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HEPARIN ADSORPTION ONTO MODEL POLY(ETHYLENE TEREPHTALATE) (PET) SURFACES MONITORED BY QCM-D

SPREMLJANJE ADSORPCIJE HEPARINA NA MODELNE POLIETILENTEREFTALATNE (PET) POVRŠINE S POMOČJO KREMENOVE MIKROTEHTNICE

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The adsorption of anticoagulant heparin onto model poly(ethylene terephtalate) (PET) film was monitored using a quartz crystal microbalance with a dissipation unit (QCM-D). Synthetic vascular grafts are usually made from PET, a material with appropriate mechanical properties but moderate haemocompatibility. Therefore anticoagulant heparin is usually used to improve haemocompatibility of PET surfaces. Heparin possesses a high negative charge and as such does not adsorb directly onto hydrophobic PET, which is also negatively charged. To increase heparin adsorption different cationic polymers were investigated as spacers and the highest adsorption was achieved using polyethyleneimine (PEI) as an anchoring agent. The heparin was adsorbed from water solution as well as from phosphate buffer saline (PBS). Heparin dissolved in PBS adsorb better onto PET than heparin from water solution. The results were characterized using Sauerbrey equation and Voigt based viscoelastic model. We found out that heparin adsorbed onto PET formed a thin and rigid film and that Sauerbrey equation is appropriate for characterization of heparin adsorption onto PET.

Keywords: poly(ethyleneterephtalate), PET, heparin, QCM-D, haemocompatibility

Adsorpcijo antikoagulanta heparina na modelni film polietilentereftalata (PET) smo merili s pomočjo kremenove mikrotehtnice s spremljanjem dušenja nihanja (QCM-D). PET, ki se uporablja za izdelavo sintetičnih žilnih vsadkov, ima sicer primerne mehanske lastnosti, vendar slabšo hemokompatibilnost. Heparin, ki izboljša hemokompatibilnost površine polimera v stiku s krvjo, ima močno negativen naboj, zato je njegova adsorpcija na hidrofobni in zato prav tako bolj negativen PET, zanemarljiva. Zato je bilo za uspešno adsorpcijo potrebno uporabiti vmesni vezivni sloj kationskega polimera. Preverili smo učinke več različnih kationskih polimerov in ugotovili, da se heparin najbolje adsorbira na sloj polietilenimina (PEI). Heparin smo adsorbirali iz vodne raztopine in iz fiziološke raztopine (PBS). Heparin se je iz raztopine PBS znatno bolje adsorbiral. Meritve smo ovrednotili z uporabo Sauerbreyeve enačbe in Voigtovega viskoelastičnega modela in ugotovili, da heparin na površini PET tvori čvrst film.

Ključne besede: polietilentereftalat, PET, heparin, QCM-D, hemokompatibilnost

1 INTRODUCTION

There are around 500 surgical treatments using vascular prosthetic material per 1 million residents per year in Western countries, and the number is increasing every year.[1] Woven or knitted vascular grafts are mainly made from polyester (poly(ethylene terephthalate) or PET) filaments of tubular shape. When foreign material is exposed to blood, the blood proteins adsorb on the surface, which triggers the activation of an intrinsic clotting system, the activation of platelets, thrombolysis, and the activation of a complementary system.[2]

Surface properties that influence blood interactions at polymer interfaces are described by surface morphology and roughness, surface chemistry and charge and, as such, by surface- free energy. Therefore, improved knowledge about chemical and physical surface characteristics can lead to improved material haemocompatibility. Intensive investigations are in progress[3–10] to define and develop polymeric materials with appropriate haemocompatibility.

It is a generally accepted theory that hydrophilic environment at the blood polymer interface is beneficial for reducing platelet adhesion and thrombus formation.[5,7,11] Several techniques have been proved to improve surface hydrophilicity and, in this way the haemocompatibility of materials.[12–14] Many techniques are based on application of highly non-equilibrium gaseous plasma.[15–24] Very high surface free energy can be obtained using such a plasma created in different gases including oxygen[25–29], nitrogen[30,31], air[32,33] and carbon dioxide.[34,35] Optimal treatments are often achieved using plasma with a very high degree of dissociation. Such plasma is usually created in high-frequency electrodeless discharges.[36,37] In such discharges the dissociation fraction exceeding 10% is often achieved although the kinetic temperature of neutral gas remains close to room temperature.[37]

Other commonly used techniques for obtaining better hemocompatible properties are surface modifications by grafting with hydrophilic materials or bioactive agents. Currently the commercially available PET vascular

grafts are coated with heparin for improving their antithrombotic properties. However, it has been proved that heparin coating on an inner graft surface remains stable only for 4 weeks after implantation, and during that time it is gradually released from the graft surface.[38,39] This effect can lead to certain adverse effects, such as abnormal bleeding of treated patients.

