Protein adsorption and oxidative properties of some cellulose-modified polyurethane membranes for medical applications

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Polyurethane (PU) materials are easily adaptable materials to many medical applications if biocompatibility characteristics can be assured. To improve the biocompatibility, PU samples obtained from poly(ethylene adipate)diol (PEGA), polytetrahydrofuran (PTHF) or poly(propylene)glycol (PPG) as soft segments and 4',4'-diphenylmethanediisocyanate were blended with hydroxypropylcellulose (HPC). The investigation of the functional integration capacity of the newly prepared materials was performed. All materials have slightly positive surface charge, the samples showed preferential adsorption of serum albumin (SA). PU_{PTHF}/HPC with ζ potential close to neutral, lowest FB adsorption properties and higher SA/FB adsorbed ratio was found to be the most bio-adaptable sample.

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1. Introduction

The need for appropriate biomaterials for medical applications is a widely discussed field. According to data of the last two decades, it is clear that, due to their wide structural availability, polyurethanes can satisfy the general requirements of "good materials" if biocompatibility is possible to ensure [1-4]. Most important properties of polyurethanes that must be considered for their appropriate functional integration are surface properties, such as hydrophilicity together with mechanical characteristics. From this point of view, segmented design of the polyurethanes or their blending with a wide spectrum of molecules makes these materials very adaptable to many medical usages. Biopolymers such as proteins, proteoglycans or polysaccharides were used for blending. To achieve new and desired properties, the cellulose derivatives like hydroxypropylcellulose (HPC) are appropriate candidates for blending due to their demonstrated biocompatibility as well as to their possible hydrogen bonding that can enhance both mechanical and surface properties of the resulted materials, which will modify nonspecific protein adsorption first [5, 6].

An example of important overlooked tissue-material interfacing phenomenon is the oxidative stress production in the live tissue. In the last decade oxidative stress cell lesions induced by some degradable biomaterials previously considered biocompatible have been demonstrated [7]. Two mechanisms can be presumed. One deals with the excessive production of reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals, hydrogen peroxide by the material constituents or by the tissues in response to the material actions. The second mechanism considers direct affecting of the tissue antioxidant capacity produced by adsorption/inactivation of the tissue antioxidant compounds [8]. Therefore, free radical production and antioxidant capacity decrease may be considered predictable parameters for foreign body reaction and material time resistance. For these reasons, this work deals with the correlation of protein adsorption and oxidative capacity with the surface charge and haemocompatibility of cellulose modified polyurethanes. We aim to highlight the functional integration possibility of HPC-modified polyurethanes previously demonstrated for their good mechanical properties [9,10].

2. Experimental

Three polyurethane (PU)/hydroxypropyl cellulose (HPC) samples were prepared according to a multistep procedure previously described [9,10]. First, isocyanate terminated urethane prepolymers were synthesized in N,N-dimethylformamide by polyaddition between 4',4'-diphenyl methane diisocyanate (MDI) and poly(ethylene adipate)diol (PEGA, Mn = 2000 g/mol), polytetrahydrofuran (PTHF, Mn = 2000 g/mol) or poly(propylene)glycol (PPG, Mn = 2000 g/mol) macrodiols. Further, the urethane prepolymers were treated with ethylene glycol (EG) chain extender. Finally, HPC (Klucel LF, Hercules Inc., the average weight molecular weight, Mw of 95 000 g/mol) was added to PU solutions and the mixtures were precipitated in warm

water. After drying under vacuum for several days, PU/HPC film samples of about 1mm thickness were obtained. Compositional weight ratio of macrodiols/MDI/EG/HPC materials was 52.24/36,57/7.27/3.92. Previously determined characteristics of PU/HPC films are given in Table 1 [9, 10]. The simplified short names like PU_{PEGA}/HPC; PU_{PTHF}/HPC and PU_{PPG}/HPC are used to identify the samples.

Table 1. Physico-chemical properties of the studied polyurethane samples [9,10].

