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# Small changes in PDMMLA structure influence the adsorption behavior of ECM proteins and syndecan-4 on PDMMLA derivative surfaces: Experimental validation by tensiometric surface force measurements

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**5209 words**

**1 Table**

**1 Scheme**

**6 Figures**

## Abstract

Cardiovascular diseases are the leading cause of death around the world according to the World Health Organization. In-stent restenosis is an inflammatory response of the immune system to endovascular stent implantation in atherosclerotic patients. Biocompatible and biodegradable polymers are of great interest in this field in order to limit the side effects of stent treatments. Poly([R,S]-3,3-dimethylmalic acid) (PDMMLA) is a new biodegradable statistical polyester which presents promising properties as a stent coating. In this work, we studied by dynamic tensiometry, the adhesion of extracellular matrix proteins (bovine serum albumin, fibronectin, fibrinogen, and vitronectin) and plasma membrane proteoglycan (syndecan-4) on three PDMMLA derivatives with different hydrophilicity levels. The results show that proteins have different adhesion profiles and affinity on these surfaces. They show similar behavior on the most hydrophilic surface, making hydrophilic, ionic and hydrogen type bonds. Then we compared each protein's individual profile to that of a mixture of all studied proteins. The comparison shows that vitronectin and syndecan-4 are

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the quantitatively dominating proteins adsorbed by specific interactions. Based on the results from previous studies, this work allowed us to identify the most important PDMMLA surface as a promising biomaterial for bioactive stent-coating.

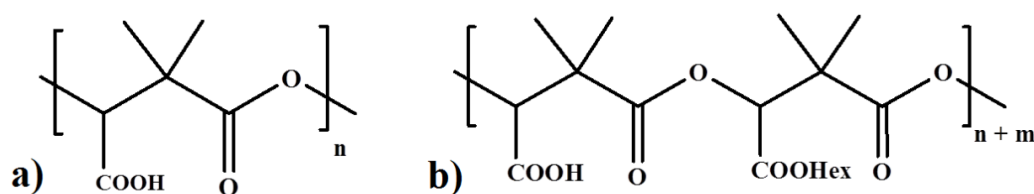
*keywords:* Protein adhesion, Biopolyesters, In-stent restenosis, Dynamic tensiometry, Syndecan-4, ECM proteins

## **1. Introduction**

Cardiovascular diseases are the main cause of mortality and morbidity in the world [1, 2]. In stenosis cases, where the arterial lumen narrows because of an atheromatous plaque formation, percutaneous devices (such as cardiovascular stents) and bypass surgery are utilized in order to re-establish a normal blood flow. These interventions cause a disruption of the vessel wall. After a stent implantation, in approximately 20 to 30% of the cases (where a bare metal stent (BMS) is used), in-stent restenosis (IR) is observed due to an inflammatory response of the immune system [3, 4]. Platelet aggregation, smooth muscle cell (SMC) proliferation and migration, and growth factor release are responsible for the neointimal hyperplasia causing a re-narrowing of the arterial lumen [5, 6].

Biocompatible and bioresorbable polymers are used in order to limit the direct contact of the BMS with the blood [7]. A favorable polymer to be used in this case would be a polymer able to inhibit the proliferation and the migration of SMCs, and promote the vessel re-endothelialization in order to re-vascularize the damaged area. The most used bioresorbable polymers for stent coating are poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA), polyesters with suitable thermal properties and degradation time [8, 9]. We present a new bioactive polyester, which would provide all the needed properties of an adequate stent coating; Poly([R,S]-3,3-dimethylmalic acid) (PDMMLA) (Scheme 1-a) is a biocompatible and biodegradable polyester with promising properties. The synthesis of amphiphilic PDMMLA derivatives takes place by incorporating on the

polymer side chain, carboxyl groups (-COOH) and hexylic ester groups (-COOHex), giving the polymers their hydrophilic and hydrophobic characters respectively (Scheme 1-b) [10]. This property is a huge advantage compared to PLA and PLGA. Indeed, the degradation kinetics of PDMMLA can be controlled by regulating its hydrophilic/hydrophobic balance [11]. All PDMMLA derivatives studied here have a glass transition temperature ( $T_g$ ) under 37°C and show interesting thermomechanical properties [12]. Also, the presence of chemically alterable functions on PDMMLA's side chain (-COOH) represents a great potential for a covalent grafting of active drugs on the polymer, which cannot be realized on the currently used biopolymers. This allows a well-managed controlled release of drug during the arterial healing process.



