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Poly(ether imide) membranes – studies on the effect of surface modification and protein preadsorption on endothelial cell adhesion, growth and function

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Abstract

Surface modifications of poly(ether imide) (PEI) membranes with carboxylic groups were tested in comparison to pure PEI and poly(ethylene terephtalate) (PET) for their ability to support attachment, growth and function of human umbilical vein endothelial cells (HUVEC) in respect to endothelization of the above materials. Flat sheet PEI membranes were modified by covalent binding of iminodiacetic acid (IDA) for different periods of time (1 to 30 min) to obtain surfaces with various content of carboxylic groups. In addition, fibronectin (FN) and fibrinogen (FNG) preadsorption on the various membranes were studied on their effect on HUVEC behaviour. Results show a decreased protein adsorption and HUVEC adhesion, growth and function in terms of prostacyclin production with an increase carboxylic groups. Preadsorption of the membranes with FN or FNG promoted activity of HUVEC, which became superior to cells on PET. FN-coated membranes were found to be better substrate for HUVEC adhesion and prostacyclin production, while on FNG-coated membranes cells grew better. Overall it can be concluded that PEI is a promising materials for endothelial cells immobilization as it is needed for improving the hemocompatibility of cardiovascular devices.

Key words: poly(ether imide), carboxylic groups, endothelial cells, cell adhesion, growth and function

1. Introduction

The replacement of large diameter blood vessels, such as the aorta by synthetic vascular grafts made of Teflon or Dacron (poly(ethylene terephtalate)) belongs to established medical therapies. Although the patency rate of these grafts is quite good, endothelization is never observed. Instead, a pseudointima is established consisting of a film of blood proteins with few adhering blood cells [1]. While large diameter vessels can be replaced all trials to develop vessels with a diameter below 7 mm, which are needed for coronary bypass operations failed due to onset of blood coagulation [2]. Endothelial cell (EC) seeding of vascular devices is a promising strategy to improve the patency rate and to decrease thrombotic complication upon implantation of synthetic vascular grafts [3]. The success of endothelization of the surface of synthetic vascular grafts, however, is highly dependent on interactions of EC (leading to functions such as adhesion and proliferation) with the material surface [4]. The presence of extra cellular matrix proteins is essential for EC attachment, proliferation, survival and function [5]. One approach to facilitate cell adhesion to biomaterials is to use adhesive proteins for pre-coating, so that cells remain viable and functional [6]. Two adhesive proteins namely fibronectin (FN) and fibrinogen (FNG) are known as proteins facilitating EC adhesion [7]. Previously we showed that these adhesive proteins enhanced HUVEC adhesion especially on poorly wettable surfaces. [8]. The functionality of EC is another important factor that has to be considered when the EC seeding is used to improve the hemocompatibility of cardiovascular implants. Since prostacyclin (PGI_2) was shown to suppress early phases of thrombosis by preventing platelet adhesion, activation and aggregation [9], the ability of seeded EC on polymer materials to secrete PGI_2 could be used as a measure for their anti-thrombotic properties. Apart from it's role in platelet aggregation, PGI_2 plays a wide spread role as a marker of various inflammatory processes [10], as prostacyclin inhibits the production of the pro-inflammatory cytokine tumor necrosis factor

α (TNF-α) by monocytes [11, 12]. On the other hand, TNF-α is an important stimulus for prostacyclin production [13, 14].

One approach to improve cell adhesion on biomaterials is surface modification of (hydrophobic) polymers by covalently binding of charged functional groups such as carboxylic (-COOH) groups [15]. It was shown that -COOH moieties on the surface can enhance adsorption of adhesive proteins [such as collagen II and III] as well as to support their fibrilar organization, which subsequently could enhance cell adhesion [FN, 16]. In addition a number of investigators have shown that polymers exposing carboxylic groups at the surface have relatively good blood-contacting features. These materials may even exhibit "heparin mimic" properties [17-19] and thus enhance the antithrombogenicity of the biomaterial.

Polyimides such as poly(ether imide) (PEI) are a new emerging class of polymeric materials for biomedical applications [20, 21]. PEI possesses a high thermal and mechanical strength, has excellent membrane forming properties and can be easily modified by wet chemical processes under mild chemical condition [22, 23]. Hence, the material seems to be promising for a variety of applications, which has been outlined in some of our previous work [24]. Other investigations have also shown a low immune response and good cell attachment and growth [25-27]. In our previous work [28] we studied also the hemocompatibility of PEI membranes. We showed that the hydrophobic PEI membrane (advancing contact angle of 83 deg.) caused a "passivation effect" on the surface, which resulted in a relatively low activation of platelets.

The objective of the current study was to compare the influence of polymer chemistry and surface properties on attachment, growth and function of HUVEC. Because of the two fold effect of carboxylic groups on blood response and cell adhesion, functionalization of PEI was performed in order to generate different quantities of -COOH groups. Since, FN and FNG

have a stimulating effect on interaction of EC with surfaces, their adsorption on the various surfaces and the subsequent behaviour of cells was studied. We observed that unmodified PEI represents an excellent substratum for HUVEC, which was comparable or better to an established material for vascular grafts poly(ethylene terephtalate) (Dacron). In contrast, introduction of carboxylic groups improved the wettability of the hydrophobic PEI with a concordant decrease of FN and FNG adsorption followed by impaired interaction with HUVEC. Details are reported herein.

