J Mater Sci: Mater Med (2009) 20:2317–2326
DOI 10.1007/s10856-009-3806-1

Immobilization of poly (ethylene imine) on poly (L-lactide) promotes MG63 cell proliferation and function

Zhen-Mei Liu · Soo-Yeon Lee · Sukhéna Sarun · Dieter Peschel · Thomas Groth

Received: 20 January 2009 / Accepted: 16 June 2009 / Published online: 30 June 2009
© Springer Science+Business Media, LLC 2009

Abstract Poly (ethylene imine) (PEI) is a polycation widely used for DNA transfection to cells but also applied as primary polycation for layer-by-layer (LBL) assembly of polyelectrolytes. The aim of the present study was to investigate the effect of modification with PEI on the biocompatibility of poly (L-lactide) (PLLA) films. PEI with different molecular weight was immobilized on PLLA by either adsorption or covalent binding. Cell morphologies, immuno-fluorescence staining, cell proliferation by lactate dehydrogenase assay and cell differentiation by alkaline phosphatase assay were utilized to assess the biocompatibility of the modified PLLA using osteoblast cell line MG63. Results revealed that PEI modification remarkably improved cell adhesion, viability, proliferation and function compared with plain PLLA. Hence, PEI-modified PLLA is acceptable as transfection vehicle for engineering of bone and other tissues, or as primary layer to allow LBL assembly to generate biomimetic surface coatings.

1 Introduction

Biodegradable materials have absorbed great interest in tissue engineering applications because of the fact that formation of new tissues must be accompanied by concomitant resorption of the scaffold [1, 2]. However, the low wettability of most synthetic biodegradable polyesters (e.g., polylactide) has been recognized to cause certain problems with regard to the biocompatibility when these polymers were used as scaffolds, in spite of their advantages such as a controlled rate of biodegradation, easy and scalable material manufacturing [3]. It has been shown in a number of studies that hydrophobic biomaterial surfaces hamper attachment of cells and/or impair their subsequent growth and function [4, 5]. The underlying reasons for this phenomenon are the altered adsorption and conformation of adhesive proteins like fibronectin, which may lead to impaired interaction with cellular adhesion receptors namely integrins [6, 7]. Surface modification is a feasible approach to make hydrophobic biomaterials more wettable or to introduce biospecific cues promoting adhesion of cells [8]. To preserve the bulk properties of the biomaterial, which are required for specific applications, various surface modification methods have been developed, including the surface chemical reaction [9], surface coating or entrapment [10], plasma treatment/glow or corona-discharge [11], surface grafting [12], chemical vapour deposition [13], ion beam irradiation [14], nanopattern fabrication [15] and so on.

Poly(L-lactide) (PLLA) is a relatively hydrophobic polymer, whose biocompatibility depends on the molecular weight besides its crystallinity [16]. To improve the biocompatibility of PLLA, plasma treatment was employed for subsequent immobilization of RGDs, which greatly promoted attachment and mineralization of osteosarcoma on modified PLLA scaffold [17]. By incorporation of natural macromolecules (e.g., fibrin) into PLLA porous scaffold by pore-filling method, both the biocompatibility and mechanical performances of the scaffold were enhanced [18]. However, most of the techniques described above

Z.-M. Liu (✉)
Institute of Polymer Science, Zhejiang University,
Hangzhou 310027, People's Republic of China
e-mail: zmliu@zju.edu.cn

S.-Y. Lee · S. Sarun · D. Peschel · T. Groth (✉)
Department of Pharmaceutics and Biopharmaceutics, Institute of Pharmacy, Biomedical Materials Group, Martin-Luther-University Halle-Wittenberg, Heinrich-Damerow-Strasse 4, 06120 Halle (Saale), Germany
e-mail: thomas.groth@pharmazie.uni-halle.de

required additional procedures during or after the formation of scaffolds, such as blending of PLLA with functional molecules, physical, plasma, chemical or photochemical treatment of scaffolds, et al.

Layer-by-layer assembly of polyelectrolytes (LBL) is a novel technique firstly developed by Decher & Hong for preparing structure-controlled multilayers on solids or semisolids for a variety of applications [19]. The ease of preparation, stability of multilayers formed, nanoscale controllability of film thickness, applicability to various charged species make it an attractive technique for the production of biomimetic structures that can find applications in biosensors, perm-selective membranes, controlled drug delivery devices, and biomaterials [20, 21]. Numerous polyelectrolytes have been used for LBL technique, among them the polycation poly (ethylene imine) (PEI) has absorbed great interest. One of the prominent features of PEI is the high cationic charge density. This makes PEI a commonly used polyelectrolyte in LBL technique [22, 23]. Another frequent application of PEI is acting as transfection agent because it readily forms complexes with negatively charged DNA [24]. However, PEI has certain cytotoxicity depending on its molecular weight [25, 26]. In the present study, both high and low molecular weight PEI were immobilized onto PLLA to generate a degradable scaffold surface, which is intended to be further utilized for LBL assembly or as transfection vehicle for cells in tissue engineering applications. The biocompatibility of PEI-modified surface was evaluated by the osteoblast cell line MG63 through cell adhesion, spreading, proliferation and differentiation.

2 Materials and methods

2.1 Materials

PLLA (Mw ~148.8 kDa, from Boehringer Ingelheim Pharma GmbH&Co.KG, Ingelheim, Germany) were prepared as film by the solvent-cast method. The obtained PLLA films were cut into discs with a diameter of 14 mm and subsequently cleaned with ethanol and water, dried in air and kept in a desiccator until use.

Surface modification of PLLA films with PEI (Mw of 750 or 25 kDa, Sigma-Aldrich, Steinheim, Germany) was conducted by adsorption or chemical methods. For samples prepared by the adsorption method, PLLA discs were incubated in 10 mg/ml PEI solution (pH 7.4 adjusted by diluted NaOH and HCl solution) at room temperature (RT) for 5 h under constant shaking. Then, the discs were washed with distilled water and dried. The samples are assigned as AH (from PEI Mw 750 kDa) or AL (from PEI Mw 25 kDa), where A stands for adsorption, H and L for high and low molecular weight PEI, respectively.