**Scheme 1,** a) Poly([R,S]-3,3 dimethylmalic acid) (PDMMLA)  
b) Statistical and amphiphilic PDMMLA copolyester with -COOH and -COOHex groups  
(PDMMLAH<sub>n</sub>-co-Hex<sub>m</sub>)

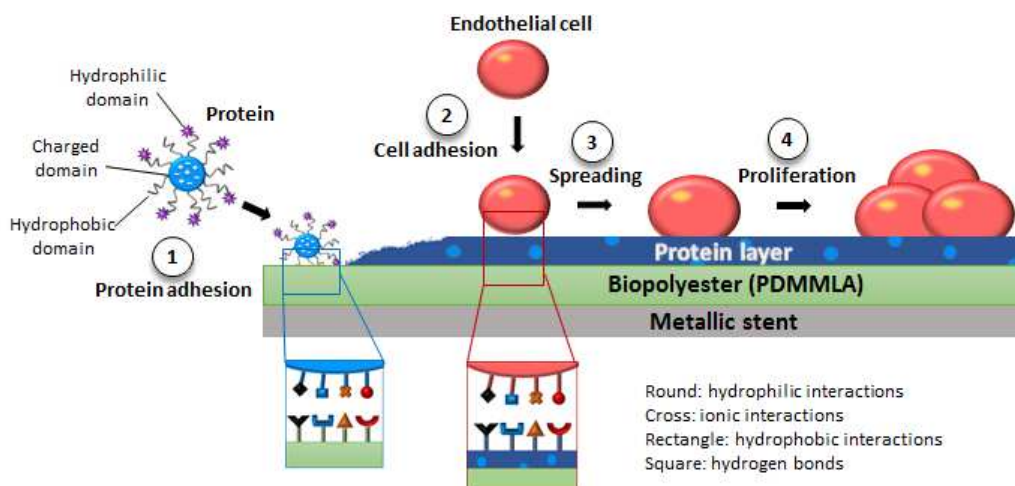
Here, we study three PDMMLA derivatives with different hydrophilic/hydrophobic ratio: 1) PDMMLAH<sub>10-co-Hex</sub><sub>90</sub>, 2) PDMMLAH<sub>20-co-Hex</sub><sub>80</sub>, and 3) PDMMLAH<sub>30-co-Hex</sub><sub>70</sub> named 10/90, 20/80 and 30/70 respectively.

When a biomedical material is implanted in the body, its surface gets covered by a layer of serum or plasma proteins. The adsorbed proteins can then freely reorient and change conformation, affecting the wettability of the material surface, and its surface force and its environment interaction [13]. Understanding surface-protein interactions is important for the prediction of biological answers (surface/cell); these interactions play a central role in cell mechanism regulation (adhesion, spreading and proliferation...) [14, 15, 16]. This surface/cell interaction depends on the physicochemical properties of the polymeric biomaterial surface (such as roughness, wettability and surface charge). Cell attachment is mediated by the adhesion proteins contained in the physiological medium (proteins/cell) [17]. Thus, the ability of materials to adsorb adhesive proteins in a favorable

conformation (surface/protein) will determine their ability to induce cell adhesion and spreading (*surface/protein/cell*), a fundamental aspect in the biocompatibility of surfaces (Fig 1).

The initial adsorption of proteins on a surface occurs very rapidly and avoids direct interactions between cells and the surface [18]. This protein adsorption can be promoted or, on the contrary, inhibited by surface modifications involving the hydration of proteins and surfaces, the redistribution of charges at the proteins interface/conformational changes [19]. The characterization of the adsorption of proteins on different surfaces makes it possible to better understand the physiological phenomena and to guide the cellular response.

DCA is a technique used for the measurement of dynamic wettability changes of a surface allowing to detect the dynamic interfacial and surface configuration changes of a biomaterial [20, 21]. DCA method characterizes the surface/interface changes during the protein adsorption by measuring the surface wetting tension. It also gives information about the proteins conformational changes and the reversibility phenomenon. [22]. Palacio and Bhushan present a schematic demonstration of protein and cell adsorption on surfaces with different characteristics [23].



**Fig 1:** Schematic presentation of protein layer formation on a biomaterial and cell adhesion on the protein layer

Four ECM proteins (Bovine Serum Albumin (BSA), fibronectin (Fn), fibrinogen (Fb), vitronectin (Vn)) are used to carry out the experiments. Then, a very interesting plasma membrane

proteoglycan, syndecan-4 (Syn-4) is studied because of its overexpression during the healing period of a damaged arterial tissue [24]. A mixture of these proteins is also studied in order to compare each protein's individual profile to that of the case where they are all present. This allows to identify the dominating adsorbed protein and its affinity with each polymer surface.