2. Materials and Methods

2.1 Polymer membranes and surface modification

PEI flat membranes were prepared from a commercial polymer (ULTEM® 100 General Electric, New York, USA) as described previously [28]. Surface modification of PEI membrane by wet chemistry was used to produce –COOH groups on the polymer surface. The dry flat membrane was mounted onto a cylinder of stainless steel (130 mm diameter) and contacted with the modifier solution under stirring at 70°C for up to 30 min. The modifier solution contained 2 wt-% of the sodium salt of iminodiacetic acid (IDA) solved in 1:1 mixture of 1-propanol and water. After quenching in cool water the membranes were demounted and stored in wet state at 4°C until use. Poly(ethylene terephthalate) (PET, Oxyphen GmbH, Dresden, Germany) tracked-etched membranes with the thickness of 23 μm and low porosity were used as a reference material.

2.2 Atomic force microscopy

The surface topography of membranes was analyzed by atomic force microscopy (AFM) with a Nanoscope III A (Digital Instruments Inc., Santa Barbara, CA). A point probe silicon cantilever tip was used in contact mode. The mean value of the surface roughness relative to

the center plan Ra was calculated by the following equation: $Ra = 1/LxLy\int_0^{Ly}\int_0^{Lx} [f(x, y)]\, dxdy$, where f (x, y) is the surface relative to the center plan and Lx and Ly are the dimensions of the surface. The evaluation of the roughness parameters of each membranes sample was based on three scanned areas.

2.3 Contact angle and streaming potential measurements

The surface wetting properties of the membranes were characterized by contact angle (CA) measurements with distilled water using the captive bubble technique with a goniometer including a microscope (Zeiss, Germany). The receding (dewetting) and the advancing (wetting) CA were taken from three different places for each membrane.

Streaming potential measurements were carried out as described previously [29] with EKA electrokinetic analyser (Anton Paar, Austria). A flat plate measuring cell with an effective area of 2 x (74 x 15) mm^2 and an effective height of 0.3 mm was used. Measurements were performed at 25.0 ± 0.5°C using aqueous KCI solution ($I = 10^{-3}$ $mole^{-1}$). The pH value was adjusted with equimolar KOH and HCI solutions, respectively. The zeta potential was calculated taking into account the surface conductivity.

2.4 Quantification of surface functional groups

The content of the generated carboxylic groups on the membrane surface was measured by binding of the fluorescent dye thionin acetate (THA) [30]. The samples (disks with 25 mm in diameter) were first washed with distilled water and then immersed into a solution of 10 mg/l THA in ethanol. The samples were incubated at room temperature (RT) for 12 h with agitation. Then, samples were washed with ethanol to remove any residual dye not bound to the membranes. Then samples were immersed in 10 ml 0.01 N HCI in water/ethanol (1:1) at RT for 2 h with agitation to replace THA bound to carboxylic groups by protons. The concentration of THA was measured with a LS50B fluorescence spectrometer (Perkin Elmer,

Beaconsfield, UK) set at an excitation wavelength of 594 nm and emission wavelength of 620 nm. The measured values were compared with that of a standard curve of THA to calculate the quantities of carboxylic groups based on a one to one stoichiometry.

2.5 Coating of membranes with FN and FNG

Human plasma fibronectin (FN, Roche Diagnostics GmbH, Mannheim, Germany) or fibrinogen (FNG, Sigma, Deisenhofen, Germany) were both dissolved in phosphate buffered saline (PBS, 137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4) to obtain a concentration of 20 µg/ml. Discs of the various membranes (13 mm in diameter) were placed in 24 well plate and immersed in protein solutions for 30 min at RT. Each membrane disc was then rinsed three times with PBS.

2.6 Measurement of FN and FNG adsorption

Adsorption of FN and FNG on the various membranes was measured by enzyme immunoassay technique, which was introduced recently [28]. As primary antibodies were used rabbit polyclonal anti-human FN (Sigma, F 3648) and goat polyclonal anti-human FNG antibody (Sigma, F 2506). As secondary antibodies were used goat anti-rabbit IgG-peroxidase conjugated (Sigma, A 4914) and rabbit anti-goat IgG-peroxidase conjugated antibodies (Sigma, A 5420).

After the washing step, the discs were blocked with 2% of BSA in PBS for 1 h at 37°C. The primary antibodies were added at a dilution of 1:1000 to the samples and incubated for 1 h at 37°C. Thereafter sample were thoroughly rinsed with PBS. The secondary peroxidase conjugated antibodies were added at a dilution of 1:10 000 for 1h at 37°C. Then, the samples were rinsed again with PBS. As substratum for peroxidase 3, 3', 5, 5'–tetramethylbenzidine (TMB) was used, which was incubated at RT for 10 min. The reaction was stopped by adding

1 M HCL. The optical density (OD) of the solution was read at 450 nm with a SPECTRA Fluor Plus plate photometer (TECAN, Crailsheim, Germany).