For samples prepared by the chemical method, PLLA discs were firstly wetted with a 50% ethanol/water solution (1:1, vol:vol) for 30 min. Then, the discs were incubated in an aqueous solution containing 3 mg/ml N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and 5 mg/ml N-hydroxysuccinimide (NHS) (Merck Schuchardt OHG, Darmstadt, Germany). After 30 min, discs were rinsed three times with water and transferred to 10 mg/ml PEI solution (pH 7.4). After 5 h incubation in PEI solution at RT, discs were washed extensively with water and dried. The obtained samples are denoted as CH (from PEI Mw 750 kDa) or CL (from PEI Mw 25 kDa), where C stands for covalent binding of PEI.

2.2 Surface characterization of substrates

The amino group density on PEI-modified PLLA films was quantified by acid orange assay as described previously [27]. Acid Orange II sodium salt was purchased from Sigma, Germany. Water contact angle measurements were performed with a contact angle device OCR 15 plus (Dataphysics, Germany) using the sessile-drop method. X-ray photoelectron spectroscopy (XPS) spectra for PLLA samples were recorded with PHI Quantum 2000 Scanning ESCA Microprobe (Physical Electronics, Inc., Chanhassen, USA) employing Al Kα excitation radiation (1486.6 eV). Zeta potentials of the different PLLA films were measured with a SurPASS electrokinetic analyzer (Anton Paar GmbH, Graz, Austria).

2.3 MG63 cells culture

The human osteosarcoma cell line MG63 was used to characterize the biocompatibility of materials. Cells were cultured in 75 cm^2 culture flasks at 37°C in a humidified atmosphere with 5% CO_2. The medium used for MG63 subculture and proliferation investigations was Dulbeccos modified Eagle medium (DMEM, Sigma), which was supplemented with 10% fetal calf serum (FCS), 1% Pen/Strep/Fungizone (PromoCell, Heidelberg, Germany), and 1% Sodium Pyruvate (Biochrom AG, Berlin, Germany). After confluence, cells were harvested with trypsin-EDTA solution (PromoCell, Heidelberg, Germany). Cells were resuspended in fresh DMEM with 10% FCS after centrifugation and counted with a hematocytometer.

Prior to cell-seeding, all films were rinsed twice with sterilized phosphate-buffered saline (PBS; 2.7 mM KCl, 137 mM NaCl, 1.4 mM KH_2PO_4, 4.3 mM Na_2HPO_4, pH 7.4). Then the samples were immersed in sterilized PBS for 1 h. The PBS solution was aspirated and the films were

incubated in the culture medium for 15 min at 37°C. Finally, the medium was removed and replaced by cell suspension at a seeding density of 10^4 cells/sample. The medium was changed every 2nd day.

2.4 Vinculin and actin staining

Fibronectin (FN) coating of surfaces was taken to model the secretion and adsorption of cellular FN on the substrata after longer culture of cells. First, samples were incubated in 5 µg/ml FN (Roche Diagnostica, Penzberg, Germany) in PBS pH 7.4 for 30 min. After rinsing three times with PBS, 1 ml of cells suspended in DMEM without serum was plated on the samples. The formation of focal adhesions and actin cytoskeleton was visualized by immunofluorescence staining after 24 h incubation. Briefly, adherent cells were fixed with paraformaldehyde solution (Roti-Histofix, Roth, Karlsruhe, Germany) for 15 min. After washing with PBS, cells were permeabilized with 0.5% Triton X-100 in PBS for 10 min. Samples were incubated with 1% BSA in PBS for 30 min for blocking non-specific binding sites. Actin (F-Actin) was stained with BODYPY-phalloidin (Molecular Probes, New Jersey, USA) for 30 min at RT. Focal adhesion plaques were stained with a primary mouse antibody against vinculin (Sigma-Aldrich, Steinheim, Germany) for 30 min, followed by 30 min incubation with Cy2 conjugated goat anti-mouse IgG (Dianova, Hamburg, Germany) as the secondary antibody. BODIPY-phalloidin and antibodies were dissolved in 1% BSA in PBS at working dilutions according to the suggestion of the producer. After washing of cells with PBS and distilled water, samples were mounted with Mowiol and viewed with confocal laser scanning microscopy (CLSM, LEICA DM IREZ TCS SP2 AOBS spectral confocal microscope, Germany).

2.5 Cell proliferation

A modified lactate dehydrogenase (LDH) assay was performed using the WST-8 kit (BioCat, Heidelberg, Germany) for cell proliferation test [28]. After 1, 3 and 6 days of culture, the medium in the wells was removed and samples were washed twice with PBS. Attached cells were lysed for 30 min at 37°C with 100 µl 0.5% Triton X-100/sample to release LDH from the cytosol of all viable cells. Then the plate was centrifuged at 1300 rpm for 10 min to separate the cell debris. 10 µl of the cell lysis solution was transferred into 96 well plates. The blank reference was taken from 0.5% Triton X-100 solution. 100 µl LDH reaction reagents were added to the lysates and incubated for 30 min at RT. The OD value at 450 nm was recorded and taken as a measure for the quantity of cells.

2.6 Alkaline phosphatase (ALP) activity

After 1, 3, 6, 9 and 14 days of culture, ALP activity was determined in cell lysates obtained as described above. ALP activity was measured colorimetrically using p-nitrophenylphosphate (pNPP) (Roth, Karlsruhe, Germany) as a substrate. Briefly, 50 µl cell lysate was incubated in a 96-well plate with 100 µl of 0.2 mg/ml pNPP solution (pH 10.2) for 1.5 h at 37°C. The absorbance of released p-nitrophenol was measured by a plate reader (BMG LABTECH, Fluostar OPTIMA, Offenburg, Germany) at 405 nm.

2.7 Histochemical analysis of glycosaminoglycan (GAG) synthesis

Alcian blue staining was carried out to visualize the synthesis of GAG as matrix components. After 3 weeks of culture, cells on each specimen were fixed for 15 min in 4% paraformaldehyde. Alcian blue (Roth, Karlsruhe, Germany) was dissolved in 3% acetic acid (pH 2.5) to obtain a final concentration of 0.5% and filtrated to remove undissolved crystals. 500 µl of alcian blue solution was added to the sample well and incubated for 1 h at RT. Excess of alcian blue was removed and samples were washed with distilled water four times before micrographs were taken. Samples cultured with inductors (DMEM containing 10% FBS, 0.2 mM L-ascorbic acid 2-phosphate, 10 mM ß-glycerophosphate and 0.1 µM dexamethasone (Sigma-Aldrich, Steinheim, Germany) from Day 6 were used for comparison.