In this research detailed analysis of heparin adsorption and interaction with model PET surfaces using a quartz crystal microbalance with a dissipation unit (QCM-D) was performed in order to define the most appropriate system and conditions for more efficient heparin binding.

2 EXPERIMENTAL

Quartz crystal microbalance with a dissipation unit (QCM-D) was used for adsorption studies of heparin onto model PET surfaces. QCM-D is one of few techniques that give direct information on the adsorption process in situ. It is based on the change in resonance frequency of a thin AT-cut piezoelectric quartz crystal disc. The crystal frequency change is in correlation to the adsorbed mass following the Sauerbrey relationship (Eq. 1), which is only valid for rigid, evenly distributed thin adsorbed layers.

$$m = \frac{C \cdot \Delta f}{n} \tag{1}$$

Where C is the mass sensitivity constant (17.7 ng Hz^{-1} cm^{-2} for a 5 MHz quartz crystal), n is the overtone number (1, 3, 5, 7, 9, 11, 13), Δm is the change in mass and Δf is the frequency change.

To obtain more accurate mass change and structural properties during adsorption, energy dissipation should be taken into account. Dissipation occurs when periodically switching the AC voltage on and off over the crystal and the energy from the oscillating crystal dissipates from the system. Dissipation is proportional to film elasticity and viscosity and is defined as:

$$D = \frac{E_{\text{lost}}}{2\pi E_{\text{stored}}} \tag{2}$$

Where E_{lost} is the energy lost during one oscillating cycle, and E_{stored} is the total energy stored in the oscillator.

The applied apparatus (QSense, Sweden) was supplied with QTools software, which includes as well the Voigt viscoelastic model. The application of this model was included in our study, and the results were compared to Sauerbrey relation. Voigt viscoelastic model takes into consideration also the dissipation change and is, as such, more suitable for the evaluation of soft adhering layers, for which the Sauerbrey equation is not valid anymore. The instrument measures frequency and dissipation at several overtones, and this enables modelling in QTools using the mentioned Voigt viscoelastic model (**Figure 1**), where Δf and ΔD are calculated according to Voinova et al. (Equations 3,4).

$$f \approx \frac{1}{2\pi\rho_0 h_0}\left\{\frac{\eta_l}{\delta_l} + h_f \rho_f \omega - 2h_f\left(\frac{\eta_l}{\delta_l}\right)^2 \frac{\eta_f \omega^2}{\mu_f^2 + \eta_f^2 \omega^2}\right\} \tag{3}$$

$$D \approx \frac{1}{\pi f \rho_0 h_0}\left\{\frac{\eta_l}{\delta_l} + 2h_f\left(\frac{\eta_l}{\delta_l}\right)^2 \frac{\eta_f \omega}{\mu_f^2 + \eta_f^2 \omega^2}\right\} \tag{4}$$

Where ρ_0 and h_0 are the density and thickness of the crystal, η_l is viscosity of the bulk fluid, δ_l is the viscous penetration depth of the shear wave in the bulk liquid, ρ_l is the density of the liquid and ω is the angular frequency of the oscillation. The adsorbed layer is the function of density (ρ_f), thickness (h_f), elastic shear modulus (μ_f) and shear viscosity (η_f).

The experimental values of Δf and ΔD of several overtones were fitted and Simplex algorithm was used to find the minimum in the sum of the squares of the scaled errors between the experimental and modelled Δf and ΔD values.[40] This model has been successfully used in many publications, however it is assumed that the coated quartz crystal was purely elastic and the bulk solution is Newtonian and purely viscous.