2.7 HUVEC attachment and growth on polymer membranes

HUVEC in EC growth medium (both Cell Lining GmbH, Berlin, Germany) were seeded at a density of $3x10^4$ cells/ml onto the membranes. The cells were incubated 2 h for cell attachment experiments or 48 h for cell growth experiments at 37°C in a humidified 5% CO_2/ 95% air atmosphere. The number of cells on the various membranes was determined by an electronic cell counter CASY Model TTC (Scharfe System GmbH, Reutlingen, Germany) after detachment with Trypsin/EDTA. The viability of HUVEC seeded on the membranes was determined by staining with 0.4% trypan blue after detachment with Trypsin/EDTA and counting with Neubauer cell chamber.

Comparison of cell counts upon seeding and after the culture periods yielded the percentage of adherent cells or the growth index.

2.8 Prostacyclin (PGI_2) assay

To measure the basal production of prostacyclin (PGI_2), the cells were cultivated for 5 days at an initial density of $7.5x10^5$ cells/well on larger membrane discs (d = 35 mm). Then the supernatant medium was collected, centrifuged (10 min, 400 g, 4°C) and stored at –20°C for later analysis. For TNF-α stimulated secretion of PGI_2, HUVEC were cultivated at the same density for 24 h and then stimulated with TNF-α (10 µg/ml) for 5 h. The supernatant was processed as described above. The PGI_2 concentration was determined using a competitive enzyme immuno assay for the stable hydrolysis product of PGI_2, 6-keto-prostaglandin F_{1a} (Amersham, England).

2.9 Statistical analysis

All statistical computations were carried out with Instat® 3.05 software (Graph Pad Software Inc., San Diego, USA). The values were considered to be significantly different if the p value was < 0.05.

3. Results and discussion

3.1 Material surface properties

The surface topography and roughness of the membranes were investigated by AFM (Fig. 1 and Tab. 1). The functionalization process with IDA resulted only in a slight increase of surface roughness. The roughness of plain PEI (see Tab. 1) was in the range of 13 nm while the roughness of PEI-1 and PEI -2 was increased to 15nm at the same size of the scanned field. The surface of PET membrane (reference material) showed approximately a three times smoother surface with a roughness of about 5.5 nm.

The success of the surface modification was evident by the increase in –COOH group content as shown in figure 2. THA assay yielded a background value of about 4.6 nmol of carboxylic groups per cm^2 of membrane area for the unmodified PEI. This result might be due to some polar interaction between the oxygen in PEI and the charged THA molecule. The values were calculated under the assumption that 1 mole of THA binds to 1 mole of carboxylic groups. Within the first 10 min of membrane modification with IDA the number of carboxylic groups increased by 4 nmol of carboxylic groups per cm^2 of membrane area and reached a plateau of about 8 nmol of carboxylic groups/ cm^2 (Fig. 2). For experiments with cells membranes modified for 1 min (PEI-1) (approx. new 2.4 nmol –COOH groups/ cm^2) and for 30 min (PEI-2) (approx. new 4 nmol –COOH groups/ cm^2) were used.

Contact angle analysis (Tab. 2) revealed that surface modification with IDA did not cause any significant differences in the advancing CA's of the PEI-1 and PEI-2 membranes in comparison to plain PEI. In contrast, a significant drop in receding CA was found for PEI-2 (28.3º). The large hysteresis of PEI indicated to some extent an increase in the heterogeneity of the material surface [31]. Since the roughness of PEI-2 membrane was just slightly higher then that of PEI only a chemical heterogeneity can be responsible indicating the existence of incomplete surface coverage with IDA.

Streaming potential measurements are shown in figure 3. The plain PEI membrane possessed an isoelectric point (IEP) at pH 3.7 and showed an increase of the magnitude of the negative zeta potential with the increasing of pH. At physiological pH (pH 7.4) the streaming potential was about –55 mV. The measured decrease of IEP to pH 3.5 for PEI-2 was correlated with the increased negative streaming potential to –61 mV at pH 7.4. The reference PET membrane, which has IEP at pH 3.9, showed the highest zeta potential value of about -42 mV at pH 7. Overall, it was evident that the introduction of carboxylic groups leads to a decrease of the zeta potential, i.e. the membrane surface becomes more negatively charged.

3.2 Protein adsorption

We studied the ability of membranes to adsorb two adhesive proteins, such as FN and FNG, which play a major role in HUVEC adhesion [7, 32]. It is well established that the organization of the adsorbed protein layer (composition, quantities and conformation) depends on the physicochemical properties of the polymer surface [33, 34]. The protein adsorption experiments showed that both PET (reference membrane) and PEI adsorbed the highest amount of FN (Fig. 4A), while PET adsorbed more FNG (Fig. 4B). These results were consistent with our previous study [28] on the ability of hydrophobic PEI to adsorbed high amount of FNG and with the data from the literature reporting for high protein adsorption on PET [15]. It is also obvious from figure 4 that the introduction of carboxylic

groups on PEI-1 and PEI-2 decreased the quantity of the proteins adsorbed. However, the observed differences in antibody binding between PEI-1 and PEI-2 were not significant ($p>0.05$) for FN adsorption, whereas a significant drop in antibody binding was detected for FNG ($p<0.01$). Our results are basically in agreement with the study of Simonovsky et al. [35], who showed that an increased quantities of carboxylic groups on polymer surfaces reduces the adsorption of negatively charged proteins like FNG.