2.8 Statistics

Three parallel experiments were performed for the measurements of LDH and ALP assay at different culture time, and the experiments were repeated three times to get similar results. Data presented are expressed as (the average ± standard derivation). Significance testing was performed with ANOVA (Tukey's test) for paired samples. Differences were considered as statistically significant at $P \leq 0.05$.

3 Results

3.1 Surface characterization of substrata

XPS is an effective method to characterize the chemical composition of surfaces. For nascent PLLA (Fig. 1), two peaks appeared in the XPS survey spectrum, which can be assigned to the elements C and O. After PEI modification, a

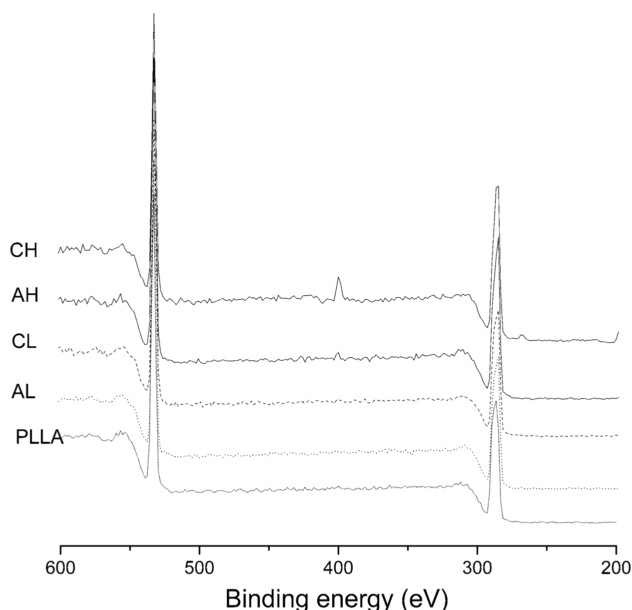

Fig. 1 XPS survey spectra for different PLLA samples. Sample illustration: *PLLA* nascent PLLA, *AL* low molecular weight PEI modified PLLA by adsorption, *CL* low molecular weight PEI modified PLLA by chemical method, *AH* high molecular weight PEI modified PLLA by adsorption, *CH* high molecular weight PEI modified PLLA by chemical method

new peak ascribed to N element became visible at the binding energy of ~400 eV. This was most pronounced for the sample CH. From the peaks of XPS spectra one can calculate the ratio of elements on the sample surface, as listed in Table 1. For blank PLLA, the contents of C and O were 61.6 and 38.4%, respectively. After PEI modification, the ratio of C:O increased since more carbon from PEI molecules was introduced onto the surface. Table 1 indicated that the amount of nitrogen increased obviously for samples modified with high molecular weight (HMW) PEI, especially for CH of which 3.7% nitrogen was detectable.

Acid orange was used to quantify surface amino group density. Data listed in Table 1 revealed that surface amino group density increased as the surface modification methods shifted from physical to chemical one and from low molecular weight (LMW) to HMW PEI. These results were consistent with XPS data as well. Static water contact angle (CA) measurements on different samples revealed that nascent PLLA was relatively hydrophobic with a CA of 72.4°. Surface modification led to decreased surface CAs. For sample AL the CA was about 60°. When HMW PEI was used for surface modification either by adsorption or covalent binding, a further decrease of CA was observed.

The results of zeta-potential measurements are depicted in Fig. 2. The extrapolated isoelectric points (IP) are also listed in Table 1 as well as the surface zeta-potential at pH 7.4. The IP for plain PLLA was as low as 3.89, which could be ascribed to the acidic end groups of PLLA molecules. After modification, the IP increased slightly due to the immobilization of positively charged PEI onto the surface. For samples AL, CL and AH, the surface modification method or molecular weight of PEI had negligible effects on the resulting IP value. However, the chemical modification with HMW PEI caused a remarkable increase of IP to 4.48. This should be due to the large quantity of available amino groups. The increased surface zeta-potential at pH 7.4 due to PEI-modification may have great effects when contacting with physiological solutions.

3.2 Cell morphology

Figure 3 shows phase contrast images of cells cultured for 24 h on different samples. Tissue culture polystyrene (TCPs) was chosen as the control, on which cells spread and acquired polar cell shape within 24 h. In contrast to this finding, cells on PLLA were less spread, many cells were still round with only a few polarized cells visible. Adsorptive binding of LMW PEI (AL) improved the cytocompatibility on which more spread and less round cells were observed. For sample AH and CL, cell adhesion was similar to those on plain PLLA. However, when HMW PEI was immobilized covalently (CH), similar to TCPs, more polarized cells were found indicating better biocompatibility on this surface compared with nascent PLLA.

Immunofluorescence staining was carried out to show the cytoskeleton developments for MG63 cells after cultured 24 h on the various PLLA surfaces. Due to practical reason, glass was used as reference material instead of TCPs to show normal expression of these structures in

Table 1 Surface properties for different PLLA films

Samples	Surface element composition (mol.%)			Amino group density (*10^{16}/cm^2)	Contact angle (°)	Isoelectric point	Zeta-potential at pH 7.4 (mV)
	C	N	O				
PLLA	61.6	–	38.4	–	72.4 ± 0.3	3.89	−94.8
AL	64.2	0.5	35.3	2.51 ± 0.19	60.5 ± 0.9	4.06	−79.8
CL	63.7	0.5	35.8	9.36 ± 0.62	59.4 ± 0.6	4.10	−94.8
AH	65.1	1.0	33.9	12.14 ± 0.40	56.8 ± 2.7	4.08	−65.7
CH	63.3	3.7	33.0	12.79 ± 0.36	56.2 ± 1.9	4.48	−53.4

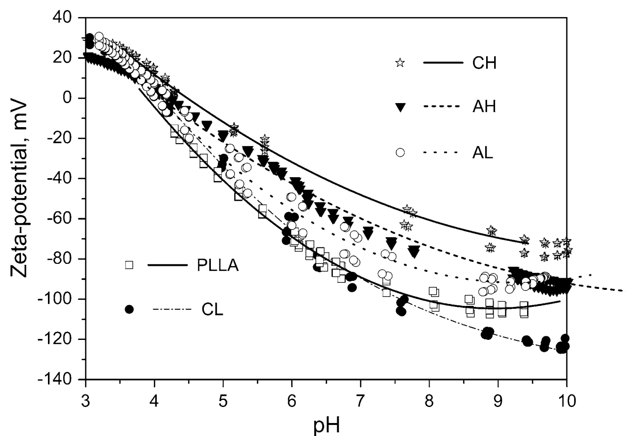

Fig. 2 Zeta potential for different PLLA samples acquired by steaming potential and streaming current method at various pH values

MG63 cells. It is evident that MG63 cells on glass possessed well developed focal adhesion plaques (Fig. 4a) and actin stress fibres (Fig. 4b). The shape of cells cultured on plain PLLA films coated with FN was irregular, focal adhesions were completely absent (Fig. 4a) and no actin stress fibres were detected (Fig. 4b). By contrast, focal adhesions were well developed in cells cultured on AL where numerous focal adhesion plaques were observed. This was also the case for sample CH though adhesions appeared to be weaker. On the other hand, no focal adhesions could be observed on sample AH and CL (Fig. 4a). When stained for F-actin, longitudinal stress fibres were visible in cells on AL and CH, and to some extend also on CL, while the absence of stress fibres in cells on AH corresponded well with their irregular shape (Fig. 4b).

