Biogenic Polyelectrolyte Multilayers on Poly(L-lactide) Films for Control of Osteoblast Adhesion

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Summary: Cell adhesion and spreading are important events during cell-biomaterial interaction, which control survival, growth and differentiation of cells. Layer-by-layer technique was used to generate multilayer coatings for regulating adhesion of primary osteoblasts on biomaterials. Polyelectrolyte multilayers (PEM) were based on poly (ethylene imine) as primary polycation layer. PEM were then prepared from chitosan (CHI) as polycation and heparin (HEP), or sulfated HA (sHA) as polyanions. It was observed that attachment and spreading of primary osteoblasts (pOB) was highly dependent on the composition of multilayers, as well as pH values of polyelectrolyte solutions. Results presented in this paper may pave the way for application of PEM surface coatings for bone-contacting implant materials.

Keywords: biocompatibility; layer-by-layer assembly; poly(L-lactide); polyelectrolytes; primary osteoblasts

Introduction

The layer-by-layer (LbL) technique introduced by Decher and colleagues represents a versatile tool to build up polyelectrolyte multilayers (PEMs) on different material surfaces.[1–3] In essence, polyelectrolytes (PEL) are deposited from an aqueous solution onto a surface of opposite charge. This leads normally to a reversal of the net surface charge due to the excess of charged groups in the polymer chain, which in turn allows the subsequent adsorption of a second, oppositely charged PEL. This process can be repeated many times to obtain a surface coating, with an overall thickness ranging from tens to hundreds of nanometers. The features of PEL such as molecular weight, polymer structure (linear, branched), charge density and type of functional groups have great impacts on multilayer formation and properties. Ionic strength and pH value of solutions can be used as additional tool to modulate PEM properties by their influence on the conformation particularly of weak PEL. The LbL technique is simple and often viewed as being applicable to virtually any pair of inorganic and organic PELs but also charged biopolymers such as proteins, enzymes, or nucleic acids.[4,5] Because of the multitude of molecules and supramolecular charged entities that can be assembled to multilayers, LbL technique has been applied in many different fields to prepare functional material coatings for sensor technology and biomedical devices.[6–8] All components of the extracellular matrix like proteins and glycosaminoglycans are PEL, which can be used to prepare multilayers by LbL technique. Since adhesion dependent cells possess specific cellular receptors that can recognize ECM components and require them for proper functioning,[9] immobilization of ECM components like GAG may be a good option to obtain biomaterial surfaces with high biocompatibility.

Glycosaminoglycans, such as heparin, chondroitin sulfate, and hyaluronic acid,

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are components of ECM, which play important roles for cell growth and differentiation. Therefore, they have been used for different biomedical applications.[10–12] Heparin (HEP) is essential in preventing blood coagulation via catalyzing and enhancing the binding of antithrombin III and thrombin. Moreover, it possesses a high binding affinity to different adhesive proteins such as fibronectin, vitronectin and laminin, but also growth factors like transforming growth factor beta (TGF-β) and bone morphogenetic protein-2 (BMP-2).[13–15] Hyaluronan (HA) is a naturally occurring member of the GAG family which ubiquitously exists in ECM.[16] HA plays a structural and mechanical role in various tissues participating in the control of tissue hydration. Furthermore HA affects numerous biological processes such as development, inflammation and tumor metastasis and development. The versatility of HA is due to its specific cellular receptor, which are termed as hyaladherins. CD 44, a significant member of the hyaladerin family, is especially noticeable because of its diverse functions in cell attachment, organization, and turnover of ECM at the cell surface as well as meditating the recruitment of lymphocytes during inflammation.[17] HA can be chemically converted into sulfated hyaluronan (sHA).[18] Due to the function of sulfate groups, sHA was reported as a promising heparin-like compound which can selectively binds to adhesive proteins such as fibronectin or fibrinogen as well.[19,20] On the other hand chitosan (CHI), as a N-deacetylated derivative of chitin by alkaline treatment, represents a natural biocompatible polycation. It is interesting to note that biodegradable chitin or chitosan provides bacteriostatic and fungistatic activities.[21,22]

