Rumen Krastev*, Alexander Rudt, Xin Xiong and Hanna Hartmann

Polyelectrolyte Coatings for Surface Modification of Medical Implants

Abstract: Polyelectrolyte multi-layer (PEM) coatings are prepared by alternative deposition of single polyelectrolyte monolayers on charged surfaces using the Layer-by-Layer (LbL) dip coating procedure. These are nanometre scaled coatings which allow fulfilling of different technical or biological requirements. The build-up process is based on self-assembly and self-organization of polycations and polyanions on different substrates including complex geometrical structures and even closed volumes, forming homogeneous layer without defects. Depending on the proper selection of the applied polyelectrolytes, coatings with different stabilities can be prepared. Some of the coatings are stable and cannot be removed from the surface. Others are degradable and can be used as systems for controlled local drug delivery. Here we summarise the results of our experience in preparation of PEM coatings with different functionalities. PEM coatings can be used as controllable delivery system for siRNA polyplexes. They can be used to control the adhesion of different cell types on the surfaces and support e.g. the endothelialisation process on cardio-vascular medical devices as e.g. stents or reduce the immunological response of the tissue after implantation. We summarise results from physical characterisation of the coatings (e.g. film thickness, roughness, electrical charge and hydrophilicity) combined with in-vitro biological studies on adhesion of HUVEC cells.

Keywords: cell adhesion; HUVEC; hydrophobicity; polyelectrolyte multilayer, roughness, stiffness

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1 Introduction

The demand for development of new materials, which are in contact with biological tissues (biomaterials), is increasing continuously. By using different coatings or other approaches, the surfaces of the biomaterials can be triggered to meet the requirements on the interface between non-viable materials and vital biological tissue. The polyelectrolyte (PE) multi-layer (PEM) coatings were intensively studied in the last two decades. These coatings are prepared by alternative deposition of single PE monolayers on charged surfaces using the Layer-by-Layer (LbL) dip coating procedure. The PEM are nanometre scaled coatings, which allow fulfilling of different technical or biological requirements. The build-up process is based on self-assembly and self-organization of polycations and polyanions on the surfaces of the materials. The deposition procedure is applicable on different substrates including complex geometrical structures and even closed volumes, forming homogeneous layer without defects. All deposition processes are performed only from water-based solutions at room temperature and do not require any further treatment in harsh chemical or physical conditions. The process guarantees biological stability and viability of different biologically active molecules (biologicals) or even cells during the deposition process. This makes the process well suited for immobilisation of active biological entities. The incorporation of various kinds of nano-particles and nano-systems in PEM coatings allows the formation of layers with different functionalities. The PEM coatings usually have a thickness of less than 1 micrometer. This thickness suffices to allow formation of surfaces with desired properties. The film thickness can be precisely controlled with an accuracy of few nanometers by changing the number of deposition cycles, electrolyte and PE concentration in the deposition solution, the deposition time or the temperature.

A large variety of PEs are already tested and show formation of excellent coatings for different medical applications. Depending on the proper selection of the PE coatings with different stabilities can be prepared. Some of the coatings are stable and can be removed from the surface only mechanically or after harsh chemical treatment. Others are degradable. The degradation can be tuned to be dependent on the surrounding conditions (e.g. pH-value, electrolyte concentration, temperature, etc.). These coatings can also be

used as drug delivery systems for controlled local drug delivery.

We demonstrated in former publications that PEM coatings can be used as controllable delivery system for siRNA polyplexes [1]. Other applications of PEM as coatings for medical implants are also reported [2]. In this work, we summarise the results of our experience in preparation of PEM coatings with different functionalities. Depending on the proper selection of the PEs, special coating systems were also developed to reduce the immunological response of the tissue after implantation. The paper demonstrates the application of the PEM as coatings for cardio-vascular medical devices as e.g. stents. The coatings can be applied for metal devices but also for degradable or non-degradable polymer materials. We summarise results from physical characterisation of the coatings (film thickness (Quartz Crystal Microbalance, QCM), roughness (Atomic Force Microscopy, AFM), surface charge and hydrophilicity) combined with in-vitro biological studies on adherence of HUVEC cells.

