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Molecular mobility of polymeric implants and acute inflammatory response: An experimental study in mice

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Abstract An overlooked factor in biomaterial research is the surface molecular flexibility for polymer based implants. The mobility of the polymer chains provides a way for the surface to adapt itself to the environment. This is relevant when the implant comes in contact with a biological fluid and its constituents. By changing the length of the alkyl side chain of poly(alkyl methacrylates) (PAMAs) an interesting opportunity is provided where it is possible to study the surface molecular mobility without changing the surface hydrophobicity, nor does it introduce any additives or any changes in the degree of polymer cross-linking. Four variants of PAMAs were implanted in the peritoneum of Balb/c mice using a well described setup. End points were taken after 18 h and estimations of inflammatory cell recruitment and implant-associated cells were studied. Relationship between surface molecular mobility and inflammatory cell recruitment as well as surface-associated cells was noted.

1 Introduction

The molecular mobility of polymer based implants seems to have important implications in the biological outcome. The common way of measuring the molecular flexibility is the glass transition temperature (Tg). This is usually measured in the bulk but it serves as a good approximation of surface mobility. Surface molecular mobility is dependant on the advancing and the receding contact angle and the ambient temperature. The biomaterial relevance of this is that the polymer will not only rearrange itself to reach low contact energy with the water, it will also, if flexible enough, accommodate to the adsorbed proteins.

In this study we are using a series of poly(alkyl methacrylates) (PAMAs) which gives an interesting opportunity to study the effect of surface molecular flexibility. By varying the different side chain in length and in structure, it is possible to varying the surface molecular flexibility with only minor variations in the advancing contact angle. A great advantage with this system is that it does not require cross-linking or any additives. The limitation is that the internal difference between the polymers is in a close range and a large sample size is needed to obtain significant differences. This polymer setup has previously been used in an *in vitro* system [1] to study the complement activation and surface induced plasma coagulation [2]. We found a linear relationship between the polymer stickiness (high hysteresis) and the biological outcome. The complement activation was smaller on the stickier polymer surfaces than on the harder (low hysteresis) ones. Interestingly, the opposite was found when studying the coagulation onset. This time the stickier surfaces induced coagulation faster than the harder polymer surfaces. Since the surface adsorbed protein layer and the early bio-interface reactions are important for the cellular response [3, 4], it is relevant to study the effect of surface molecular flexibility in an acute *in vivo* model. In this study we are aiming at building a model for understanding the foreign body response to polymeric implants.

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2 Materials and methods

2.1 Surface preparation and surface characterization

The PAMA substrates was coated on thin circular aluminium discs, $\varnothing = 10$ mm. The discs were carefully polished in order to remove any sharp edges. After that, the discs were cleaned in ultrasonic bath with milli-Q and EtOH (99%). The surfaces were also UV-ozone treated and finally rinsed thoroughly in milli-Q. Four types of PAMA coatings were used; poly(isobutyl methacrylate) (PIBMA), (Sigma-Aldrich, cas# 9011-15-8), poly(butyl methacrylate) (PBMA), (was made in-house by radical polymerisation, monomer purchased from Fluka cas# 96-05-9), poly(hexyl methacrylate) (PHMA), (Scientific Polymer Products, cas# 25087-17-6) and poly(lauryl methacrylate) (PLMA), (Sigma-Aldrich, cas# 25719-52-2). PAMA coating of the surfaces were done by dip-coating the discs in the corresponding polymer solutions (1% w/v in toluene) twice followed by ventilation in vacuum. Advancing and receding contact angle was determined with the Wilhelmy plate method [5] using de-ionized water ($\gamma = 72{,}8$ mJ/m^2) at 37°C. The instrument used was a DCA-322 from Cahn operating at 100 μm/s. Data was evaluated with WinDCA version 1.03 (Cahn, WI, USA).

All surfaces was tested with regard to hydrophobicity by using sessile water drops [6] to ensure complete coating coverage. All surfaces were carefully cleaned in alcohol and water before implantation.