Adhesion and spreading of osteoblast is a prerequisite for subsequent growth and bone formation. Polylactides represent degradable polymers with modest biocompatibility, which is partly related to impaired adhesion of cells. In this paper, the formation of PEMs composed of HEP and sHA onto poly (L-lactide) (PLLA) films was conducted by LbL technique to control adhesion of primary osteoblasts. Two different pH values (pH 4 and pH 7) were applied for polyanions during the multilayer formation process. Since, CHI is insoluble above a pH 6, the pH value of the polyanion solutions was changed to modulate charge density and conformation of CHI previously adsorbed onto the surface. It was found that PEM prepared at pH 7 promoted adhesion of pOB in comparison with plain PLLA, particularly when cells were plated on the surface terminated with HEP or sHA.

Experimental Part

Materials

PLLA (Mw ~ 148,800) was a commercial product from Boehringer Ingelheim Pharma GmbH&Co. KG (Ingelheim, Germany). PEI (Mw of 750,000 or 25,000) was purchased from Sigma (Deisenhofen, Germany). *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Merck Schuchardt OHG (Darmstadt, Germany). All water used was fresh MilliQ water with a conductivity below 0.055 S/cm except specially mentioned. Chitosan (CHI) with a deacetylation degree of 85% was obtained from Heppe GmbH (Halle, Germany). Heparin sodium salt from porcine mucosa was a commercial product from AppliChem GmbH (Darmstadt, Germany). Hyaluronan and sulphated hyaluronan with averaged 2.8–3.1 sulphate groups per repeating unit was kindly supplied by Dr. Schnabelrauch (INNOVENT e.V., Department Biomaterials, Jena, Germany).

Formation of Multilayers on PEI-Modified PLLA Films

LbL technique was utilized to incorporate glycosaminoglycans onto the surface of PLLA films. To accomplish that, positive charges were firstly generated on the surface of PLLA by covalent binding of PEI on

the carboxyl groups from PLLA molecules.[23] The LbL process was conducted by alternative dipping of PEI-modified PLLA films into the polyanion and polycation solutions for 20 min at room temperature (RT) under constant shaking, followed by water rinsing for 15 min.[24] Heparin (HEP) or sulphated hyaluronan (sHA) (2 or 1 mg/mL in water supplemented with 0.14 M NaCl, respectively, pH adjusted to either 4.0 or 7.0) were utilized as polyanion, and chitosan (CHI, 2 mg/mL in 0.05 M acetic acid supplemented with 0.14 M NaCl, pH adjusted to 5.0) as the polycation. The dip-rinsing steps were repeated several times to produce PEM with 10^{th} polycation or 11^{th} polyanion as the terminal layer. PEM coated PLLA films were finally rinsed with water and dried prior to further experiments such as cell adhesion studies.

Characterization of Multilayer Formation

Quartz crystal microbalance (QCM, LiquiLab 21, IFAK e.V., Magdeburg, Germany) following the mass change with each adsorption step by a change of resonance frequency (Δf) of the sensor, and surface plasmon resonance (SPR, IBIS-iSPR, IBIS Technologies B.V., Hengelo, the Netherlands) detecting the angle shift (m^o) against time, were utilized to monitor the LbL process. To mimic the modification on PLLA surface, a PLLA thin film was generated on either QCM or SPR sensor by spin coating from PLLA/CH_2Cl_2 solution (2 wt.%). After drying, the surface-coated sensors were subjected to chemical modification with PEI prior to QCM or SPR measurements. After Sensors being fixed to QCM or SPR devices, solutions of HEP or CHI were injected alternatingly into the flow cells connected to the sensors, washing steps with water were conducted in between to remove unbound PEL and continue the multilayer formation process.

Cell Adhesion Studies with Primary Osteoblasts (pOB)

Cell adhesion studies were conducted with human primary osteoblasts (pOB) on various surfaces to evaluate their biocompatibility. Cells were cultured in 75 cm^2 culture flasks, at 37 °C in a humidified atmosphere with 5% CO_2. Dulbecco's modification of Eagle's 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) was used for cell culture. The medium was changed twice per week.