2 Experiment

PEM were prepared by LbL dip coating procedure either on QCM Au crystals (film thickness), on Si wafers (AFM, contact angle), SiO2 nano particles (surface potential respectively surface charge) or on microtiter plates (biological tests). Two couples of PEs were used. PEM1 were prepared from the natural polyelectrolytes Chitosan and Hyaluronic acid. PEM2 were prepared from the synthetic polyelectrolytes Poly(sodium styrenesulfonate) and Poly(allylamine hydrochloride). The deposition time was always kept constant. The concentration of the PEs and electrolytes in the deposition solutions were also constant.

Figure 1 shows the change of the films thickness with the number of the deposition steps for both used PEM systems. All tests were performed at least in triplicate with PEMs with a thickness of 50 nm for the PEM1 system and 40 nm for the PEM2 system. The coatings were characterised using different physical methods. The contact angle was analysed by Young-Laplace equation. The thickness was evaluated from QCM data using the Sauerbrey and Voight modelling [3].

Atomic force microscopy (AFM) was utilized to access the topography and the elastic modulus of the PEM in liquid environment. The elastic modulus was calculated using the Hertz equation [4]. The roughness of the coatings was calculated from topographic profile images. Clear difference in the roughness was visible. The roughness was numerically characterised counting peaks and valley per distance – peak per distance method.

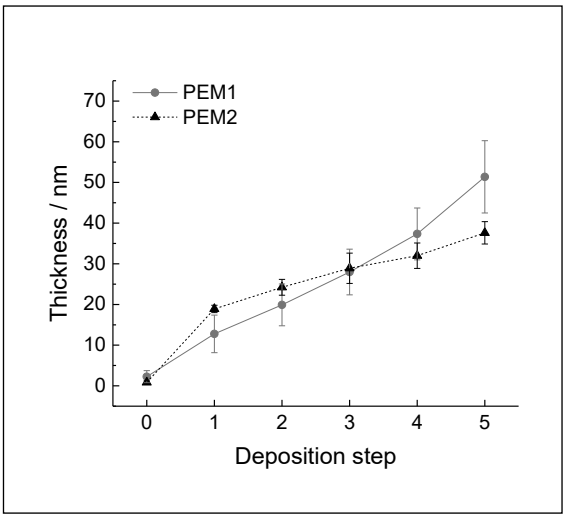

Figure 1: Change of the film thickness with the number of the deposited single PE layers.

Biological tests were conducted to obtain the number of cells, which adhere to the surfaces. The tests were performed with Human Umbilical Vein Endothelial Cells (HUVEC). Defined amount of cells were seeded and incubated on non-treated (non-coated) microtiter plates as a negative control and on microtiter plates coated with PEM1 respectively PEM2. The samples were fixed with paraformaldehyde and stained with fluorescent dyes after 24 and 48 h. Cytoskeleton was stained by Phalloidin (green), cellular nucleoli were stained by DAPI (blue). After these procedures, the samples were microscopically observed.

3 Results

PEM1 and PEM2 coatings charge the surfaces positively. The PEM build-up for PEM1 and PEM2 is similar. The hydrophobicity of the surfaces was determined via contact angle measurement. Both tested PEM are slightly hydrophilic with a contact angle of 75 deg and 60 deg for PEM1 and PEM2 respectively (Fig. 2a). The film roughness and stiffness are differed for the both studied PEM (Fig. 2b, 2c). PEM1 has a roughness of 1.5 nm^{-1} and PEM2 that of 0.5 nm. PEM2 is much stiffer than PEM1. Taken into account, that both PEMs have comparable thickness, the strong differences in roughness and stiffness are a result of different adsorption behaviour correlated with the used polyelectrolytes. The PEs of PEM2 interact stronger electrostatically resulting in a tighter adsorption with formation of thin rigid structures at the surface. In contrast, the PEs used for PEM1 are loosely

adsorbed with weaker interaction, resulting in softer film, that are able to realise stress relaxation forming wider softer surface structures (Fig. 3).