3.3 Attachment of HUVEC on investigated polymers

Adhesion of EC to biomaterial surface is an important prerequisite for improving the hemocompatibility of synthetic vascular grafts [36]. Overall, attachment of HUVEC after 2 h showed that significantly more cells adhered to protein coated membranes than to uncoated ones (Fig. 5). For all groups the highest adhesion was observed on unmodified PEI, followed by PEI-1 and the lowest HUVEC attachment showed PEI-2 in each groups of different protein coatings ($p<0.05$). A slight, but significant ($p<0.05$) increase in the cell attachment was observed on FN-coated PEI when compared to FNG-coated one (Fig. 5). The difference between attached HUVEC on FN- or FNG-coated PEI-1 was not significant, while the number of cells attached to FN-coated PEI-2 was higher then the one found for FNG-coated PEI-2, respectively ($p<0.001$). The viability of HUVEC (Tab. 3) attached to FN- and FNG-coated PEI membranes was above 95%. The viability of the cells attached to uncoated PEI membranes was in the range of 89-93%. The relatively low cell attachment observed for PET despite the high protein adsorption is in accordance with results from Engle and coworkers [37], who showed poor adhesion and spreading of smooth muscle cells on PET. The poor adhesion of HUVEC on –COOH bearing (negatively charged) membranes shown in this study is in accordance with results of others showing that attachment and spreading of bovine aortic endothelial cells was reduced on surfaces of self-assembled monolayers with terminal carboxylic groups [38].

3.4 Cell proliferation

The rate and the quality of endothelization of the synthetic vascular grafts, however depends on proliferation and migration of EC on the substrate. Since cell adhesion and growth are receptor-ligand dependent processes [5, 6], it was assumed that coating of membranes with FN or FNG should have a positive impact on growth of HUVEC. The growth index in figure 6 resembles the ratio between the number of attached HUVEC at the beginning and after 48 h of incubation. The presented results showed that the uncoated membranes were poor substrata for HUVEC growth. The results after 48 h incubation revealed that the number of cells on these membranes remained approximately the same when compared with the time zero ($p>0.05$) (Fig. 6). In contrast, there was a significant increase in cell growth on protein coated membranes compared to uncoated ones ($p<0.05$). Overall, HUVEC growth was higher on protein coated PEI and PET, followed by PEI-1 and PEI-2. For each membrane, FNG-coating provoked an enhanced cell growth when compared with FN-coating ($p<0.001$). This fact might be correlated with the enhanced cell motility and lower strength of cell adhesion in the same time on FNG-coated substrata as we described previously [8]. Many authors [39-41] reported dependence between adhesion strength and cell migration, too. The viability of HUVEC (Tab. 4) on FN-coated PEI and FNG-coated PEI was about 93-95 %. For all other membranes, the cell viability was above 90 %.

3.5 Functionality of seeded HUVEC (prostacyclin production)

Seeding of EC on artificial substrates, however, may affect the functionality of the endothelium from anticoagulant to pro-coagulant, from anti-inflammatory to pro-inflammatory state [3]. The PGI_2 production is an important prerequisite for anti-coagulant properties of the newly established endothelium [11]. Its role in hemostasis can be associated with down regulation of tissue factor synthesis and inhibition of platelet adhesion and activation [11]. The basal production of PGI_2 by HUVEC revealed the significant increase on

the protein coated membranes in comparison to non-coated ones (Fig. 7A). The type of protein coating showed a slight effect on PGI_2 production as only for PEI-2 the difference between the two coatings was significant ($p<0.05$) (Fig. 7A). Overall, the PGI_2 production for FN-coated PEI and a PET membrane was higher, followed by FNG-coated PET and FNG-coated PEI. PEI-1 showed the same PGI_2 production on FN-coating and even higher production on FNG-coated PEI-1 when compared to the same coatings on PEI. In contrast, PEI-2 caused the lowest PGI_2 production for both protein coatings. The rate of EC proliferation caused an influence on PGI_2 production as the lowest proliferated HUVEC's on PEI-2 (Fig. 6) exhibited the lowest anti-thrombotic activity (Fig. 7A). PGI_2 plays also a role in inflammation by inhibiting the production of TNF-α by monocytes [11, 12] and in turn, proinflamatory cytokine TNF-α is an important stimulus for prostacyclin production [13, 14]. Exploring that fact, we observed PGI_2 production after a stimulation of the seeded HUVEC with TNF-α. After stimulation with TNF-α only HUVEC seeded on FN-coated PET and FN-coated PEI retained a high level of PGI_2 production, as this level was highest for PET followed by PEI. (Fig. 7B). The FNG-coating on the same membranes resulted in a significant lower amount of PGI_2 ($p<0.001$). The production of PGI_2 by HUVEC seeded on –COOH bearing membranes PEI-1 and PEI-2 was very low (fewer than 10 pg/cells x 10^5 PGI_2). Thus, the results revealed that FN-coated PET and FN-coated PEI produced significantly higher amount of PGI_2 then more negatively charged (-COOH bearing) membranes ($p<0.001$). An interesting finding was that FN is the better coating then FNG for inducing PGI_2 production from seeded HUVEC. That fact was confirmed by ESEM analysis of the extent of platelet retention and activation on PET membranes coated with FN or FNG and seeded with HUVEC [non-published data, dissertation of Rumiana Tzoneva-Velinova, University of Potsdam, Germany, 2003, URL: http://opus.kobv.de/ubp/volltexte/2005/121/]. We showed that the EC layer seeded on FN-coated membrane repels platelets to adhere since

HUVEC cultivated on FNG-coated attract many platelets, which were well spread, and even platelet aggregates can be achieved.