3.3 Cell proliferation

The growth of MG63 on sample surfaces was quantified by a modified LDH assay. Results shown in Fig. 5 indicated that after a culture period of 24 h, the highest number of viable cells was detected on sample AL, while on plain PLLA surface the lowest cell number was observed. Up to day 6 the quantity of viable MG63 cells was significantly lower on PLLA ($P \leq 0.05$) than on PEI modified surfaces. There was a slightly enhanced cell growth on AL, though no remarkable differences were found among the PEI-modified samples.

3.4 Cell differentiation

ALP assay was used to quantify the osteogenic activity of MG63 cells on different substrates. As is shown in Fig. 6, there was no ALP activity of MG63 on the tested surfaces at day 1. After 6 days, the ALP activity of MG63 cells increased dramatically. It kept increasing up to day 9. Among PLLA samples, cells on the PEI-modified samples expressed significantly higher ALP activity ($P \leq 0.05$) than those growing on plain PLLA. No distinct differences could be observed among the PEI-modified surfaces concerning ALP activity.

To visualize the differentiation of cells cultured on PLLA samples, they were stained with alcian blue, the micrographs observed by transmitting light were shown in Fig. 7. It is worth noting that in the absence of osteogenic inductors, a more pronounced blue staining, which indicates deposition of extracellular matrix related to glycosaminoglycans, was

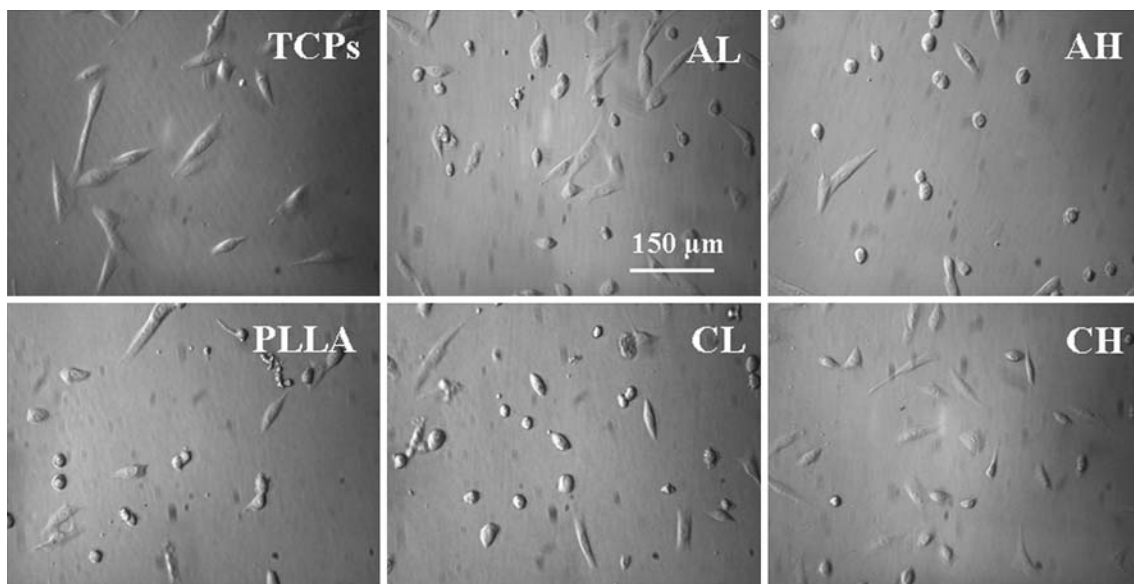

Fig. 3 Cell morphology of MG63 on different samples taken by phase contrast microscopy after 24 h incubation