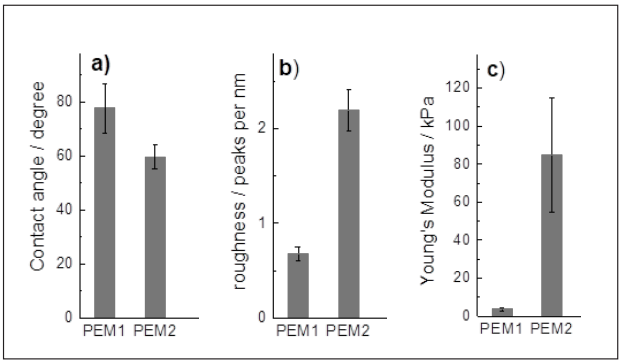

Figure 2: Physical characterization of the PEM: a) Contact angle of water; b) Roughness (peak per distance method); c) Stiffness - Young's modulus

The effect of the PEM functionalization on the cell adhesion and proliferation was accessed growing a HUVEC cell culture in PEM coated polystyrene microtiter plates. The non-treated plates were previously tested as non-suitable for cultivation of HUVEC cells and were used as a negative control. A clear difference in cell numbers was observed after 24 h cultivation as shown in Fig. 4c. Only few cells were observed on the non-treated and PEM1 coated plates.

The number of cells adhered on PEM2 coated surface is higher. This difference was further confirmed by the images after 48 h of cultivation. On PEM1, almost no living cells were found. Cells on PEM2 showed progressing proliferation. The PEM2 functionalized surface therefore show a clear beneficial

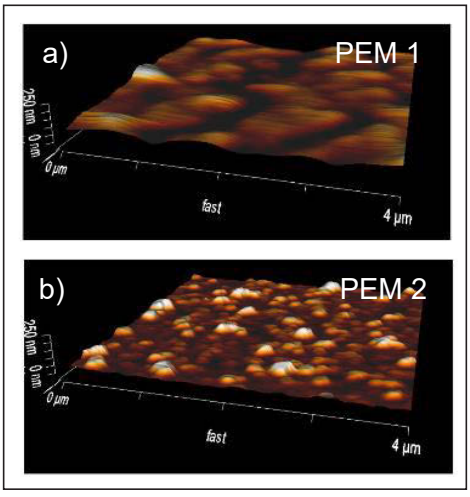

Figure 3: AFM images of the PEM on Si wafers as substrates: a) PEM1; b) PEM2

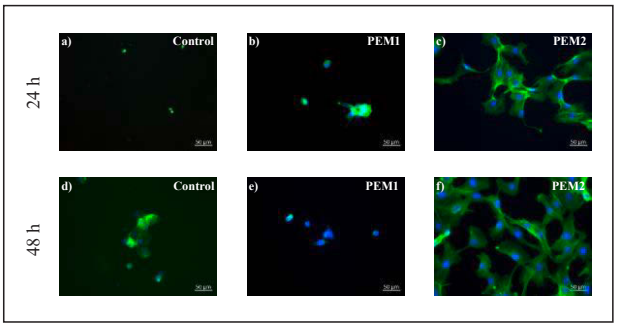

Figure 4: Effect of PEM coatings on adhesion and proliferation of HUVEC after 24 and 48 hours of cultivation:
a), d) Control: polystyrene non-treated non-cell adherent surface; b), e) PEM1 coated surface; c), f) PEM2 coated surface;
Cytoskeleton staining by Phalloidin (green), nucleolus staining by DAPI (blue)

stiffness effect on UVECs which is most likely correlated to the higher and roughness of PEM2.

4 Summary

Polyelectrolyte multilayer coatings show great potential in medical application such as implantology. Amongst other advantages, the surface properties of these surface modifications can easily be tuned to meet requirements for biocompatibility and enable the regulation of biological tissue. In this work, two different polyelectrolyte coating systems were physically characterized and their biological effect on endothelial cells was studied. Physical parameters such as hydrophobicity, nano-roughness, elasticity and topography are different for the both coating systems. According to the desired application, these parameters can be controlled and adjusted. Most probably, the observed difference in the cell adhesion and proliferation is due to the higher stiffness and roughness of the PEM. This promotes the growth of the endothelial cells. Further studies are needed to be carried out for in depth analysis of these effects on cellular behaviour.

Author Statement

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