After confluence, the cell monolayer was washed once with PBS and incubated in trypsin-EDTA solution (PromoCell, Heidelberg, Germany) for 3–5 min at 37 °C to detach the cells. Cells were resuspended in fresh DMEM without serum after centrifugation and counted with a hemacytometer to adjust the cell density to 2×10^4 cells/mL. 1 mL of cell suspension was seeded onto different films, which were placed in 24 well tissue culture plates. Samples with cells were incubated at 37 °C in a humidified 5% CO_2 / 95% air atmosphere for 6 h to allow cell attachment. The adhered pOB were fixed by incubation in 3% (w/v) paraformaldehyde (Roti-Histofix, Roth, Karlsruhe, Germany) in phosphate buffered saline (PBS, 150 mM NaCl, 5.8 mM Na2HPO4, 5.8 mM NaH2PO4, pH 7.4) for 15 min. Morphology of cells was observed with phase contrast microscopy (Leica DM4000B, Leica Microsysem AG, Germany) equipped with a Motic camera MC 1000. The number of adhering cells was evaluated with image processing and analysis software (ImageJ 1.38e, released by National Institutes of Health). For that purpose, 5 images at different locations per well (2 wells for each sample, 10 images in total) were selected. Data presented are expressed as (average $\pm$ standard derivation). Significance testing was performed with ANOVA (Tukey's test) for paired samples. Differences were considered as statistically significant at $p \leq 0.05$ and denoted in the corresponding figures as "*".

Vinculin and Actin Staining

The formation of focal adhesions and actin cytoskeleton was visualized by immuno-

fluorescence staining after 24 h incubation. pOB were adhered as described above on multilayers prepared from polyanion solutions at pH 4 or pH 7. To increase the adhesivity part of the samples were precoated with fibronectin (FN). 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, cells suspended in DMEM without serum were seeded to the samples and incubated for 24 hr prior to immunofluorescence staining.

After incubation for 6 h, cells were first rinsed with PBS. Adherent cells were fixed with paraformaldehyde solution for 15 min. After washing with PBS twice, cells were permeabilized with 0.5% Triton X-100 in PBS for 10 min. Samples were incubated with 1% bovine serum albumin (BSA) in PBS for 30 min for blocking non-specific binding sites. Filamentous 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, and centrifuged at 12,000 rmp for 5 min at 4 °C prior to use. After washing of cells with PBS and distilled water, samples were mounted with Mowiol and viewed with confocal laser scanning microscopy (LSM 710 Carl Zeiss, Oberkochen, Germany).

Results and Discussion

Preparation of Polyelectrolyte Multilayers

To accomplish the assembly of PEM on the surface of PLLA, this substrate was first subject to modification with PEI to generate a positive surface charge, which is denoted as the precursor layer. The procedure of chemical binding has been described elsewhere by us in more detail.[24] The effect of the molecular weight of PEI on the formation of HEP/CHI multilayers is depicted in Figure 1. It is visible that the molecular weight of PEI has a remarkable impact on the multilayer formation of HEP and CHI. QCM measurement is based on the change of resonance frequency of a quartz sensor, which is according to Sauerbrey proportional to the mass change.[25] The observed changes of resonance frequency, as shown in Figure 1, can be ascribed to the adsorption or desorption of PELs or water. It is interesting to note that on PLLA modified with high molecular weight PEI (HMW PEI), a steeper increase of resonance frequency change was observed compared to LMW PEI after the buildup of 4^{th} layers. It has been demonstrated that higher molecular weight of PEI resulted in a higher amount of immobilized PEI on the surface resulting in a higher quantity of amino groups.[24] This leads also to higher surface charge density, and facilitates the LbL process in comparison to PLLA modified with LMW PEI. Based on this observation, the following experiments were conducted with HMW PEI-modified PLLA films.