From adsorbed masses (ng/cm^2) is also possible to obtain the thickness (Equation 5) of the adsorbed layer, however in this case the effective density of adsorbed layer ρ_{ef} should be estimated.

$$h_f = \frac{10^7\left(\frac{nm}{cm}\right)\Delta m\left(\frac{ng}{cm^2}\right)}{10^9\left(\frac{ng}{g}\right)\cdot\rho_{ef}\left(\frac{g}{cm^3}\right)} \tag{5}$$

In our study the quartz crystals covered with a thin gold film (QSX 301, QSense, Sweden) were used as substrates for model PET surface preparation. The model PET surfaces were prepared by a "spin coating" technique from a 1 wt % PET solution in 1,1,2,2-tetrachloroethane. Detailed procedure is described elsewhere.

Since heparin possesses a highly negative charge at pH of around 7.0[41] and the PET is negatively charged as well[42,43], the repulsion forces between them are too high and heparin did not adsorb onto the surface (not shown here). Therefore, different cationic polymers were studied as anchoring layers in order to achieve sufficient

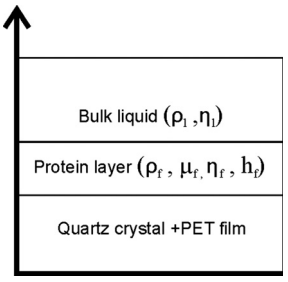

Figure 1: The Voigt based visoelastic film model
Slika 1: Voigtov model viskoelastičnega filma

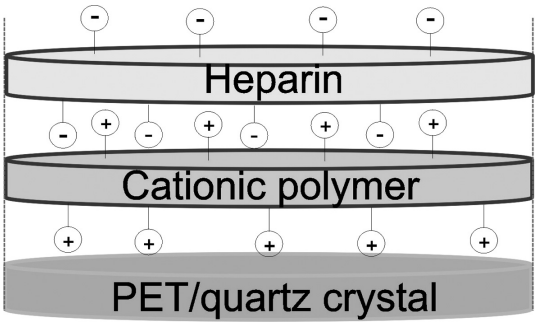

Figure 2: Schematic presentation of layer-by-layer polymer film formation onto quartz crystal coated with model PET surface

Slika 2: Shematski prikaz formiranja sloj-na-sloj polimernega filma na kremenovem kristalu z modelno PET površino

adsorption and binding of heparin. After rinsing of the model PET film with MilliQ water for 20 min at 0.25 mL/min, the cationic polymers (0.02 wt %, dissolved in MilliQ water) were adsorbed on PET surfaces with solution flow rate 0.1 mL/min. Thereafter the solution of heparin in MilliQ water and phosphate buffer saline (PBS) at 200 mg/L was used with the same flow rate (0.1 mL/min) to form layer by layer film as shown in **Figure 2**.

3 RESULTS AND DISCUSSION

In the **Figure 3** typical diagrams of frequency change during the heparin adsorption are represented for PET films previously coated with different cationic polymers (PEI – Poly(ethylene imine), PAH – poly(allylamine hydrochloride), pDADMAC – Poly(diallyl dimethyl ammonium chloride), and polylysine). The anchoring polymer and heparin additions as well as rinsing steps

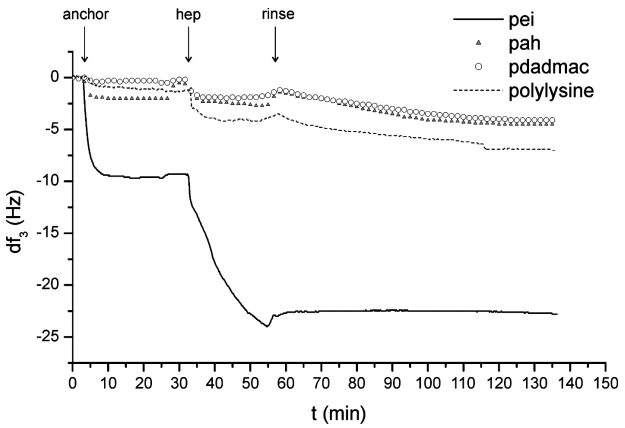

Figure 3: Frequency change (at 3rd overtone) during the adsorption of heparin (hep) from the water solution onto the model PET surfaces coated with different cationic polymers (PEI – Poly(ethylene imine), PAH – poly(allylamine hydrochloride), pDADMAC – Poly(diallyl dimethyl ammonium chloride), and polylysine)