## 2. Experimental section

### 2.1. Polymer synthesis and polymer film preparation

Three statistical PDMMLAs were synthesized through an anionic ring opening polymerization of  $\beta$ -lactone monomers. Two different  $\beta$ -lactones were used in order to obtain the wanted amphiphilic copolymers. (**10/90**:  $M_{n(10/90)} = 148060$  g/mol,  $M_{w(10/90)} = 153800$  g/mol,  $D_{(10/90)} = 1.038$ ; **20/80**:  $M_{n(20/80)} = 214420$  g/mol,  $M_{w(20/80)} = 231800$  g/mol,  $D_{(20/80)} = 1.081$ ; **30/70**:  $M_{n(30/70)} = 354460$  g/mol,  $M_{w(30/70)} = 364800$  g/mol,  $D_{(30/70)} = 1.029$ ) The protocols for the synthesis of the copolymers,  $\beta$ -lactones as well as different characterization techniques are described in the literature [25, 26]. The mean surface roughness values ( $R_a$ ) for each copolymer are:  $R_{a(10/90)} = 0.106$  nm;  $R_{a(20/80)} = 0.184$  nm;  $R_{a(30/70)} = 0.216$  nm [20].

For the preparation of polymer films, glass slides (Objektträger,  $10 \times 13.3$  mm, 0.99 mm thick, MENZEL-GLASSER, Braunschweig, Germany) were cleaned in water, chloroform, and acetone, then, dried at  $37^\circ\text{C}$  in an oven. Polymers were dissolved in a small quantity of acetone in order to obtain a viscous solution. The polymers solution was loaded on both sides of the glass slides with a micropipette ( $n = 3$  for each experiment). The covered slides were dried at room temperature, then at  $37^\circ\text{C}$  overnight in order to obtain visible, smooth and homogeneous films.

### 2.2. Protein solution preparation

All proteins (BSA, Fn, Fb, Vn, and Syn-4) were purchased from Sigma Aldrich and employed as received. All protein solutions were prepared in Phosphate Buffered Saline (PBS), pH = 7.4, at 0.2% of total plasma proteins' concentration, representing the physiological concentration for each protein (BSA at  $86 \mu\text{g/mL}$ , Fb at  $6 \mu\text{g/mL}$ , Fn and Vn at  $0.6 \mu\text{g/mL}$ , and Syn-4 at  $1 \mu\text{g/mL}$ ) [21]. The

“Mix” solution was prepared in accordance with the physiological proportions, by mixing equal volumes of each previously prepared protein solution.

**Bovine Serum Albumin (BSA)** is used as a model protein for albumin. It is a globular protein, with a plasma concentration range of 35 to 50 mg/mL; it is thus the most abundant protein in the plasma. Regulating the oncotic pressure of blood is the main function of this protein [27].

**Fibronectin (Fn)** is a multi-domain glycoprotein. Fn regulates cell adhesion, migration and differentiation [28] and provides structural support for cell survival, gene expression, growth factor signaling, etc [29]. This protein is soluble in a globular form in the plasma (concentration range of 0,2 to 0,4 mg/mL), and insoluble in a fibrillary pattern in the ECM [30].

**Vitronectin (Vn)** is a cell adhesion promoting glycoprotein present in the ECM with a concentration range of 0.25 to 0.45 mg/mL. Although it has a similar activity to Fn in terms of cell attachment and spreading it remains biochemically and immunologically different from the latter [31, 32].

**Fibrinogen (Fb)** is a glycoprotein that cleaved with fibrin, plays an important role in the process of hemostasis and coagulation, which is essential in limiting blood loss and wound healing. It is present in the plasma with a concentration range of 1.5 to 4.5 mg/mL [33].

It is also known that Fb and its breakdown products promote processes like angiogenesis, migration and proliferation of various cells such as fibroblasts, smooth muscle cells (SMCs) and lymphocytes, which are important events in tissue repairing [34].

**Syndecan-4 (Syn-4):** Syndecans are transmembrane heparan sulfate proteoglycans (HSPGs) synthesized by many cells. Syndecans bind extracellular ligands via their covalently attached heparan sulfate chains and influence intracellular events through transmembrane signaling pathways [35]. There are four syndecan family members: syn-1, syn -2, syn -3, and syn -4 [36]. Here, we study Syn-4 thanks to its major role in biological functions such as regulation of blood coagulation, cell adhesion, and cell growth [37]. Studies show that Syn-4 activates flow-dependent atheroprotective pathways by sensing flow direction and promoting endothelial cell alignment [38].