Figure 2 shows results of measurements with SPR recording the multilayer formation process with sHA and CHI at different pH combinations. As shown in Figure 2 (left), the assembly of PEM from sHA and CHI results in stepwise increase in resonance angle when the sensor was alternatively injected with polyanion or polycation solution. After PEI modification, positive charges will be generated on the surface. When the solution of polyanion flows across the sensor surface, the electrostatic attraction between the positively charged surface and polyanion molecules happens, which is reflected by the angle shift. The complexation of polyanion with water in the surrounding solution is another factor leading to the angle shift. Rinsing with water will remove the unstably adsorbed polyanions, resulting in moderate recovery of angle shift. When this sensor is injected with a polycation solution, the

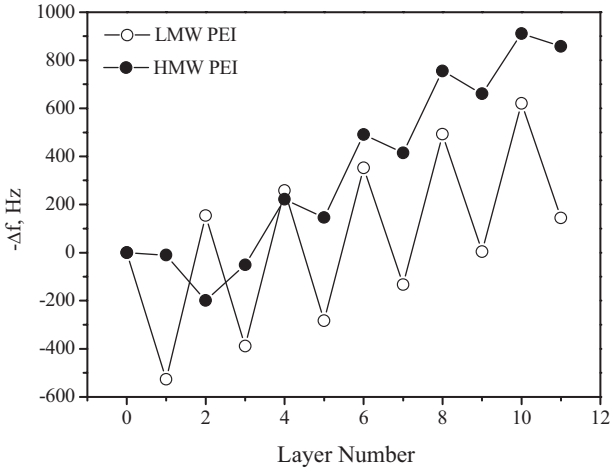

Figure 1.
Effect of PEI modification on the frequency shift during formation of PEM from HEP and CHI on the surface of PEI-modified PLLA film measured with QCM. LMW PEI: Mw 25000, HMW PEI: Mw 750000.

interaction between the previous-assembled polyanion and polycation in the solution takes place. The angle shift will increase or decrease depending on the stability of previously assembled layer.

The pH value of solution affects the disassociation status of PELs, which leads to their different behaviors on LbL process. As shown in Figure 2, the LbL process triggers a more remarkable increase of SPR angle shifts when the polyanion solution was prepared at acidic condition (Figure 2, right), compared with those assembled at pH 7.0 (Figure 2, left). The polyanion used in this paper bears sulphate groups, the averaged number of sulphate group per repeating unit is 2.8∼3.1.[26] According to the literature, this polyelectrolyte is well dissociated at pH values higher than 3. On the other hand, chitosan is a weak polyelectrolyte of which the pKa value is around 6.5. Although the pH value of CHI solution was adjusted to pH 5.0 due to solubility reason, the charge and conformation of adsorbed CHI can be influenced by the pH changes of surrounding liquids, which vice versa affects the incorporation of sHA, as well as stability of previously adsorbed CHI. The precursor PEI layer is supposed to be highly protonized when contacting the sHA solution at pH 4.0. In other words, a high surface charge can be obtained, which in turn enhance the adsorption of sHA. In addition, the excess of sHA on surface is helpful for the later incorporation of CHI, leading to the overcompensation of surface charge, as well as higher angle shifts.[15,27] This allows for a strong complexation of sHA on the positively charged surface for the next LbL step.

The slope of linear fit from the angle shift versus time curve is listed in Table 1. It reveals that for the polyelectrolyte pairs (sHA/CHI), the slope obtained at sHA (pH 4.0) is much higher than that at sHA (pH 7.0). The difference between slopes for HEP/CHI PEM is more pronounced, which also confirms this observation. It can be concluded that immobilisation of polyelectrolytes on the surface of PLLA films can be accomplished by LbL process. It is possible to control the amount of adsorbed PELs simply by adjusting the pH value of polyelectrolyte solutions.