Slika 3: Sprememba frekvence tretjega nadtona med adsorpcijo heparina (hep) v vodni razopini na modelne PET površine predhodno obdelane z različnimi kationskimi polimeri (PEI – Poly(ethylene imine), PAH – poly(allylamine hydrochloride), pDADMAC – Poly(diallyl dimethyl ammonium chloride), and polylysine)

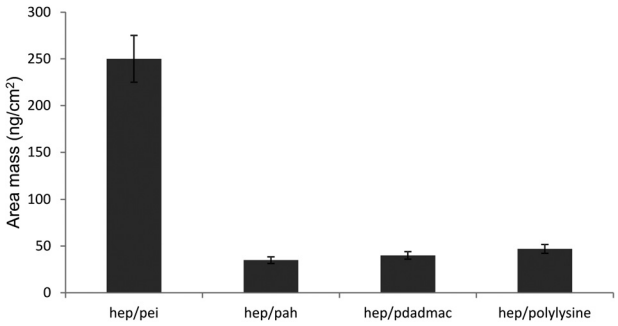

Figure 4: Area mass of adsorbed heparin calculated using the Sauerbrey equation from frequency change at 3rd overtone for PET films covered by different cationic polymers (PEI, PAH, pDADMAC, polylysine)

Slika 4: Površinska masa adsorbiranega heparina, določena s pomočjo Sauerbreyeve enačbe, pri spremembi frekvence tretjega nadtona za PET film, prekrit z različnimi kationskimi polimeri (PEI, PAH, pDADMAC, polylysine)

are marked with arrows. It can clearly be seen from the diagram that the largest frequency change was achieved in the case of PEI followed by heparin adsorption.

In **Figure 4** area masses of adsorbed heparin calculated using the Sauerbrey equation (1) from frequency change at 3rd overtone are represented for the PET films with adsorbed different anchoring agents. In the case of PEI application as an anchoring layer, the area mass of adsorbed heparin was approximately 5-times higher in comparison with other anchoring agents (**Figure 4**).

In the **Figures 5 and 6** the frequency and dissipation changes at 3rd overtone during the adsorption of heparin dissolved in MilliQ water (hep/w) and heparin dissolved in phosphate buffer saline PBS (hep/pbs) are represented. From the figures it can clearly be seen that the decreases in frequencies are significantly higher in the case of adsorption from buffer solution in comparison to the adsorption from pure water. From the extreme minimum of the frequency during the heparin adsorption from water solution it can be assumed that the

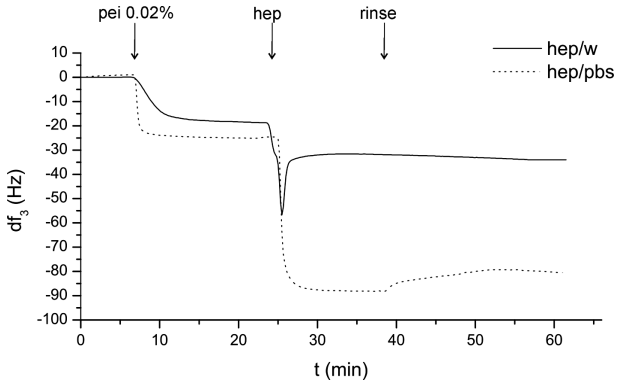

Figure 5: Frequency change (at 3rd overtone) during the adsorption of heparin, dissolved in MilliQ water (w) and in phosphate buffer saline (PBS) onto the polyethyleneimine (PEI) layer on the PET film

Slika 5: Sprememba frekvence tretjega nadtona med adsorpcijo heparina, raztopljenega v MilliQ vodi (w) in v fosfatnem pufru (PBS) na sloj polietilenimina (PEI) na PET filmu