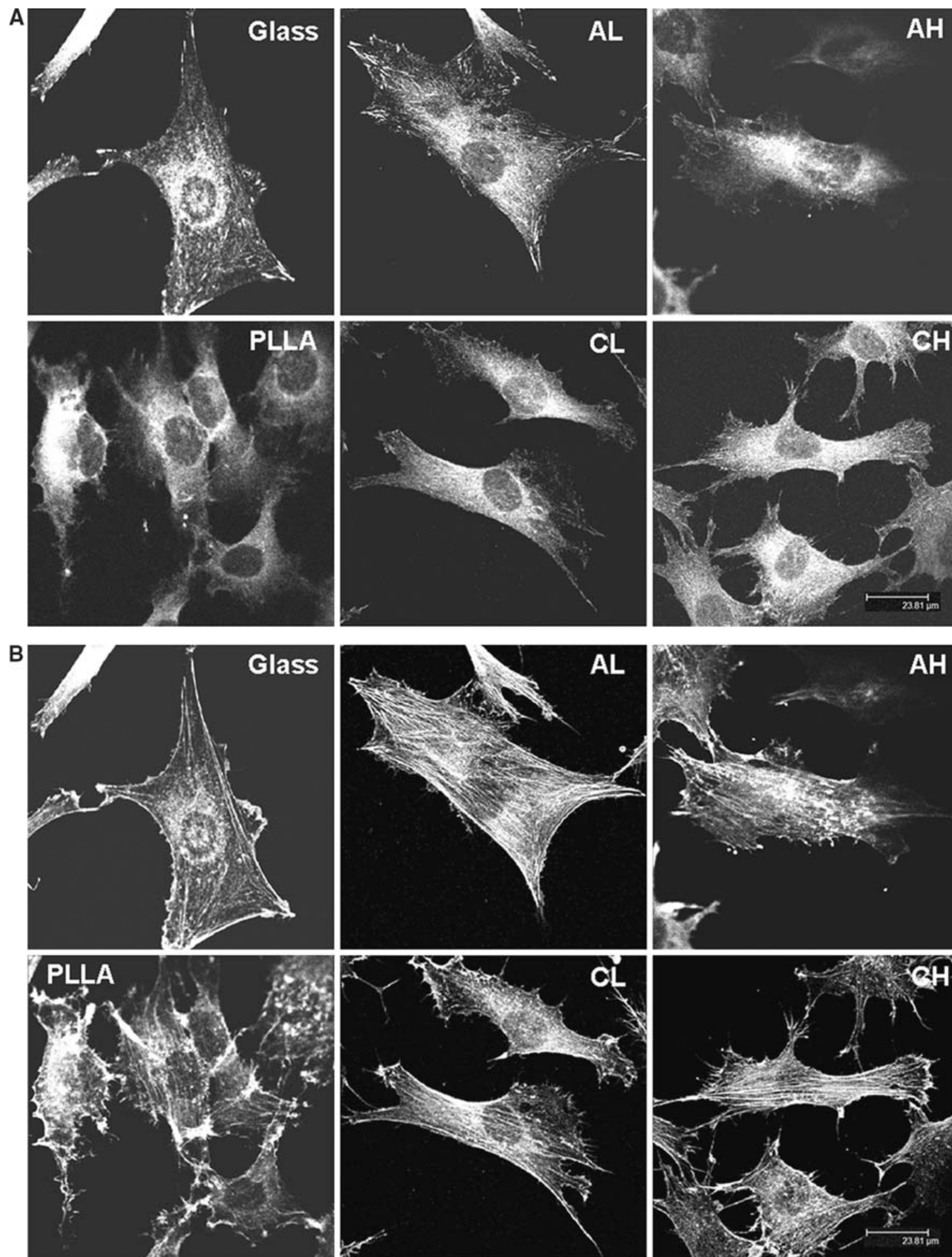

Fig. 4 Immunofluorescence staining for the visualisation of cellular structures, such as vinculin (**a**) and actin (**b**) for MG63 seeded onto FN-precoated PLLA films after incubation for 24 h. Scale bar = 23.81 μm

observed on AL and CH if compared with other PLLA surfaces. When MG63 cells were cultured in the presence of osteogenic inductors, no strong differences were detectable (images not shown here).

4 Discussions

It was the aim of this study to investigate the effects of PEI modification on the biocompatibility of PLLA films. The

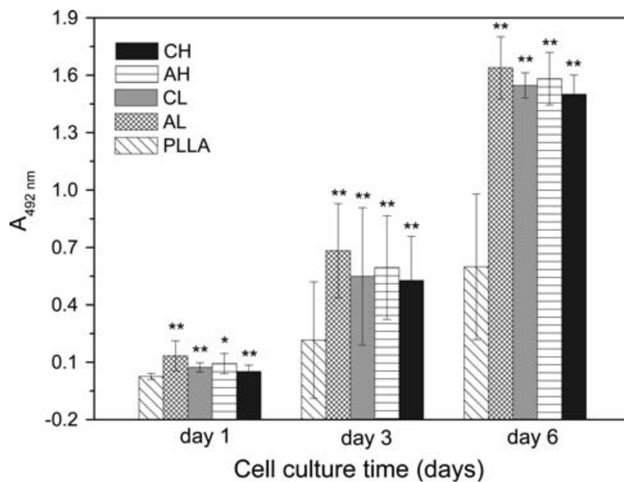

Fig. 5 LDH assay for cell number measured as optical density function of culture time on different types of surfaces. * $P < 0.01$; ** $P < 0.05$

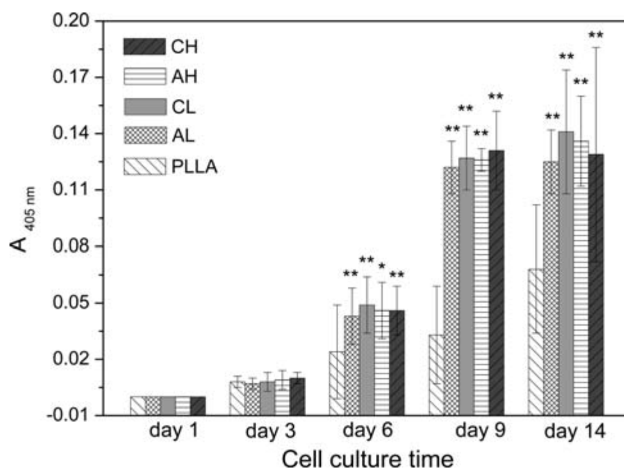

Fig. 6 ALP assay for MG63 differentiation measured as optical density function of culture time on different types of surfaces

osteoblast cell line MG63, which is derived from a human osteosarcoma, was used for these studies. MG63 cells are supposed to express a number of characteristics typical for osteoblasts with a reduced ability to produce calcified tissue, but have also a poor growth control [29]. Despite the toxic effects of PEI described in the literature, a distinct result of the present study was that modifications with PEI by either adsorption or covalently binding improved the biocompatibility of PLLA significantly.

Physicochemical investigations demonstrated that PEI can be feasibly immobilized on PLLA. Since PEI has a certain cytotoxicity which is dependent on the molecular weight, both HMW (750 kDa) and LMW (25 kDa) PEI were used in this study. Results of XPS and acid orange assay revealed that the application of HMW PEI is superior to induce larger quantities of amino groups on the surface, particularly for chemical method. Streaming potential measurements confirmed this, which revealed that sample CH provoked the greatest increase in surface zeta potential. Though the change in contact angle after modification of PLLA with PEI was only moderate, previous studies showed that amino groups promoted the adhesion of a variety of cells [30, 31]. Therefore improved cell contacting properties of PEI modified surfaces were anticipated. On the other hand, cytotoxic effects of PEI are dependent on its molecular weight, but maybe also the way how is PEI attached to substrata. In a recent paper we could show that LMW PEI promoted adhesion and growth of keratinocytes, while HMW PEI inhibited it [27]. Hence, distinct effects of the molecular weight of PEI and its way of attachment on adhesion, growth and function of MG63 cells were expected.