Cell Adhesion Studies

Cell adhesion studies with human primary osteoblasts were conducted with the various types of surfaces to investigate their biocompatibility. The morphology of pOB

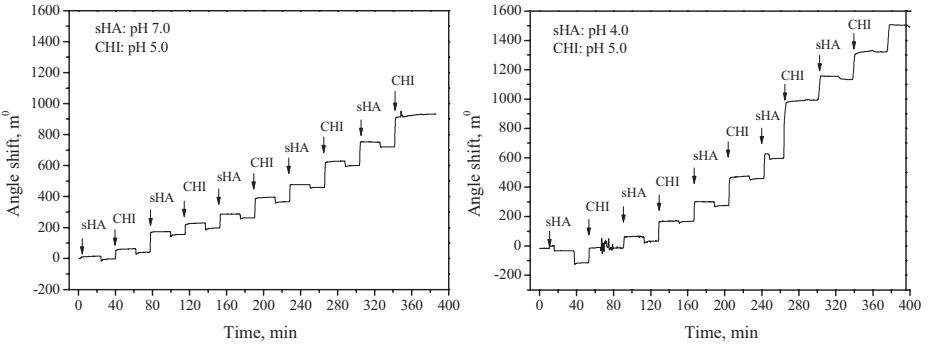

Figure 2.
Effect of pH values on the SPR curves for formation of sHA-CHI multilayers on the surface of HMW-PEI modified PLLA films. Arrows in the images represents the injection of sHA or CHI solutions.

adhering on different surface after 6 h incubation is shown in Figure 3 and 4. After the initial attachment stage, most cells adhering on the tissue culture polystyrene (TCPs) display an elongated, spreading phenotype. By contrast, on plain PLLA films, mostly round cells are observed with few cells starting to flatten. Both round and spread cells are detected on PEM. For surface prepared from HEP (pH 7.0)/CHI (pH 5.0) combination, as shown in Figure 3, cells display triangular and spherical shapes, where an initial tendency of aggregation can also be detected. More spread cells are present on HEP-terminated surface compared with CHI terminated one. On PEM prepared from sHA (pH 7.0) and CHI (pH 5.0), more flat cells are observed on sHA-terminated PEM, of which some round cells are still detectable. On CHI-terminated surface, the majority of cells are round, evenly distributed, with no contact among cells.

Table 1.
Slopes for the linear fit curves of different polyelectrolyte pairs (angle shift versus layers number). Data obtained from SPR results.

Polyelectrolyte pair	Slope	
	pH 7.0	pH 4.0
HEP-CHI	52.81 ± 8.68	122.42 ± 11.48
sHA-CHI	103.22 ± 0.71	182.01 ± 0.90

It is also obvious that the pH value of polyanion solutions during the multilayer formation process has an impact on the adhesivity of the resulting surfaces. As shown in Figure 4, the number of adhered cells on surface prepared from HEP (pH 4.0)/CHI (5.0) combination is less than those from polyanion (pH 7.0)/polycation (5.0). In addition, more round cells presents on HEP-terminated surface prepared from lower pH value as compared with those on HEP (7.0/5.0) one, although there is minor differences among CHI-terminated surfaces. Nevertheless, on the surface prepared from lower pH values, more spread cells can be observed on the polycation-terminated surface.

Quantitative analysis of pOB adhesion is depicted in Figure 5. Overall, the adhesion data confirm the observation of cells with phase contrast microscopy. Among surfaces investigated in this paper, number of cells adhered on PEM is enhanced compared with that on blank PLLA, except for the CHI-terminated surface prepared from sHA/CHI polyelectrolytes on which the lowest amount of cells are adhered (Figure 5). For surface prepared from HEP and CHI, however, the effects of pH for polyelectrolyte solutions are complicated. Data presented in Figure 5 reveals that higher pH leads to more adhered pOBs on polyanion-terminated surface than that on CHI-terminated one.