Characteristics/Samples	**PU_{PEGA}/HPC**	**PU_{PTHF}/HPC**	**PU_{PPG}/HPC**
Structural characteristics			
Mn, polyurethane, g/mol	134520	73950	70290
Mw/Mn, polyurethane	1.86	1.59	1.67
Mechanical testing (Dry / Conditioning in saline water 0,9% for 24h)			
Young modulus, M Pa	90/113	70/30	75/39
Elongation at break, %	71/84	72/159	53/56
Tensile strength at break, MPa	19/22	14/10	15/9
Toughness, MJ/m^3	9.3/13.1	7.7/11.8	5.6/3.5
Dynamic Contact angle / Water uptake			
θ_{adv} (advanced)	84.9±1.1	77.4±1.1	85.6±1.1
θ_{rec} (receding)	44.2±0.5	42.9±0.5	44.8±0.5
Hysteresis, %	47.9	44.6	47.7
Water uptake, %	140±4	167±3	92±6

The zeta-potential (ζ) measurement was performed by using a commercial electrokinetic analyzer SurPASS, (Anton Paar GmbH, Graz, Austria). For each sample zeta potential has been measured in 0.1 M NaCl solution at physiological 7.4 pH value, a 300 mbar electrolyte pressure and a 80 ml/min flow rate. For statistical reasons, four streaming potentials were measured. The mean value of these data was used for potential calculation by Fairbrother–Mastin equation, considering also the effect of surface conductivity [11].

The protein adsorption on material surface was measured in different conditions: (a) on individual protein solutions of fibrinogen (FB 95% clotable from Sigma-Aldrich; 3 mg/ml) and bovine serum albumin (BSA from Sigma-Aldrich; 45 mg/ml) of normal physiological concentration; (b) FB and BSA mix physiological solution (3 mg/ml for BSA and 45 mg/ml for FB); (c) complex protein solution - platelet poor blood plasma (PPP). Prior the adsorption experiment, the PU/HPC films were brought to equilibrium with phosphate buffer saline (PBS) up to reaching maximum hydration, for about 72 h. The PPP and protein solutions were always freshly prepared before every adsorption experiment. PPP was prepared by standard clinical method [12]. In order to perform the adsorption experiments, PU/HPC films with 50 mm^2 surface were covered with 0.25 ml of one of the protein solutions or with blood plasma and kept at 37 ^oC for 30 min. A turbidimetric method based on insoluble complex formation with Na_2SO_4 was used for FB assay [12]. For albumin assaying, a turbidimetric grade antibody, monospecific for albumin (Dialab kit, Austria) was employed. The antigen-antibody insoluble immune complex was formed after monospecific albumin – antibody reaction. FB and BSA reaction products were assessed on a Piccos 05 UV–VIS spectrophotometer at λ = 530 nm and 340 nm, respectively. The adsorbed amount of proteins was calculated from equation:

$$Adsorbed\ protein\ (mg/mm^2) = (Co - Ce)\ x\ V / S;$$

where *Co* and *Ce* are the initial and equilibrium concentrations of protein solution (mg/ml), *V* is the incubated volume of the protein solution (ml) and *S* is the surface of the incubated PU/HPC sample.

Total antioxidant capacity (TAS) in blood plasma was determined after 24 h incubation of 0.25 ml freshly heparinized human plasma with 0.5x0.5cm^2 well hydrated PU/HPC samples. The TAS measurement was made by standard Randox TAS kit protocol. Control serum was used for data validation.

The thrombogen potential of the film surface was judged by the blood clot formation test modified in our laboratory [13]. To choose an appropriate duration for blood clot formation, the evaluation in normal recalcified blood was made first. A duration of 240 s was chosen for clot weight relevance reason. To PBS-swollen sample, 0.2 ml of human blood, collected on 3.8 % sodium citrate anticoagulant (9:1v/v) from a healthy donor, was added. The thrombus formation was started by 0.05 ml $CaCl_2$ solution (0.025 mol L^{-1}) and after 240 s it was stopped by diluting with 1 ml of distilled water. The formed thrombus was blotted with filter paper and then weighed. The thrombus weight of the PU/HPC film was compared with control experiment performed without polymeric material. Collagen film was used as procoagulant material reference.

3. Results and discussion

ζ potentials of the PU/HPC films measured in 0.1 M NaCl solution are presented in Table 2.

Table 2. Surface zeta potential (ζ) of PU/HPC samples.