When adhesion of MG63 cells was studied by phase contrast microscopy, TCPs was used as a control surface to visualize normal morphology of MG63 cells, which was represented by a spread, polarized shape [32]. By contrast, MG63 cells adhered to the PLLA surfaces with remarkable differences in morphology. These morphological differences decreased after longer culture of cells, which is consistent with the malignant character of sarcoma cell line [33]. However, short term cultures gave interesting insights into the biocompatibility of the investigated surfaces. It was obvious that plain PLLA did not provide sufficient attachment sites since cells remained round. This seems to be related to the lower wettability of PLLA, which may lead to conformational changes and stronger binding of non-specifically adsorbed adhesive proteins [34, 35]. Results in this paper revealed that modification of PLLA with PEI (both LMW and HMW) improved the adhesion of cells by their better spreading with less round cells adhered. The presence of different amine functionalities may be beneficial because amino groups promote adsorption of fibronectin and do not disturb its conformation and activity [37], nevertheless HMW PEI is normally cytotoxic [26, 36]. Hence, the biological activity of such functionalized surfaces might be improved.

Also immunofluorescence microscopy supported these findings. Focal adhesion formation requires sufficient quantities of adsorbed adhesive proteins like fibronectin [38, 39], and is related to signal transduction processes regulating actin polymerization, cell growth and differentiation [40]. It was visible that even after coating with fibronectin, plain PLLA did not support focal adhesion formation and actin polymerization. Hence it was not surprising that growth of MG63 cells was delayed on this substratum as found with the modified LDH method. Among the PEI modifications, sample AL seemed to provide best conditions for focal adhesion formation and actin polymerization. If one compares this finding with the results of growth experiments, it seems to be correlated

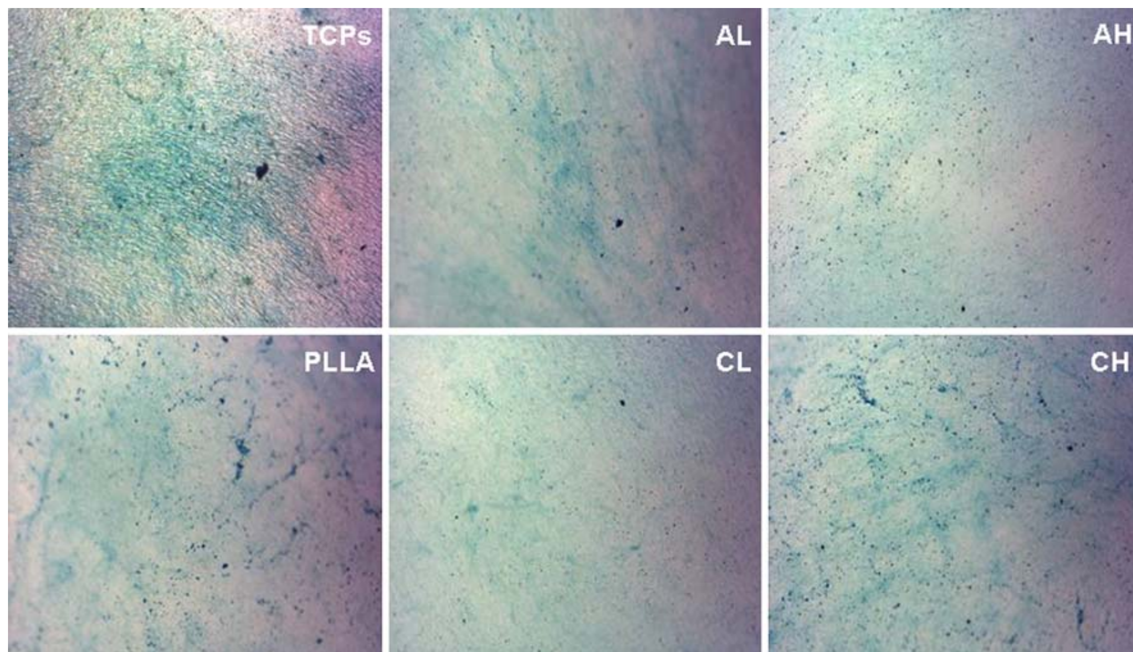

Fig. 7 Micrographs taken by transmitting light for alcian blue stained MG63 cells. The cells were cultured on different types of surfaces for 3 weeks in normal medium

with the slightly enhanced growth of MG63 cells on AL surface, which corresponds well to our recent finding showing enhanced growth of keratinocytes on poly (ether imide) membranes modified with LMW PEI [27].

The functional activity of MG63 cells was measured by the release of alkaline phosphatase which is one of the early markers of osteogenic activity [29]. Investigations to detect calcified tissue by a histochemical approach were carried out, but failed to show any significant quantities of calcified material, which seems to be related to the reduced ability of MG63 cells to form a calcified matrix after longer culture time [32, 41]. In concordance with other studies it was demonstrated that ALP activity was low during the initial cell culture period [42]. To find notable quantities of ALP, experiments were conducted for 14 days instead of one week. The quantitative results with ALP revealed that plain PLLA did not provide favorable conditions for functioning of osteoblasts, on which the ALP activity of cells was significantly lower from day 6 until day 14 compared with that on surface modified samples. On the other hand, the differences among PEI modified PLLA were negligible. These findings are basically in line with the results of alcian blue staining. A reduced synthesis of GAG by MG63 cells on blank PLLA could be observed if compared to AL and CH. Overall, one can state that modification of PLLA in either way—adsorptive or covalently bound, low or high molecular weight, improves the biocompatibility of PLLA in terms of MG63 cell adhesion, growth and function obviously. Reasons for the observed effects can be deduced particularly from the presence of amino groups and concomitant increase of surface charge for most of PEI modifications. Less negatively charged surfaces attract proteins and cells more strongly [7, 37]. Also the improved wettability of PLLA after modification with PEI is in line with those findings [4–6]. Moreover, it has been demonstrated by us and many other groups that the amino group carrying surfaces can promote adsorption of adhesive proteins such as fibronectin and vitronectin, which retain their biological activity [7, 8, 37–39]. This may also explain why such a small change of surface properties after minor adsorption of LMW PEI had a pronounced effect on adhesion, growth and function of MG-63 cells.

5 Conclusions

High and low molecular weight PEI were immobilized onto the surface of PLLA films by either physical or chemical methods. The morphologies of MG 63 cells visualized by phase contrast microscope, and immunofluorescence staining investigated by CLSM revealed that cell adhesion was remarkably enhanced upon PEI modification compared with nascent PLLA. Results from LDH and ALP assay demonstrated that PEI modification could further improve the proliferation and function of MG 63, nevertheless the surface derived from LMW PEI was slightly better. Hence, method introduced in this paper may

be explored for the modification of scaffolds for tissue engineering of bone [18, 43]. It is worth noting that methods presented here is simple and applicable to scaffolds with various morphologies. The choice of immobilization method (adsorptive or covalent) and the molecular weight of PEI (low or high) depends on the desired application. The adsorptive immobilization with LMW PEI may find applications for in situ gene transfection [44], while covalently binding with HMW PEI are promising to prepare polyelectrolyte multilayers on tissue engineering scaffolds, which will be shown by us in future investigations.