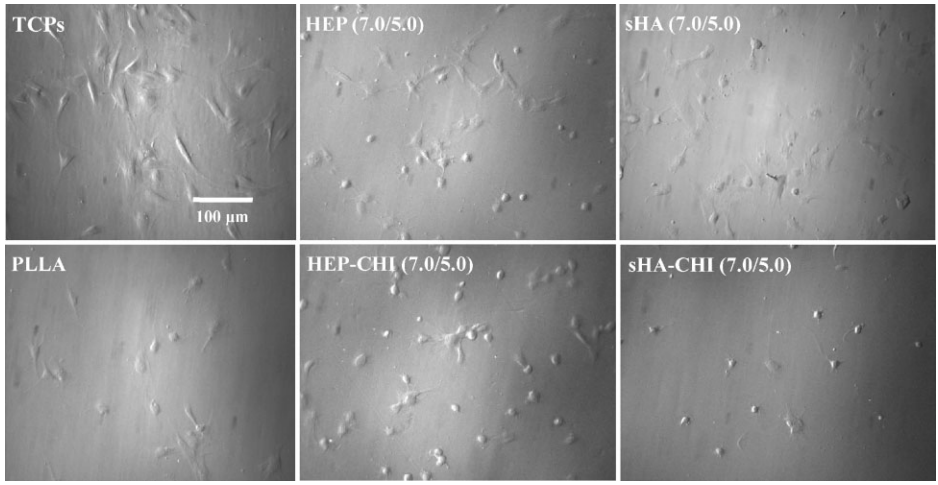

Figure 3.
Morphology of pOB plated on PEM assembled at polyanion (pH 7.0) and polycation (pH 5.0) after 6 h incubation. TCPs: tissue culture polystyrene; PLLA: plain PLLA; HEP: surface prepared from HEP/CHI polyelectrolyte pair with the 11th HEP as terminal layer; HEP-CHI: surface prepared from HEP/CHI polyelectrolyte pair with the 10th CHI as terminal layer; sHA: surface prepared from sHA/CHI polyelectrolyte pair with the 11th sHA as terminal layer; sHA-CHI: surface prepared from sHA/CHI polyelectrolyte pair with the 10th CHI as terminal layer. Numbers in the brackets represent the pH values of polyanion/polycation solutions.

While CHI-terminated PEM prepared from polyanion (pH 4.0)/polycation (pH 5.0) is more helpful for the adhesion of pOBs.

Cell adhesion and subsequent growth are influenced greatly by surface properties of substrates. It is well-known that cell adhesion is dependent on physical substrate properties that direct adsorption of proteins.[28] Cell adhesion requires interaction between cellular adhesion receptors and specific ligands that are present on the

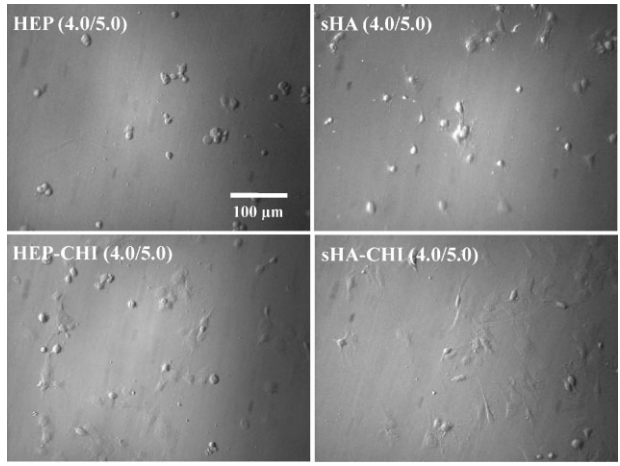

Figure 4.
Morphology of pOB adhering on surfaces assembled at polyanion (pH 4.0) and polycation (pH 5.0) after 6 h incubation. Sample definition is the same as for Figure 3; Numbers in the brackets presents the pH values of polyanion/polycation solutions.

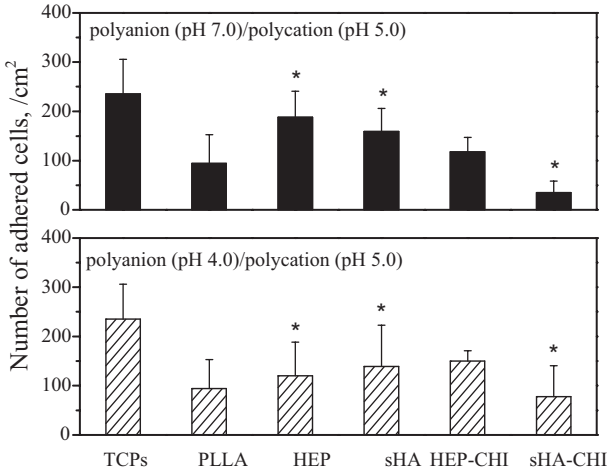

Figure 5.
Number of primary osteoblasts adhered on various surface after incubation for 6 h.