2.2 Animal model

The animal model has earlier been used for hard surfaces that has been coated with inflammatory relevant proteins [3]. In this investigation, we did not pre-coat the surfaces with any protein. Instead, we focused on the inflammation caused by the protein layer adsorbed to the different materials found in the intra-peritoneal cavity. Twenty Balb/c mice from the same batch with a body weight of 20–25 g were given two intraperitoneal implants (one disc at each side). The values shown in all graphs represent the results of single implantation experiments using five animals per treatment. The animals where sacrificed 18 h after implantation which is sufficient time to obtain an inflammatory response in the peritoneum [7]. The implants were harvested and the peritoneal cavity was irrigated with phosphate buffered saline (PBS, 4 ml, pH 7.3). The cell rich lavage was collected for enzyme activity evaluation.

2.3 Measurement of enzyme activities and inflammatory cell estimations

2.3.1 Estimation of surface-associated cells

Cytosolic and granular enzymes were released from surface associated cells by incubation of each disc with 0.5 ml of 1.0% Triton X-100 for one hour. Activities of myeloperoxidase (MPO) and non-specific esterase (NSE) in the triton X-100 solution were used to estimate the number of surface-associated polymorphonuclear neutrophils (PMNs) and monocytes/macrophages (M∅), respectively. More than 95% of implant associated peroxidase activities represent MPO, total peroxidase activity was taken as a measure of surface-associated PMN cells. MPO was measured spectrophotometrically at 470 nm with guaiacol as substrate [8]. Control studies on purified mouse PMN is about 23 nano units/cell [3]. NSE is relatively restricted to M∅, and the activity of this enzyme was measured to assess the number of adherent cells [9]. NSE activity was determined by following the rate of hydrolysis of *o*-nitrophenyl butyrate [10] in the presence of eserine (10 mM), which will eliminate possible interference by cholinesterase [11]. Enzyme assays on mouse resident peritoneal M∅ is about 11 nano units/cell [3].

2.3.2 Estimation of non-bound phagocytes

The cell rich lavage that was collected during the explantation procedure was concentrated by centrifugation ($900 \times g$ for 10 min). The cell pellet was incubated with 0.5 ml of 1.0% Triton X-100 solution for one hour to release cytosolic and granular enzymes. Previously described enzyme assays were conducted to estimate the number of non-bound phagocytes in the peritoneal cavity.

3 Results

3.1 Surface characterization

Results from the Wilhelmy plate measurements are displayed in Table 1 together with the glass transition temperature which is comparable to the hydrophobic hysteresis. PIBMA has a lower hysteresis due to the iso-conformation which limits the molecular mobility.

3.2 Enzyme activity and cell estimation

The PMN cell recruitment and surface-association was estimated from the MPO enzyme activity. The results are

Table 1 General polymer characteristic in relation to biological effect. The first column contains data of the acronum of the polymers and the number of carbon atoms in the alkyl group. The name of the substituting alkyl groups is given in the second column. The glass transition temperature (Tg) is given in the third column. The forth and fifth column contains data wettability data obtained by the Wilhelmy plate method

Polymer (n carbons in alkyl side chain)	Alkyl group	Glass transition temperature (°C)	Advancing contact angle (°)	Receding contact angle (°)
PIBMA (4)	Isobutyl	66	94,1	78,4
PBMA (4)	Butyl	17	94,5	72,3
PHMA (6)	Hexyl	− 6	100,1	75,0
PLMA (12)	Lauryl	−70	106,6	72,0