Conclusion

We have studied cell attachment, growth and function of HUVEC seeded on PEI and two surface modifications of PEI membrane with various content of carboxylic groups. In addition, adsorption of FN and FNG onto the various membranes was studied with respect to HUVEC behavior. Physico-chemical characterization of the membranes showed that the introduction of carboxylic groups makes the membranes more negatively charged, but no significant difference in water CA among the PEI membranes was detected, which might be an indicator for an incomplete surface coverage with -COOH groups. The protein adsorption study reveals that the introduction of carboxylic groups on PEI-1 and PEI-2 decreased the quantity of the proteins adsorbed, which fact reflected on the decreased number of cells adhered and grown on –COOH modified membranes. FN-coating was better for cell adhesion, since FNG-coating enhanced cell growth. Prostacyclin production was higher for HUVEC seeded on PEI and significantly diminished for PEI-1 and PEI-2. From the results presented it is evident that PEI shows measurable features with Dacron membrane for adhesion and growth of EC (even with better cell viability) and can be used as a promising material for immobilization of endothelial cells as it is needed for improving the hemocompatibility of cardiovascular devices.

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Figure Legends

Figure 1: AFM micrographs of the membranes obtained by scanning three 25 x 25 μm^2 fields.

Figure 2: Content of carboxylic groups measured with THA assay. PEI membranes were treated for 1 (PEI-IDA1), 5 (PEI-IDA5), 10 (PEI-IDA10), 20 (PEI-IDA20) and 30 min (PEI-IDA30) with sodium salt of iminodiacetic acid (IDA).

Figure 3: Zeta potential and isoelectric point (IEP) of PEI (○), PEI-2 (♦) and PET (▼) membranes estimated by streaming potential measurements.

Figure 4: Protein adsorption to PEI, PEI-1, PEI-2 and PET measured by binding of polyclonal antibodies. **A.** FN adsorption and **B.** FNG adsorption. Data are means ± SD of ten replicates. The statistic was performed by one way analysis using Tukey-Kramer post test. n.s. – not significant, (**) - $p<0.01$ (compared to PEI-2 in the same experiment) and (***) - $p<0.001$.

Figure 5: Attachment of HUVEC after 2 h of incubation as percentage of cells attached from suspension. HUVEC were attached to plain (white columns), FN- (striped columns) or FNG- (black columns) coated PEI, PEI-1, PEI-2 and PET. Results are the means ± SD of two independent experiments, each performed in triplicate. For statistics one way analysis with unpaired Tukey-Kramer multiple post test was used.

Figure 6: Proliferation of HUVEC after 48 h of incubation. HUVEC were incubated on plain (white columns), FN- (hatched columns) and FNG- (black columns) coated PEI, PEI-1, PEI-2 and PET. Results are the means ± SD of two independent experiments each performed in triplicate. For statistics one way analysis with Tukey-Kramer post test was used.

Figure 7: PGI_2 production by HUVEC at basal and stimulated conditions. **A.** Basal PGI_2 production: HUVEC were incubated for 6 days on plain (white columns), FN- (striped columns) or FNG- (black columns) coated PEI, PEI-1, PEI-2 and PET. **B.** TNF-α stimulation: The cells were incubated 24 h on the same materials, and then TNF-α (10 μg/ml) was added to all samples for 5 h. Results are the mean ± SD of two independent experiments each performed in triplicate. The statistics was performed by one way analysis using Tukey-Kramer post test.

Table Legends

Table 1: The average roughness (nm) of the surfaces calculated by scanning 25 x 25μm^2 fields in triplicate.

Table 2: Advancing and receding water contact angles (deg.) for PEI, PEI-1, PEI-2 and PET.

Table 3: Viability of HUVEC attached after 2 h on PEI, PEI-1, PEI-2 and PET.