Its presence is also essential in neointimal formation and preventing cardiac rupture after vascular injuries [39, 40]. Studies show that in patients with myocardial infarction (MI), human Syn-4 expression is elevated following MI in repair region of the damaged cardiac tissue [41]. Our biology research team demonstrated that Syn-4 is positively involved in this process of arterial healing [42].

### **2.3. Dynamic protein adsorption by DCA**

Surface wetting forces of PDMMLA derivatives (10/90, 20/80, and 30/70) were registered in the presence of different proteins in order to study their interaction with different polymer surfaces. The experiments were carried out using a Wilhelmy balance tensiometer (K100MK2 from Krüss GmbH), connected to a computer for sorption and contact angle measurement and analysis. The relation between surface tension and force is established by the Wilhelmy method using the following equation:

$$F = \gamma_{lv}.L.\cos\theta$$

where  $F$  is the force in mN,  $\gamma_{lv}$  the surface tension of the liquid,  $L$  the wetted length, and  $\theta$  either the advancing or the receding contact angle [22]. The contact angle hysteresis is determined by calculating the difference between the advancing and receding contact angles. Surface chemistry, morphology and roughness are the main factors affecting thermodynamic hysteresis [14].

To perform the blank experiment, 10 wetting/dewetting cycles of the polymer films were realized in a PBS solution (pH = 7.4). To perform the protein experiments 5 cycles were realized for adsorption (5 wetting/dewetting of the polymer film in the protein solution), followed by 5 cycles for desorption/rinsing (5 wetting/dewetting of the same polymer film in PBS). For all samples, immersion depth and the immersion/emersion rate were set to 10mm and 6mm/min respectively. All experiments were carried out at 37°C.

### **3. Results and discussions**

Three statistical PDMMLA surfaces (10/90, 20/80 and 30/70) were studied in contact with five proteins (BSA, Fn, Fb, Vn and Syn-4). Three kinds of interaction forces were considered in this



study: ionic interactions, hydrogen bond and hydrophobic interactions (Fig1). Ionic and hydrogen bond interactions are dominant in carboxyl groups, while hydrophobic interactions are dominant in the hexylic ester groups of the polymers side chains.

In a parallel work, *in vitro* cell adhesion, spreading and proliferation are performed in order to evaluate the human umbilical vein endothelial cells (HUVEC) behavior on different polymer-based films (10/90, 20/80 and 30/70). First results are very encouraging (results will shortly be published).

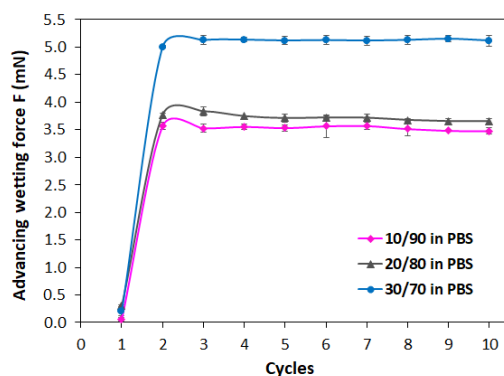
In order to complete this work and explain the obtained results, we study in this work, the protein adhesion on these PDMMLA surfaces using DCA technique with the intention of designating the future bioactive stent coating.

In this work, we are interested in the advancing wetting force, “F” of PDMMLA derivatives in the presence of different proteins, as well as a mixture of all these studied proteins.

### **3.1. Blank experiments**

10 cycles of wetting/dewetting in PBS (no proteins) were realized on copolymers 10/90, 20/80, and 30/70. The measured advancing wetting force values for each polymer are represented as a function of cycle numbers (Fig 2). The increase of a wetting force indicates the increase of hydrophilic character of the surface.

Blank experiments show that F increases with the increase of carboxyl groups on the polymer chain. Copolymers 10/90 and 20/80 reveal a baseline between 3 and 4mN ( $3.18 \pm 0.07\text{mN}$  and  $3.38 \pm 0.04\text{mN}$  respectively). F values are slightly higher for copolymer 20/80 due to the existence of 20% of hydrophilic groups versus 10% in 10/90 ( $p > 0.05$ ). This non-significant increase of F confirms the increase of the hydrophilicity of the surface but is not sufficient to make a considerable difference between these two surfaces.