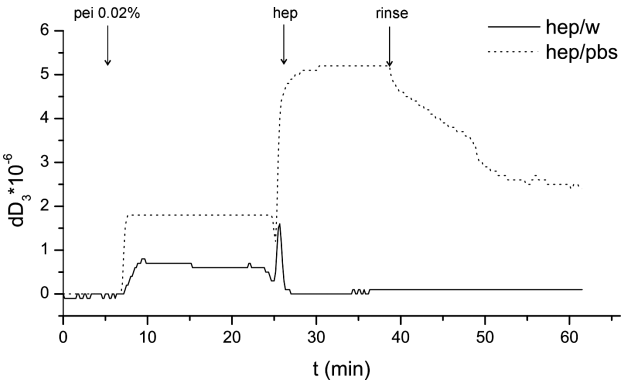

Figure 6: Dissipation change (at 3rd overtone) during the adsorption of heparin, dissolved in MilliQ water (w) and in phosphate buffer saline (PBS) onto the polyethyleneimine (PEI) layer on the PET film
Slika 6: Sprememba disipacije tretjega nadtona med adsorpcijo heparina, raztopljenega v MilliQ vodi (w) in v fosfatnem pufru (PBS) na sloj polietilenimina (PEI) na PET filmu

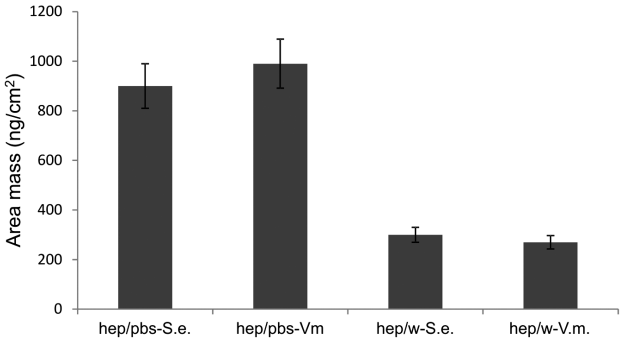

Figure 7: Area mass of heparin adsorbed from water solution (hep/w) and adsorbed from PBS (hep/pbs), calculated using the Sauerbrey equation (S.e.) or Voigt based viscoelastic model (V.m.)
Slika 7: Površinska masa adsorbiranega heparina, raztopljenega v vodi (hep/w) in v PBS (hep/pbs), določena s pomočjo Sauerbreyeve enačbe (S.e.) in s pomočjo Voigtovega viskoelastičnega modela (V.m.)

migration/desorption of heparin molecules at the beginning is rather intensive. After that the adsorbed layer is relatively thin and firm (**Figure 6**).

In water solution, which is slightly acidic (pH=5.7), most of the functional groups of heparin molecules are deprotonated and the molecule is negatively charged. This is the reason the molecules are in more or less unfolded conformation. As such they relatively quickly cover the positive charges on the PET/PEI surface. Ionic strength of buffer solution is high and deprotonated functional groups of heparin are occupied by counter ions from the buffer. Therefore, heparin molecules in such a solution obtain a more folded conformation and much higher amounts of such molecules are needed to cover positive PET/PEI surface (**Figure 7**).

From the **Figure 7** it is obvious that different models (Suerbrey eq. and Voight model) had no influence on the calculated mass area in the case of adsorption from water solution. It can be assumed that the adsorbed layers in these cases are more or less firm. The evaluations using these two models, however, gave different results in the case of adsorption from the buffer solution, which indicates formation of much softer adhering layers, for which Suerbrey equation is not valid anymore.

Assuming that density of adsorbed layer of heparin is around 1 g/cm^2 one can easily get the thickness of the adsorbed heparin, just dividing the estimated area mass with 10^2 (according to Eq. 5). That means that layer thickness of heparin on PET surface is around 2.5 nm in case of water solution and the heparin layer thickness is almost 10 nm when heparin in PBS was used.

4 CONCLUSION

In order to find out most appropriate system for heparin binding in the present research heparin adsorption onto PET model surfaces was investigated using QCM-D. Several cationic polymers were analysed as an anchoring layer for better heparin binding onto PET surfaces.

The results showed that quartz crystal microbalance was a suitable tool for the analysis of the adsorption of heparin onto PET surfaces. Furthermore, from the QCM measurements, important data about adsorbed polymer masses and adsorbed film thicknesses onto surfaces can be achieved using the Sauerbrey equation and Voigt viscoelastic model.

Poly(ethyleneimine) (PEI) showed best results as an anchoring layer, thus in that case the area mass of adsorbed heparin was approximately 5-times higher as for other anchoring agents.

The adsorbed layers of heparin were much thicker and adsorbed amounts much higher in the case of heparin adsorption from PBS solution in comparison to the adsorption from pure water solution. Folded conformation of heparin molecules in the solution with high ionic strength form thicker and softer adsorbed layer on the PET/PEI surfaces.

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