Polyurethane samples	**PU_{PEGA} without HPC**	**PU_{PEGA}/HPC**	**PU_{PTHF}/HPC**	**PU_{PPG}/HPC**
Zeta potential (mV)	-4.31	+3.14	+0.78	+ 4.84

Contrary to a pure PU_{PEGA} sample, all studied PU/HPC films showed slightly positive ζ potential values in the range very close to neutral and depending on the sample hydrophiliciy. Most positive ζ potential was measured for PU_{PPG}/HPC (+4.84 mV), characterized by a low polarity of the polyether soft segment, lower hydrophilicity and lower water swelling (see Table 1). Almost neutral ζ potential value (+0.78 mV) was measured for the most hydrophilic PU_{PTHF}/HPC with higher swelling properties. The intermediate value of ζ potential (+3.14 mV) was measured for PU_{PEGA}/HPC having a water contact angle close to PU_{PPG}/HPC sample, but higher swelling capacity. These findings are in agreement with other data, showing that more hydrophilic surfaces are characterized by lower zeta potential [14]. There are very few data concerning zeta potential on polyurethanes membranes for medical applications. Some of them reported very negative (– 25 mV) ζ potential values for poly(ether)urethanes, while for poly(ester)urethanes less negative (- 12 mV) values were registered at neutral pH. The authors demonstrated that positively charged PU surfaces are more suitable for cells attachment and proliferation than negatively charged surfaces [15]. Our results showed that introduction of HPC in PU structure shifted the surface potential to netural or even slightly positive values compared to control PU (PU_{PEGA} without HPC) (-4mV).

The results on protein adsorption in noncompetitive condition are shown in Fig. 1 while Figs. 2 and 3 compare the adsorption capacity of PU/HPC samples for BSA and FB, respectively, in noncompetitive (individual protein solutions) and competitive conditions (mix solutions).

As one can see from Fig. 1, in idealized conditions (individual solutions), the adsorbed BSA is significantly higher than the adsorbed FB for all PU/HPC samples. In competitive conditions (Figs. 2 and 3), we found that PU_{PPG}/HPC has significant lower protein adsorption capacity for both BSA and FB as compared to the other two samples. PU_{PTHF}/HPC adsorbs a smaller amount of FB but a highest amount of BSA. The highest amount of FB was adsorbed on PU_{PEGA}/HPC. From these results one can resume that BSA adsorption is strongly depending on hydrophilic and swelling properties of surfaces, while FB adsorption has more complex criteria and, unlike albumin, its adsorption seems to be more correlated with ζ potential (see Table 2).

Fig. 4 presents the adsorbed albumin/fibrinogen ratio in complex competitive condition. We found that this parameter increases with increased sample hydrophilicity and decreased ζ potential. The highest albumin/fibrinogen ratio (18/1) was found for the most hydrophilic and neutrally charged PU_{PTHF}/HPC film.

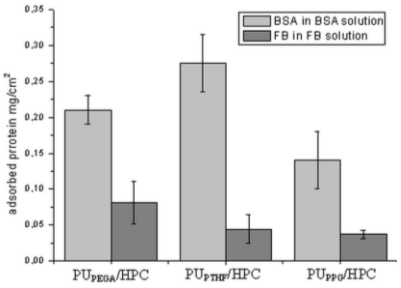

Fig. 1. Noncompetitive albumin (BSA) and fibrinogen (FB) adsorption.

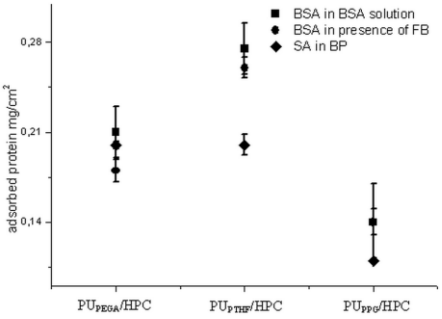

Fig. 2. Serum albumin adsorption on PU/HPC samples in different conditions: (B)SA –(bovine) serum albumin; FB – fibrinogen.

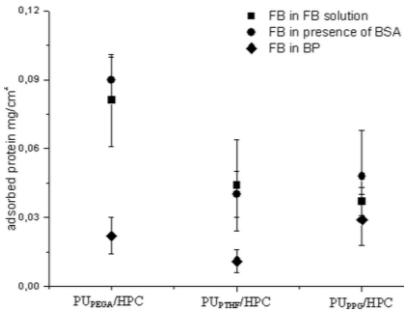

Fig. 3. Fibrinogen adsorption on PU/HPC samples in competitive conditions: FB – fibrinogen; BSA – bovine serum albumin; BP – blood plasma.

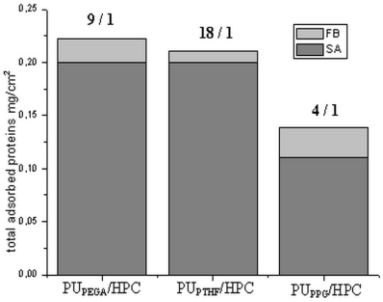

Fig. 4. Total amount of adsorbed albumin and fibrinogen (albumin/fibrinogen adsorbed ratio).