**Fig 2:** blank experiments of copolymers 10/90, 20/80, and 30/70 in PBS at 37°C (n = 3)

It can be explained that in the case of copolymer 10/90, due to the presence of 90% of hexylic ester groups, the carboxyl functions are hidden and less exposed by self-organization of these hydrophobic side chains.

In contrast, a significant gap is observed between copolymers 20/80 and 30/70 ( $4.62 \pm 0.07$  mN) ( $p < 0.05$ ). This indicates that with 30% of carboxyl functions, the polymer has more opportunities to expose these groups to the interface between the polymer surface and aqueous medium. These chains are further exposed to the surface compared to previous cases. Indeed, the percentage of hydrophilic chains has a significant impact on the chain mobility effect.

### 3.2. Protein adsorption/desorption

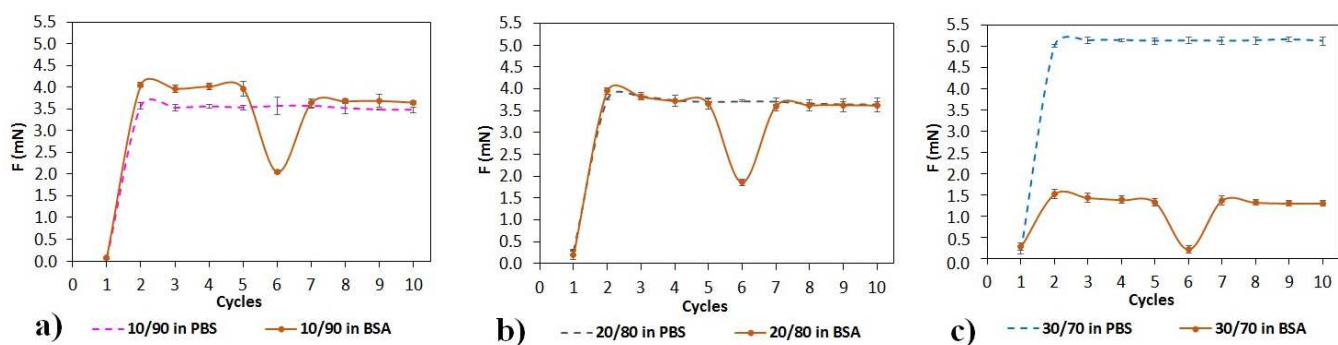
The first 5 cycles of wetting/dewetting are realized in a protein solution (cycles 1 to 5 for adsorption experiment) and the last 5 cycles are realized in a PBS solution (cycles 6 to 10 for rinsing/desorption experiment). Each profile is compared to that of PBS alone obtained with corresponding polymer film. If the adsorption curve is superimposed with the baseline (PBS curve), there is no adhesion. Above the baseline, the protein is adsorbed via hydrophobic interactions, exposing its hydrophilic parts to the surface. If the curve is below the baseline, adsorption takes place via hydrophilic interactions, exposing hydrophobic parts of the protein to the surface. As for the rinsing stage, three cases can be observed: if the rinsing curve is on the baseline, it means that either the protein is desorbed ( $\Delta F = 0$ ), or reoriented ( $\Delta F \neq 0$ ). If the force remains stable with no change, it means that the protein is adsorbed without reorientation or desorption. If the force

changes (increase or decrease), it means that the protein is adsorbed and changes conformation/orientation.

In all studied cases, a very low value of  $F$  is observed for the first immersion cycle (cycle 1 for adsorption and cycle 6 for rinsing). This first cycle determines the surface pressure. The surface pressure decreases with increasing of interfacial tension during the first contact (polymer-air-solution interfacial tension). The stabilizing cycles are then established and form a polymer-solution contact from the second immersion cycle (cycle 2 for adsorption and cycle 7 for rinsing).

