

5. Conclusion

This study demonstrated that a strictly controlled and profound *in vitro* evaluation of stem cell interaction with polymeric materials is required before the materials are used for stem cell expansion and eventually translated into clinical setting. Multiple parameters have to be examined including cell viability, morphology, apoptosis and proliferation to monitor the stem cell behaviour. Here we could show the cell compatibility of different polymers with hMSCs. Among the tested polymers, PEI was the best suited one for hMSCs culture and supported long term maintenance of the stem cell state. In contrast, PS was not very compatible for hMSCs culture with the poor cell attachment and high apoptotic rate. Moreover, these observations demonstrated the potential of using PEI with hMSCs for investigations in cell therapy. Further mechanistic studies on signalling pathway and surface molecule expression, which were involved in the interaction of stem cells and polymer surfaces may help to better understand the interplay between stem cells and biomaterials.

Competing interests

The authors declare that they have no competing interests.

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