Acknowledgments This work was supported by the Marie-Curie Incoming International Fellowship (Contract No. MIF1-CT-2005-021854) of European Commission to Dr. Z.-M. Liu. The authors are grateful to Prof. Shui-Ju Wang from State Key Laboratory of Physical Chemistry of Solid Surfaces, Xiamen University, Xiamen, P. R. China for XPS measurements.

References

1. Navarro M, Aparicio C, Charles-Harris M, Ginebra MP, Engel E, Planell JA. Development of a biodegradable composite scaffold for bone tissue engineering: physicochemical, topographical, mechanical, degradation, and biological properties. Adv Polym Sci. 2006;200:209–31. doi:10.1007/12_068.
2. Silva GA, Ducheyne P, Reis RL. Materials in particulate form for tissue engineering. 1. Basic concepts. J Tissue Eng Regen Med. 2007;1:4–24. doi:10.1002/term.2.
3. Nieminen T, Rantala I, Hiidenheimo I, Keränen J, Kainulainen H, Wuolijoki E, et al. Degradative and mechanical properties of a novel resorbable plating system during a 3-year-follow-up in vivo and in vitro. J Mater Sci: Mater Med. 2008;19:1155–63. doi:10.1007/s10856-007-3082-x.
4. Tzoneva R, Groth T, Altankov G, Paul D. Remodeling of fibrinogen by endothelial cells in dependence on fibronectin matrix assembly. Effect of substratum wettability. J Mater Sci: Mater Med. 2002;13:1235–44. doi:10.1023/A:1021131113711.
5. Webb K, Hlady V, Tresco PA. Relative importance of surface wettability and charged functional groups on NIH 3T3 fibroblast attachment, spreading, and cytoskeletal organization. J Biomed Mater Res. 1998;41:422–30. doi:10.1002/(SICI)1097-4636(19980905)41:3<422::AID-JBM12>3.0.CO;2-K.
6. Altankov G, Groth T. Reorganization of substratum-bound fibronectin on hydrophilic and hydrophobic materials is related to biocompatibility. J Mater Sci: Mater Med. 1994;5:732–7. doi:10.1007/BF00120366.
7. Faucheux N, Schweiss R, Lützow K, Werner C, Groth T. Self-assembled monolayers with different terminating groups as model subtrates for cell adhesion studies. Biomaterials. 2004;25:2721–30. doi:10.1016/j.biomaterials.2003.09.069.
8. Pompe T, Keller K, Mothes G, Nitschke M, Teese M, Zimmermann R, et al. Surface modification of poly (hydroxybutyrate) films to control cell-matrix adhesion. Biomaterials. 2007;28(1):28–37. doi:10.1016/j.biomaterials.2006.08.028.
9. Sperling C, Maitz MF, Talkenberger S, Gouzy M-F, Groth T, Werner C. In vitro blood reactivity to hydroxylated and non-hydroxylated polymer surfaces. Biomaterials. 2007;28:3617–25. doi:10.1016/j.biomaterials.2007.04.041.
10. Van Den Dolder J, Jansen JA. The response of osteoblast-like cells towards collagen type I coating immobilized by p-nitrophenylchloroformate to titanium. J Biomed Mater Res A. 2007;83:712–9. doi:10.1002/jbm.a.31428.
11. Kamath S, Bhattacharyya D, Padukudru C, Timmons RB, Tang LP. Surface chemistry influences implant-mediated host tissue responses. J Biomed Mater Res A. 2008;86:617–26. doi:10.1002/jbm.a.31649.
12. Liu Z-M, Xu Z-K, Wan L-S, Wu J, Ulbricht M. Surface modification of polypropylene microfiltration membranes by the immobilization of poly(*N*-vinyl-2-pyrrolidone): a facile plasma approach. J Membr Sci. 2005;249(1–2):21–31. doi:10.1016/j.memsci.2004.10.001.
13. Klee D, Ademovic Z, Bosserhoff A, Hoecker H, Maziolis G, Erli H-J. Surface modification of poly(vinylidenefluoride) to improve the osteoblast adhesion. Biomaterials. 2003;24:3663–70. doi:10.1016/S0142-9612(03)00235-7.
14. Silvan MM, Valsesia A, Hasiwa M, Gilliland D, Ceccone G, Rossi F. Surface characterization of biopolymer micropatterns processed by ion-beam modification and PECVD. Chem Vapor Depos. 2007;13:211–8. doi:10.1002/cvde.200606580.
15. Engel E, Michiardi A, Navarro M, Lacroix D, Planell JA. Nanotechnology in regenerative medicine: the materials side. Trends Biotechnol. 2008;26:39–47. doi:10.1016/j.tibtech.2007.10.005.
16. Salgado AJ, Wang Y, Mano JF, Reis RL. Influence of molecular weight and crystallinity of poly (L-lactic acid) on the adhesion and proliferation of human osteoblast like cells. Mater Sci Forum. 2006;514–516:1020–4. doi:10.4028/www.scientific.net/MSF.514-516.1020.
17. Ho MH, Hou LT, Tu CY, Hsieh HJ, Lai JY, Chen WJ, et al. Promotion of cell affinity of porous PLLA scaffolds by immobilization of RGD peptides via plasma treatment. Macromol Biosci. 2006;6:90–8. doi:10.1002/mabi.200500130.
18. Zhao H, Ma L, Gong Y, Gao C, Shen J. A polylactide/fibrin gel composite scaffold for cartilage tissue engineering: fabrication and an in vitro evaluation. J Mater Sci: Mater Med. 2009;20:135–43. doi:10.1007/s10856-008-3543-x.
19. Ariga K, Hill JP, Ji Q. Biomaterials and biofunctionality in layered macromolecular assemblies. Macromol Biosci. 2008;8:981–90. doi:10.1002/mabi.200800102.
20. Hammond PT. Form and function in multilayer assembly: new applicaitons at the nanoscale. Adv Mater. 2004;16:1271–93. doi:10.1002/adma.200400760.
21. Tang Z, Wang Y, Podsiadlo P, Kotov NA. Biomedical applications of layer-by-layer assembly: from biomimetics to tissue engineering. Adv Mater. 2006;18:3203–24. doi:10.1002/adma.200600113.
22. Neu M, Fischer D, Kissel T. Recent advances in rational gene transfer vector design based on poly (ethaylene imine) and its derivatives. J Gene Med. 2005;7:992–1009. doi:10.1002/jgm.773.
23. Burnot C, Ponsonnet L, Lagneau C, Farge P, Picart C, Grosgogeat B. Cytotoxicity of polyethyleneimine (PEI), precursor base layer of polyelectrolyte multilayer films. Biomaterials. 2007;28:632–40. doi:10.1016/j.biomaterials.2006.09.026.
24. Bertschinger M, Backliwal G, Schertenleib A, Jordan M, Hacker DL, Wurm FM. Disassembly of polyethylenimine-DNA particles in vitro: Implications for polyethylenimine-mediated DNA. J Control Release. 2006;116:96–104. doi:10.1016/j.jconrel.2006.09.006.
25. Fisher D, Bieber T, Li Y, Elsässer HP, Kissel T. A novel non-viral vector for DNA delivery based on low molecular weight, branched polyethylenimine: effect of molecular weight on transfection efficiency and cytotoxicity. Pharm Res. 1999;16:1273–9. doi:10.1023/A:1014861900478.