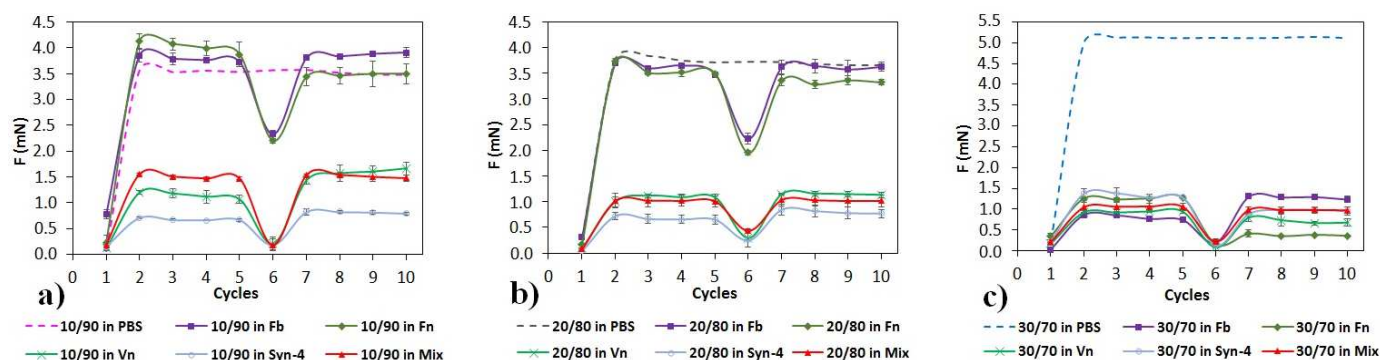
Figure 3 shows the BSA adsorption/desorption profiles on three polymer surfaces. BSA is strongly adhered to the 10/90 surface via hydrophobic interactions at the first contact with the polymer. The surface becomes slightly more hydrophilic during rinsing ( $\Delta F = 0.46 \pm 0.005 \text{ mN}$ ). Adsorbed BSA changes conformation during rinsing, it decreases the hydrophobic interaction but leaves the electrostatic interaction. The surface becomes less hydrophilic compared to adsorption case ( $\Delta F = 0.15 \pm 0.01 \text{ mN}$ ). This is confirmed by the case of 20/80 copolymer containing more hydrophilic groups. The adhesion of BSA takes place by more hydrophilic interactions compared to 10/90, but its adhesion does not change the surface hydrophilicity. The adsorption/desorption interfacial force is very close to that of PBS alone ( $\Delta F = 0.03 \pm 0.07$  and  $-0.05 \pm 0.08 \text{ mN}$ , respectively). Regarding the 30/70, the surface is suitable for making hydrophilic interactions as well as hydrogen bonds and ionic interactions. All adhered BSA remains attached to the surface, and does not change conformation during the rinsing step ( $\Delta F = -3.67 \pm 0.02$  (adsorption) and  $-3.81 \pm 0.01 \text{ mN}$  (rinsing)).

In conclusion, BSA adsorption power increases as hydrophilic character increases, which means that the BSA affinity is reduced by hydrophobic interactions.



**Fig 3:** adsorption and desorption of BSA on a) 10/90, b) 20/80, c) 30/70 copolymer surfaces ( $n = 3$ )

The results of the tests on the other studied proteins are represented in the Figure 4.



**Fig 4:** comparative behavior of different proteins on a) 10/90, b) 20/80, c) 30/70 copolymer surfaces ( $n = 3$ )

According to the experimental results, it is clearly visible that from the copolymer 10/90 (the most hydrophobic surface) to the copolymer 30/70 (the most hydrophilic surface), the surfaces become more and more hydrophobic after the adhesion of different proteins (table 1 and Fig 5). Only the copolymer 30/70 has the surface on which all proteins expose their hydrophobic side after adhesion, meaning that increasing the hydrophilicity of the polymer surface promotes hydrophilic, ionic interactions and hydrogen bond type adhesions. Concerning Vn, which presents a hydrophobic curve for all three surfaces 10/90, 20/80 and 30/70 ( $\Delta F = -2.40 \pm 0.02$ ,  $-2.69 \pm 0.02$  and  $-4.17 \pm 0.01$  mN, respectively), we can say that the latter makes only one type of interaction on all surfaces. Syn-4, which is not a plasma protein, shows a similar behavior on all three copolymers with small difference of F values ( $\Delta F = -2.87 \pm 0.04$ ,  $-3.09 \pm 0.04$  and  $-3.76 \pm 0.002$  mN, respectively).

In addition, using the whole set of studied polymers and proteins, we can see the overall trend between interfacial force and acidity percentage (%A) of polymers. Figure 5 histograms show the variations of proteins profiles on different polymer surfaces during adsorption/desorption cycles and

linear correlation of  $F$  with %A. The slope corresponds to the sensitivity of the correlation and  $R^2$  is the correlation coefficient.  $R^2$  and slope are calculated for the interfacial force  $F$  (the straight line is drawn only from the three points corresponding to the three studied copolymers).