material surface.[29] Formation of multilayers from HEP or sHA with CHI on PLLA will increase the hydrophilicity of the surface due to the hydrophilic nature of the polyelectrolytes applied in this study. Certainly, there are two different mechanisms that affect cell adhesion here. First, the increasing hydrophilicity, particularly for polyanion-terminated surfaces, can reduce non-specific adsorption of proteins. On the other hand, both HEP and sHA bind specifically adhesive proteins that possess heparin-binding domains such as fibronectin.[20] Hence cell adhesion might be promoted on polyanion-terminated PEM, which is observed particularly on PEM prepared at pH 7 (see Figure 5, upper part), the reduced cell adhesion on PEM at pH 4 seen in Figure 5 as well should be related to increasing layer mass and probably water binding of PEM under these circumstances.[30]

The different behavior of pOBs adhered on PEM surface prepared from sHA and CHI was further confirmed by immunofluorescence staining of focal adhesions and development of actin cytoskeleton (Figure 6). Focal adhesion formation requires the presence of adsorbed adhesive proteins like fibronectin or vitronectin on the material surfaces.[31] For CHI-terminated surface, it is obvious that there are no striking differences in cell spreading, and only weak focal adhesion are detectable (Figure 6, upper panel). When considering pH effects, however, focal adhesions and actin cytoskeleton are better developed on CHI-terminated surface prepared from sHA (pH 4.0)/CHI (pH 5.0). For sHA-terminated surface, cells show striking differences in spreading (Figure 6, middle panel). Larger cells are presented at PEM prepared from pH 7, which also confirms results of cell adhesion studies discussed above. Similarly, focal adhesion plaques and much stronger formation of longitudinal actin stress fibres can be observed on PEM formed at pH 7 while those structures are almost absent on PEM prepared at pH 4. It is also interesting to note that pre-adsorption of 5 μg/mL fibronectin on sHA as terminal layer does not lead to a large change in focal adhesion formation and actin cytoskeleton formation (Figure 6, lower panel). The spreading of pOB on sHA formed at pH 4 is reduced with absence of focal adhesions and only weak actin stress fiber formation, which indicates that few fibronectins are adsorbed on this surface. By contrast, on surface prepared from sHA at pH 7 a strong focal adhesion and actin stress fiber formation