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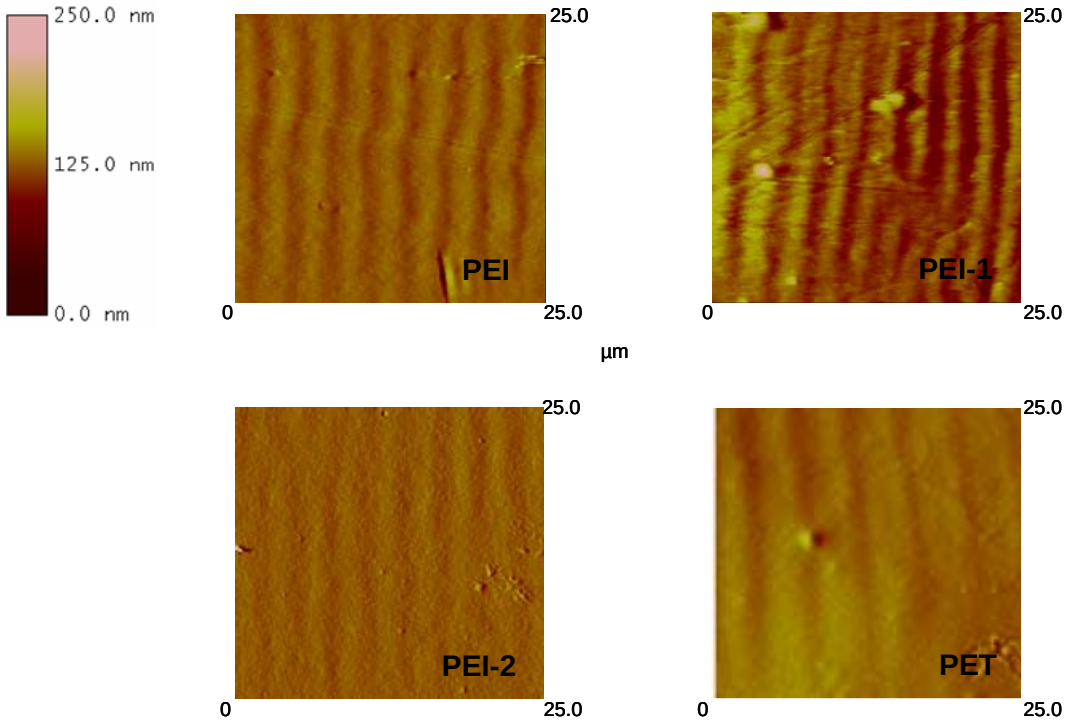

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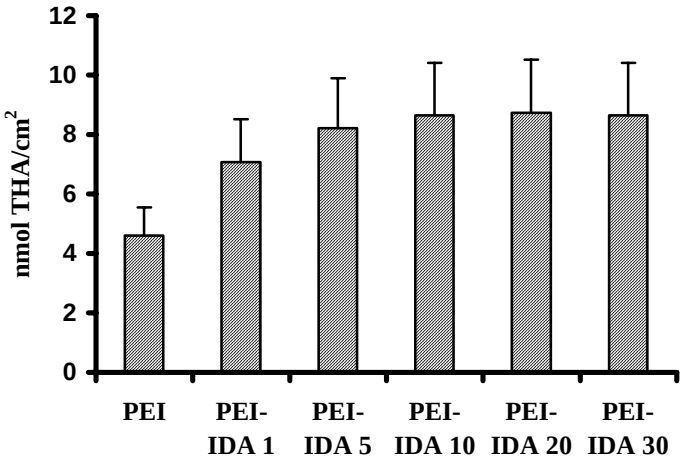

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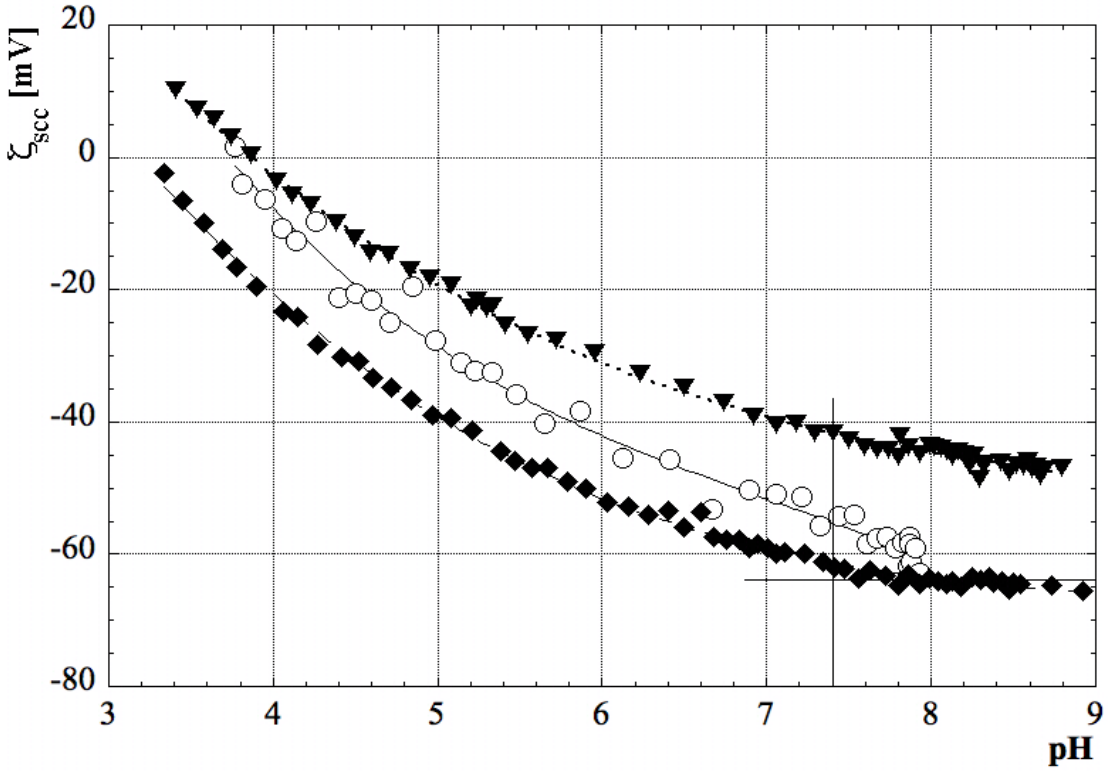