With the exception of membrane protein Syn-4 which gives the same profile as blank (increase of  $F$  with the increase of acidity), the hydrophobicity of surface increases linearly with %A (decrease of  $F$ ) for all studied plasma proteins. Indeed, Vn exhibits the highest sensitivity associated to an excellent  $R^2$  correlation coefficient which is very close to 1 for adsorption ( $R^2 = 0.97$ ) and equal to 1 for desorption. BSA and Fn are also interesting ( $R^2 = 0.81$  and  $0.87$ , respectively) whereas Mix is not well fitting to %A ( $R^2$  far from 1).  $R^2$  of Fb and Syn-4 are close to 0.80.

Then, the “Mix” curve represents the overall behavior of the mixture of all studied proteins, giving information about affinity to the surface and competitiveness between the proteins. As observed with plasma proteins, from the most hydrophobic polymer (10/90) to the most hydrophilic one (30/70), surfaces become more hydrophobic after the adhesion of different proteins (Mix) (Table 1 and Fig 5).

In the case of the copolymers 10/90 and 20/80, the closest curve to that of the “Mix”, is the Vn's, meaning that this protein has an important affinity to these surfaces. It adheres the first when all the studied proteins are present in the medium, making the surface more hydrophobic. Regarding the copolymer 30/70 though, we can see that all of the curves are gathered around the “Mix” curve. This means that in the presence of all of the studied proteins, the surface becomes hydrophobic and all of the proteins show a similar affinity to the surface with poor competitiveness even though the domination of Vn is visible.

The mobility of the carboxyl groups on the 30/70 copolymer surface results in exposing these groups further to the surface, leading to further possible hydrophilic interactions between the surface and the proteins. Furthermore, at neutral pH these groups exist in both carboxyl ( $-\text{COOH}$ ) and carboxylate anion ( $-\text{COO}^-$ ) forms, which favor also the formation of ionic and hydrogen bonds.

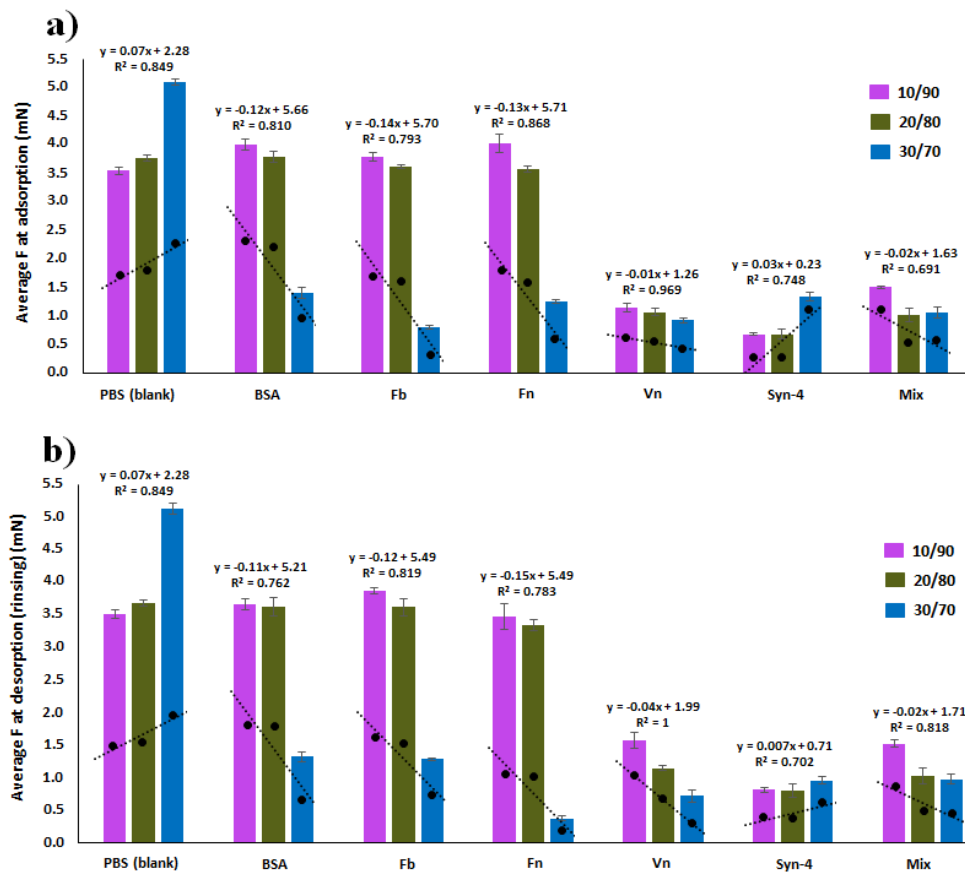
The adhered proteins thus expose their hydrophobic parts to the surface and reduce the hydrophilicity.