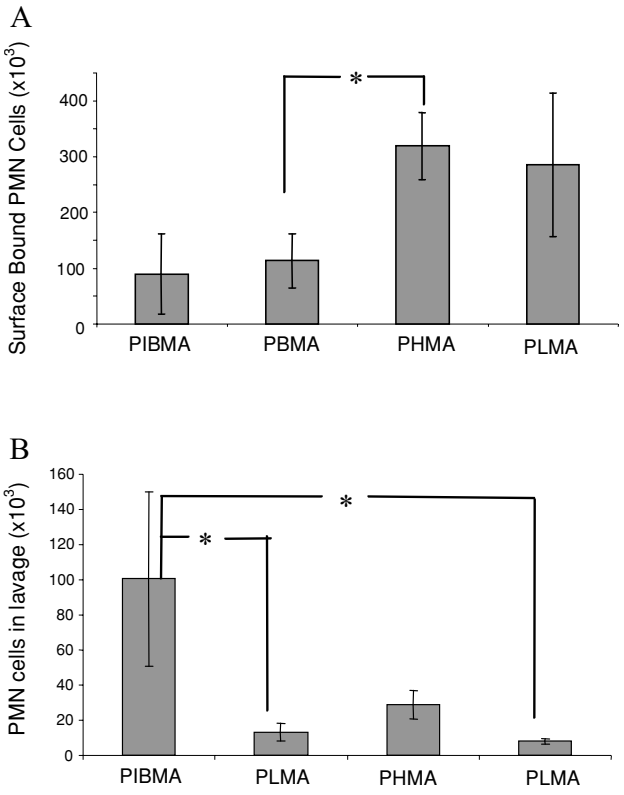

Fig. 1 The PMN cell number on PAMA substrates estimated from the MPO enzyme activity. Number of PMN's found associated to the surface (A) and in the lavage (B). Figure (A) shows that the degree of cell association is higher on the more sticky surfaces (PLMA and PHMA), which correlates well with the total cell recruitment in figure, although the effect is not as prominent. However, there is a difference in cell association that is connected to the surface molecular flexibility. * marks statistic significance ($p < 0.05$)

displayed in Fig. 1. There is a clear change in the amount of cells that has been associated to the protein layer (Fig. 1(A)). The Sticky PLMA together with its closest relative PHMA shows a much higher degree of cell association than PIBMA and PBMA. The effect can also be seen in the total cell

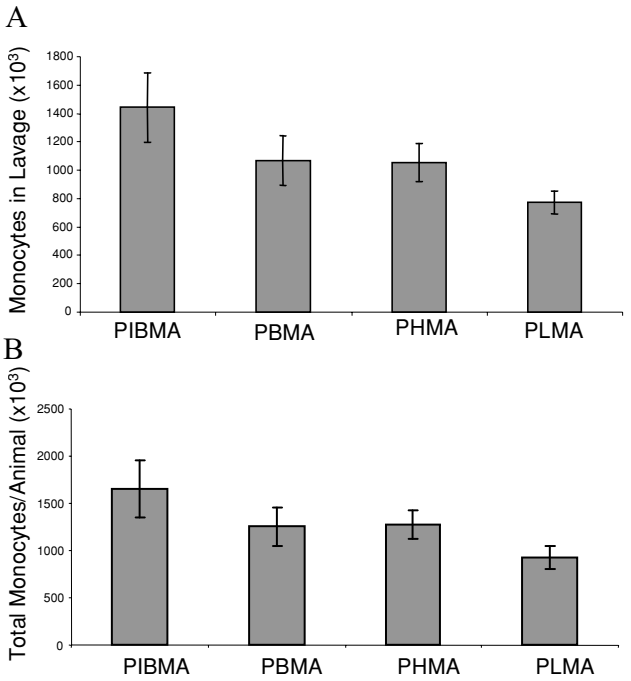

Fig. 2 Number of monocytes found in the lavage (A) and total number of cells present (B) as estimated from the NSE activity. Although no significant difference could be found, there is a noticeable trend that the hard non-flexible surfaces recruited more monocytes. PLMA has about 40% less monocytes present compared to PIBMA

recruitment (Fig. 1(B)), although not as prominent. Figure 1 shows that there is a difference in cell association that is connected to the surface molecular flexibility.