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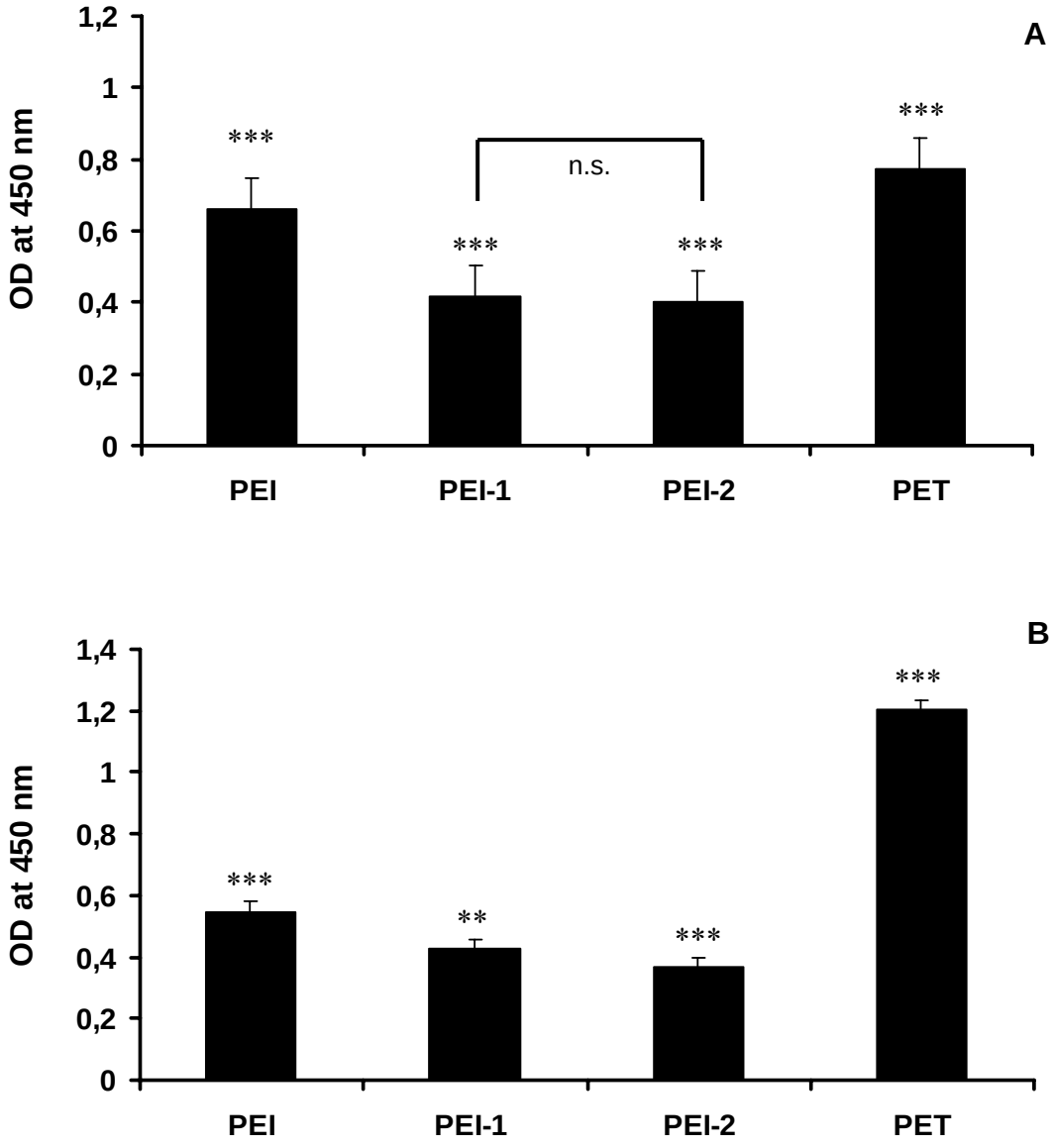

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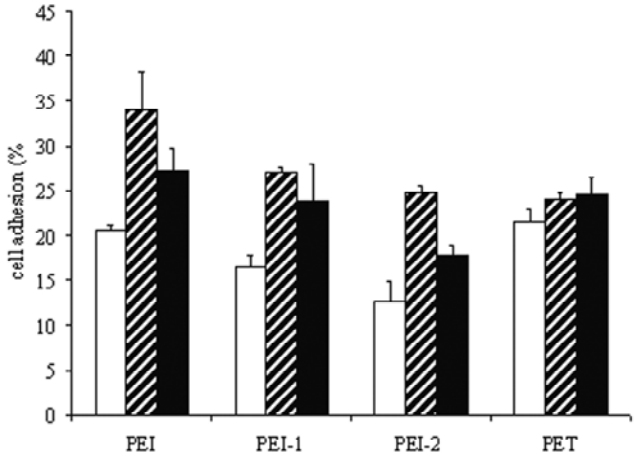

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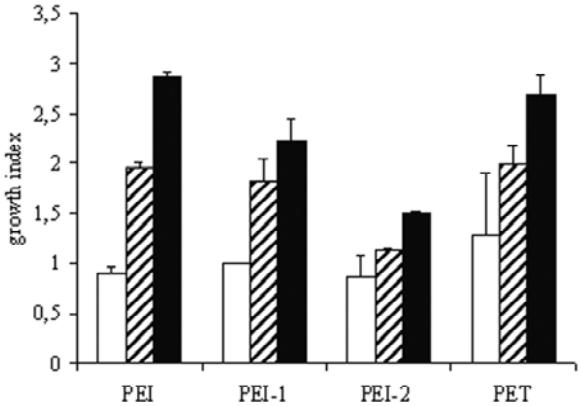