**Table 1**

Hydrophilicity of copolymer surfaces compared to the baseline (PBS alone) after protein adhesion.  $\Delta F$  is the difference between F of adsorption/desorption and F of blank.

	$\Delta F$ (mN) 10/90		$\Delta F$ (mN) 20/80		$\Delta F$ (mN) 30/70	
	Adsorption	Rinsing	Adsorption	Rinsing	Adsorption	Rinsing
<b>BSA</b>	++ ( $0.46 \pm 0.005$ )	+ ( $0.15 \pm 0.01$ )	0 ( $0.03 \pm 0.07$ )	0 ( $-0.05 \pm 0.09$ )	---- ( $-3.69 \pm 0.02$ )	---- ( $-3.81 \pm 0.01$ )
<b>Fn</b>	++ ( $0.47 \pm 0.09$ )	0 ( $-0.03 \pm 0.13$ )	- ( $-0.20 \pm 0.02$ )	- ( $-0.34 \pm 0.04$ )	---- ( $-3.85 \pm 0.04$ )	---- ( $-4.76 \pm 0.05$ )
<b>Fb</b>	+ ( $0.24 \pm 0.01$ )	+ ( $0.35 \pm 0.02$ )	- ( $-0.16 \pm 0.04$ )	0 ( $-0.06 \pm 0.08$ )	---- ( $-4.29 \pm 0.04$ )	---- ( $-3.85 \pm 0.06$ )
<b>Vn</b>	--- ( $-2.40 \pm 0.02$ )	-- ( $-1.94 \pm 0.06$ )	--- ( $-2.70 \pm 0.02$ )	--- ( $-2.53 \pm 0.001$ )	---- ( $-4.17 \pm 0.01$ )	---- ( $-4.41 \pm 0.01$ )
<b>Syn-4</b>	---- ( $-2.87 \pm 0.04$ )	---- ( $-2.70 \pm 0.03$ )	---- ( $-3.09 \pm 0.04$ )	--- ( $-2.87 \pm 0.06$ )	---- ( $-3.76 \pm 0.001$ )	---- ( $-4.17 \pm 0.02$ )
<b>Mix</b>	--- ( $-2.04 \pm 0.03$ )	-- ( $-1.99 \pm 0.01$ )	--- ( $-2.75 \pm 0.06$ )	--- ( $-2.65 \pm 0.07$ )	---- ( $-4.04 \pm 0.01$ )	---- ( $-4.15 \pm 0.01$ )

The surface: (+) becomes hydrophilic, (-) becomes hydrophobic, and (0) does not change due to the protein adhesion.



**Fig 5:** protein profile variations on different polymer surfaces during **a)** adsorption, **b)** desorption (rinsing), and linear correlation of F with %A

#### 4. Conclusion

DCA technique was used to measure surface forces between three PDMMLA derivatives (10/90, 20/80, and 30/70) in contact with different protein solutions, allowing to determine different protein adhesion profiles on each of these surfaces. Studying protein adhesion on biomaterial surfaces is important because of its considerable role in inducing cell adhesion on these surfaces.

This work shows that there is a strong correlation between the surface hydrophilicity and protein adhesion on the studied copolymers.

The “Mix” experiment is realized in order to obtain data about the affinity and competitiveness between the different proteins. In the case of copolymer 30/70, all proteins show similar adhesion profiles with low competitiveness. All studied proteins are adhered to the surface via hydrogen bonds, hydrophilic and ionic interactions making the polymer surface more hydrophobic than the starting point.

Vn gives a hydrophobic sorption curve for all three surfaces, meaning that the latter makes only one type of interaction with all surfaces.

Syn-4, a transmembrane plasma protein, shows also similar behavior on all studied surfaces.

First results of parallel work show that the HUVECs are more likely to adhere on more hydrophobic surfaces (i.e. copolymer 10/90) than hydrophilic ones. However, the spreading and growth of these cells might demand a moderately hydrophilic surface (i.e. copolymer 30/70) (results will shortly be published). On the other hand, study shows that even on hydrophobic surfaces, there is a chance for the endothelial cells to grow constantly in a time-dependent manner [13]. Therefore, to validate the choice of the customized polymer 30/70, further study is needed to determine cell attachment, spreading, proliferation and growth on the surfaces covered by the protein layer, in the presence and absence of growth factors and other parameters affecting cell growth. These experiments will shortly be carried out and the results will be communicated in a next publication.

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