Table 3. Antioxidant status of blood plasma incubated with polyurethane samples.

Samples	24 h (Mmol/l)	72 h (Mmol/l	Decrease	Physiological value
Plasma control	1.30±0.17	0.84±0.05	0 %	1.30 – 1.77
PU_{PEGA}/HPC	1.23±0.06	0.90±0.08	5.4%	
PU_{PTHF}/HPC	1.20±0.11	0.84±0.06	7.7%	
PU_{PPG}/HPC	1.03±0.09	0.75±0.03	20.5%	

*difference with plasma control at 24 hours of incubation

The results on TAS modification of blood plasma are shown in Table 3.

As one can see from Table 3, the more hydrophilic PU_{PEGA}-HPC and PU_{PTHF}/HPC samples do not affect the antioxidant capacity of blood plasma after 24 h of incubation as compared to the relatively more hydrophobic PU_{PPG}/HPC which decreases the blood TAS by about 20%.

These preliminary results do not allow the access to the involved mechanisms. However, as blood plasma without cells was used (PPP), we can suppose that the mechanism involves the antioxidant enzymes adsorption and/or inactivation on the material surface. At prolonged incubation (72 h), the antioxidant capacity decreases in both control and experimental plasma samples with highest modification on hydrophobic PU_{PPG}-HPC sample. Thus, these results showed a higher exposition of PU_{PPG}-HPC to oxidative degradation and higher risks for foreign body reactions.

As functional test for bio-integration capacity of PU/HPC samples, the amount of blood clot formed in contact with a material was determined. The results for clot weight are shown in the Table 4. As shown by the data in Table 4, PU_{PTHF}/HPC film characterized by less adsorbed fibrinogen, no charge on surface and greater water uptake has little contribution to clot formation, while the other two materials with a roughly similar charge but different hydrophilic properties contributed to blood clotting proportional to their hydrophilicity. These results suggest that the oxidative stability and haemocompatibility properties are depending more on surface neutrality and hydrophilic properties in good correlation with serum albumin (SA)/FB adsorption ratio.

Table 4. Amount of blood clot formed in contact with PU/HPC samples.

Incubated surface	Clot weigh at 240 sec (mg)
Clot without material	24.8±2.0
Collagen membrane	42.5±3.5
PU_{PEGA}/HPC	31.9±2.6
PU_{PTHF}/HPC	28.4±2.9
PU_{PPG}/HPC	46.8±2.2

Our results are in accordance with other studies showing less thrombogenic activity of some hydrophilic segmented PU materials with preferential albumin adsorption characteristics or pre-albuminated [16,17]. Oppositely to PU_{PTHF}/HPC in terms of thrombogenicity and oxidative stability, is situated PU_{PPG}/HPC sample. This material had lowest SA/FB adsorption ratio, lowest water swelling and highest ζ potential.

4. Conclusions

The investigated cellulose modified polyurethanes possess surface properties that can be adaptable for blood-contact applications. Studied samples showed slightly positive surface charges in the range between +0.78 mV and + 4.84 mV at physiological pH that can be advantageous for this kind of application. However, depending on the nature of the soft segment, the surface charge increases in the following order: PU_{PTHF}/HPC < PU_{PEGA}/HPC < PU_{PPG}/HPC, in a reversed order of their hydrophilicity. The serum albumin adsorption was found to be less depended on small positive surface charge, but highly depended on hydrophilic properties, in both noncompetitive and competitive conditions. Unlike serum albumin, fibrinogen adsorption is more depended on surface charge.

The best functional bio-integration oxidative capacity was shown by PU_{PTHF}-HPC and PU_{PEGA}-HPC materials. This means that these materials do not affect the functionality of the tissue antioxidant defence mechanisms and consequently they are more oxidative stable. The thrombogenicity testing highlighted that only PU_{PTHF}/HPC sample possesses high haemocompatibility properties. Thus, considering all these observations we can conclude that nature of the PU soft segment strongly influence the functional capacity integration due to its implication in the hydrophilic/hydrophobic balance, while HPC adjusts the surface biocompatibility through the beneficial positivation of the surface potential. Between the three studied samples, PU_{PTHF}/HPC sample is characterised by enhanced biocompatibility and functional blood-contact adaptability due to its cumulative properties such as elasticity, hydrophilicity, surface neutrality, higher albumin/fibrinogen ratio adsorption as well as due to its low oxidative capacity.

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