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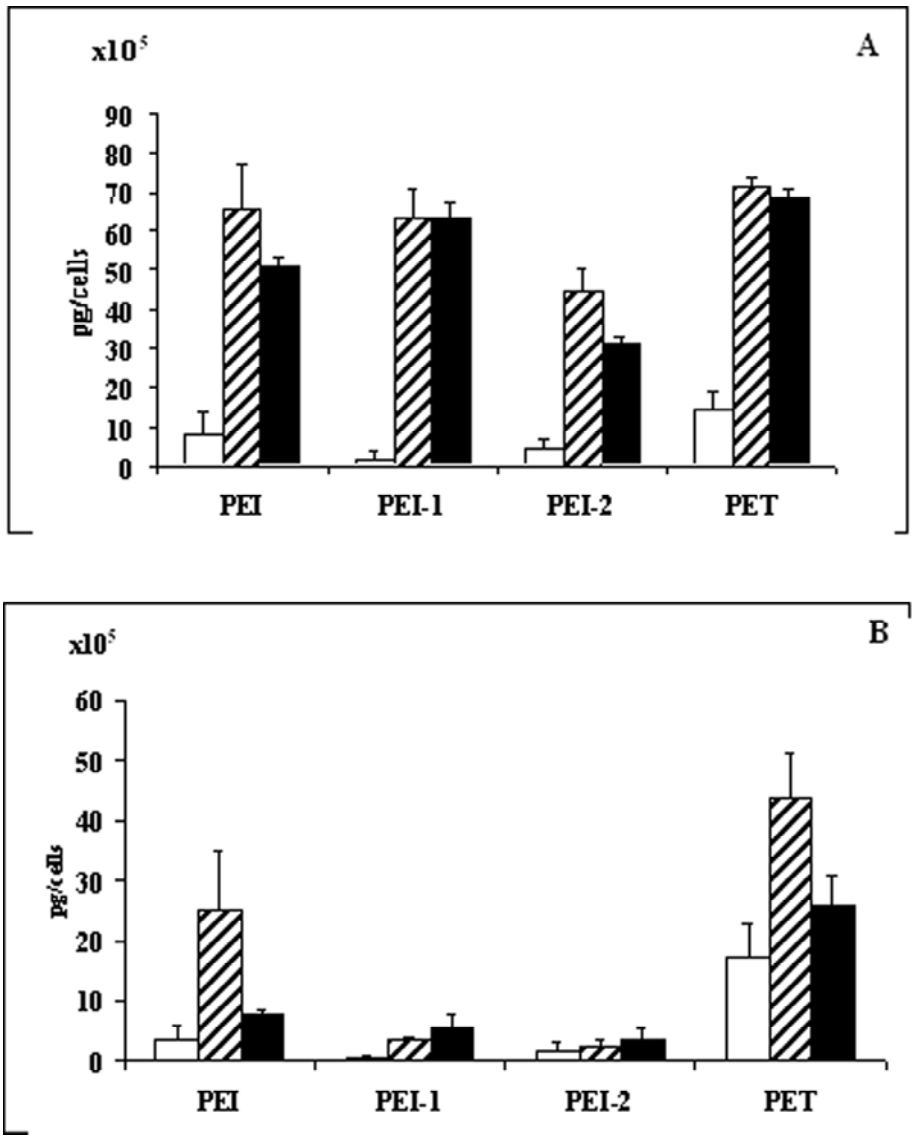

Table 1: Average roughness (nm) of the surfaces calculated by scanning 25 x 25μm^2 fields in triplicate.

Surface	**Average Ra (Mean ± SD)**
PEI	13.5 ± 5.2
PEI-1	15.3 ± 4.3
PEI-2	15.3 ± 2.5
PET	5.5 ± 1.6

Table 2: Advancing and receding water contact angles (CA in degree) for PEI, PEI-1, PEI-2 and PET.

Surface	**Advancing CA** (Mean ± SD)	**Receding CA** (Mean ± SD)
PEI	78.1 ± 0.92	39.1 ± 0.49
PEI-1	81.0 ± 1.70	45.2 ± 0.49
PEI-2	82.1 ± 0.14	28.3 ± 0.57
PET	80.2 ± 0.11	48.8 ± 0.49

Table 3: Viability of HUVEC attached after 2 h on PEI, PEI-1, PEI-2 and PET.

Surface	**PEI**	**PEI-1**	**PEI-2**	**PET**
plain	93.5	93.5	89.0	93.0
FN-coated	95.0	95.5	96.5	95.0
FNG-coated	95.5	97.0	95.5	95.5

Table 4: Viability of HUVEC on PEI, PEI-1, PEI-2 and PET after 48 h of incubation.

Surface	**PEI**	**PEI-1**	**PEI-2**	**PET**
plain	78.9	87.8	79.3	80.0
FN-coated	93.0	82.2	86.3	88.5
FNG-coated	95.0	80.7	84.0	85.0