The amount of M∅ that was estimated from the NSE activity is presented in Fig. 2. The differences were not significant but the results from the four polymers show a noticeable trend. It appears as if the hard non-flexible surfaces recruited more monocytes. In that respect it could be said that less molecular flexibility are more pro-inflammatory. The total cell number of both cell types (recruited and adhered) did not differ that much between the PAMA substrates and no trend is seen (Fig. 3(A)). However, the fraction of bound cells from the total cell number shows a plateau-like distribution (Fig. 3(B)). The percentage of surface associated cells is much lower on PIBMA (approximately 18%) compared to PLMA (about 35%).

4 Discussion

As seen in Fig. 1, the PMN cells adhere to the PAMA surfaces in different degrees. The stickier PLMA and PHMA has much more cells adhered to it than the harder polymers. Since all implants has a protein layer adsorbed on it and the cells are attached to the proteins and not directly on the polymer, it can be ruled out that the polymer stickiness itself governs the cell adhesion. It is most likely a question of the conformation

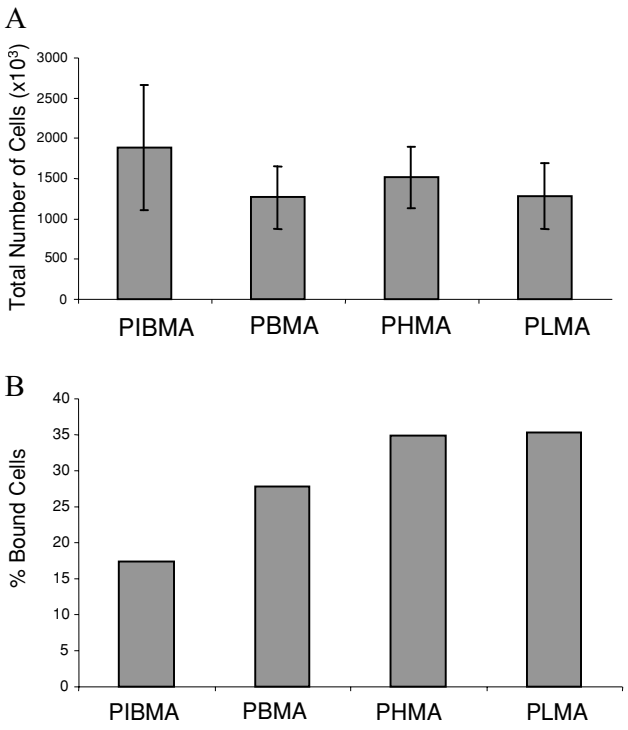

Fig. 3 No difference can be found for the total cell number for each polymer (A). Cell bound fraction (%) of total cell number (B). The percentage of surface associated cells is much lower on the hard non flexible PIBMA (∼18%) compared to the sticky PLMA (∼35%)

or surface concentration of protein that defines if the cells are adhering or not. In the case of the stickier polymers (PHMA and PLMA), it has previously been shown that they has a much faster coagulation onset and a more rapid fibrin deposition rate than PIBMA and PBMA. If this has anything to do with the cell association to the surfaces is not yet known. However, the importance of fibrin(ogen) being present on the surface is well known and phagocytes can bind to the P1 and P2 epitopes on the D-domain of fibrin(ogen) via the integrin Mac-1 (CD11b/CD18) [12–14].

Another comparison that can be made with the previous study [2] is that the complement activation is significantly lower on the stickier surfaces. The lower amount of chemotactic peptides C3a and C5a appears to have resulted in a lower recruitment of M∅ as seen in Fig. 2. This can however be disputed since they all showed relatively low complement activation compared to previous studies made on other surfaces [1, 15].

The percentage of total number of adhered cells can be seen in Fig. 3(B). It seems as the trend is reaching a plateau. This could mean that the stickier surfaces are saturated with cells.

5 Conclusion

We conclude that surface molecular flexibility has an impact on the acute phase of foreign body reaction. Further studies will focus on long term effects of molecular flexibility.

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