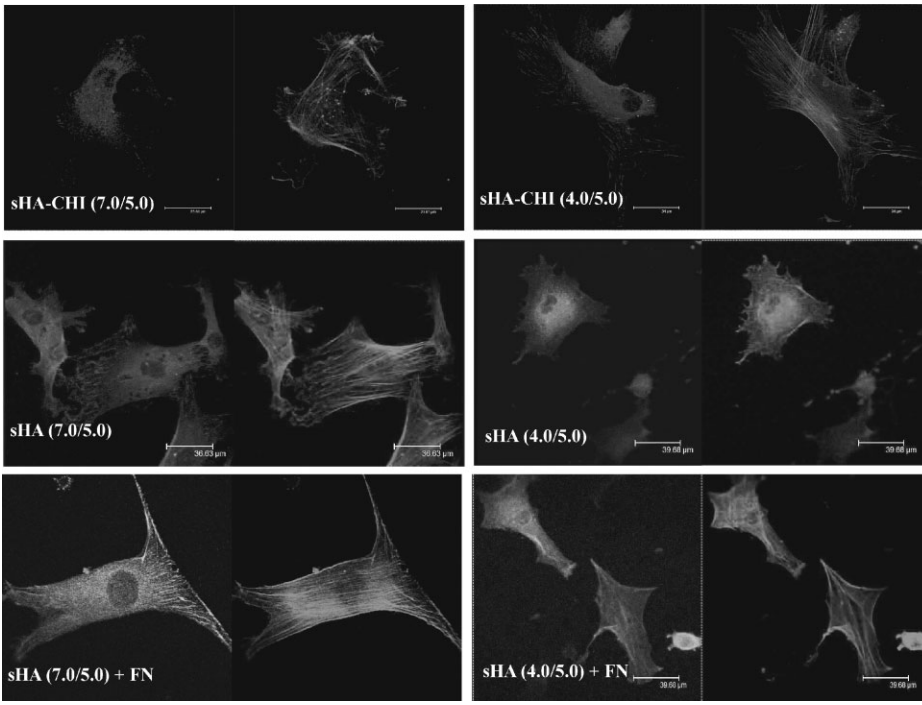

Figure 6.
Immnofluorescence images of primary osteoblasts stained for vinculin and actin on various types of surfaces. Surface definition is the same as for Figure 3. Left images are from polyanion (pH 7.0)/CHI (pH 5.0) combination, while right ones are from polyanion (pH 4.0)/CHI (pH 5.0) combination.

are observed, indicating higher quantities of adsorbed fibronectin. Overall, findings of immunofluorescence studies confirm the results of adhesion studies with phase contrast microscopy that terminal polyanion layers prepared at pH 7 are more adhesive that those prepared at pH 4.

Cell adhesion and spreading are influenced by factors such as surface hydrophilicity, chemical composition, viscoelastic properties, surface charge, surface roughness, and so on.[28] HEP and sHA assume a stretched conformation under both pH values due to the presence of strongly-charged sulphate groups. By contrast, the conformation and charges of CHI as weak polyelectrolyte depends on pH value even after its adsorption on the multilayer surface, which in turn will have an impact on the subsequent adsorption of polyanions like HEP and sHA. Due to this reason a higher layer mass is observed for the low pH set-up, which leads to polyanion-terminated layers with higher charge density and probably wettability. Similar results have been obtained for the combination of heparin and chitosan, where it was shown that layer mass and wettability of HEP-terminal layer were higher at low pH value.[27] In this context the higher number of adhering and spread cells at higher pH value was related to increased adsorption of fibronectin,[15,27] which fits also to the observations made in this study. Beside the specific interaction with adhesive proteins, sulfated hyaluronan can interact with specific cellular receptors, such as CD44.[32] The conformations variations of sHA molecules may also have affects. Similar to HA, since the intracellular domain of CD44 isoforms can selectively interact with cytoskeletal proteins and regulate specific signaling,[33] its interaction with sHA molecules influences the cytoskeleton

developments. As one of the most common receptors for hyaluronan or sulphated hyaluronan in adhesive processes, CD44 needs an oligomer of least 10 repeating units for binding to the polysaccharide,[32] which requires suitable 3D organization of sHA molecules. It indicates a different adhesive mechanism on sHA, which might be related to CD 44 receptor. This interplay may be promoted at pH 7, while at pH 4 the stronger electrostatic interactions between CHI and sHA may somehow decrease recognition of sHA due to the inhibition of its molecular mobility. The higher hydrophilicity of polyanion-terminated multilayers formed at lower pH value, which can be anticipated from previous studies,[15,27] may be also responsible for lower cell adhesion and absence of focal adhesion and actin stress fibers on sHA(4.0/5.0) and (sHA(4.0/5.0) FN) surfaces, because it inhibits adsorption of adhesive proteins that are important for cytoskeleton development.[15] Overall, it was shown that subtle differences of pH value during multilayer formation may have a great impact on adhesion of primary osteoblasts, which will influence cellular growth and function.

Conclusion

Heparin and sulphated hyaluronan are successfully used to form polyelectrolyte multilayers on the surface of PLLA films by LbL technique. It is shown that primary modification of PLLA with PEI affects greatly the following LbL process. HWM PEI is more helpful for the subsequent adsorption of polyelectrolytes. The pH values of polyelectrolyte solutions are another key factor influencing multilayer formation process, which in turn affects cell adhesion. For the polyelectrolytes combination of polyanion (pH 4.0)/polycation (pH 5.0), about twice of angle shift is detected with SPR measurements compared with those assembled at polyanion (pH 7.0)/polycation (pH 5.0). Adhesion studies with human primary osteoblasts reveal that coating of PLLA with these polyelectrolytes improves the surface biocompatibility as compared with plain PLLA. Results presented in this paper supply a versatile method to adjust the properties of scaffolds for tissue engineering of bone.

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