26. Moghimi SM, Symonds P, Murray JC, Hunter AC, Debska G, Szewczyk A. A two-stage poly(ethylenimine)-mediated cytotoxicity: implications for gene transfer/therapy. Mol Ther. 2005;11:990–5. doi:10.1016/j.ymthe.2005.02.010.
27. Trimpert C, Boese G, Albrecht W, Richau K, Weigel T, Lendlein A, et al. Poly(ether imide) membranes modified with poly (ethylene imine) as potential carriers for epidermal substitutes. Macromol Biosci. 2006;6:274–84. doi:10.1002/mabi.200500238.
28. Kirchhof K, Hristova K, Krasteva N, Altankov G, Groth T. Multilayer coating on biomaterials for control of MG-63 osteoblast adhesion and growth. J Mater Sci: Mater Med. 2009;20:897–907. doi:10.1007/s10856-008-3639-3.
29. Charles-Harris M, Koch MA, Navarro M, Lacroix D, Engel E, Planell JAA. PLA/calcium phosphate degradable composite material for bone eittue engineering: an in vitro study. J Mater Sci: Mater Med. 2008;19:1503–13. doi:10.1007/s10856-008-3390-9.
30. Nakayama Y, Takahagi T, Soeda F, Hatada K, Nagaoka S, Suzuki J, et al. XPS analysis of NH_3 plasma-treated polystyrene films utilizing gas phase chemical modification. J Polym Sci A. 2003;26:559–72. doi:10.1002/pola.1988.080260219.
31. Ren TB, Weigel T, Groth T. Microwave plasma surface modification of silicone elastomer with allylamine for improvement of biocompatibility. J Biomed Mater Res A. 2008;86:209–19. doi:10.1002/jbm.a.31508.
32. Clover J, Gowen M. Are MG63 and HOS TE 85 human osteosarcoma cell lines representative models of the osteoblastic phenotype? Bone. 1994;15:585–91. doi:10.1016/8756-3282(94)90305-0.
33. Schwartz Z, Lohmann CH, Oefinger J, Bonewald LF, Dean DD, Boyan BD. Implant surface characteristics modulate differentiation behavior of cells in the osteoblastic lineage. Adv Dent Res. 1999;13:38–48. doi:10.1177/08959374990130011301.
34. Grinnell F, Feld MK. Adsorption characteristics of plasma fibronectin in relationship to biological activity. J Biomed Mater Res. 1981;15:363–81. doi:10.1002/jbm.820150308.
35. Renner L, Pompe T, Salchert K, Werner C. Fibronectin displacement at polymer surfaces. Langmuir. 2005;21:4571–7. doi:10.1021/la046801n.
36. Tryoen-Toth P, Vautier D, Haikel Y, Voegel JC, Schaaf P, Chluba J, et al. Viability, adhesion, and bone phenotype of osteoblast-like cells on polyelectrolyte multilayers films. J Biomed Mater Res. 2002;60:657–67. doi:10.1002/jbm.10110.
37. Michael KE, Vernekar VN, Keselowsky BG, Meredith JC, Latour RA, Garcia AJ. Adsorption-induced conformational changes in fibronectin due to interactions with well defined surface chemistries. Langmuir. 2003;19:8033–40. doi:10.1021/la034810a.
38. Kowalczyńska HM, Nowak-Wyrzykowsk M, Dobkowski J, Kołos R, Kamiński J, Makowska-Cynka A, et al. Adsorption charcteristics of human plasma fibronectin in relationshsip to cell adhesion. J Biomed Mater Res. 2002;61:260–9. doi:10.1002/jbm.10151.
39. Pompe T, Kobe F, Salchert K, Jørgensen B, Oswald J, Werner C. Fibronectin anchorage to polymer substrates controls the initial phase of endothelial cell adhesion. J Biomed Mater Res A. 2003;67:647–57. doi:10.1002/jbm.a.10130.
40. Hynes RO. Integrins: versatility, modulation and signaling in cell adhesion. Cell. 1992;69:11–25. doi:10.1016/0092-8674(92)90115-S.
41. Bilbe G, Roberts E, Birch M, Evans DB. PCR phenotyping of cytokines, growth factors and their receptors and bone matrix proteins in human osteoblast-like cell lines. Bone. 1996;19:437–45. doi:10.1016/S8756-3282(96)00254-2.
42. Shirosaki Y, Tsur K, Hayakawa S, Osaka A, Lopes MA, Santos JD, et al. In vitro cytocompatibility of MG63 cells on chitosan-organosiloxane hybrid membranes. Biomaterials. 2005;26:485–93. doi:10.1016/j.biomaterials.2004.02.056.
43. Budyanto L, Goh YQ, Ooi CP. Fabrication of porous poly (L-lactide) (PLLA) scaffolds for tissue engineering using liquid-liquid phase separation and freeze extraction. J Mater Sci: Mater Med. 2009;20:105–11. doi:10.1007/s10856-008-3545-8.
44. Li WZ, Ma N, Ong LL, Kaminski A, Skrabal C, Ugrlucan M, et al. Enhanced thoracic gene delivery by magnetic nanobead-mediated vector. J Gene Med. 2008;10:897–909. doi:10.1002/jgm.1208.