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Surface charge-bias impact of amine-contained pseudozwitterionic biointerfaces on the human blood compatibility

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ABSTRACT

This work discusses the impact of the charge bias and the hydrophilicity on the human blood compatibility of pseudozwitterionic biomaterial gels. Four series of hydrogels were prepared, all containing negatively-charged 3-sulfopropyl methacrylate (SA), and either acrylamide, *N*-isopropylacrylamide, 2-dimethylaminoethyl methacrylate (DMAEMA) or [2-(methacryloyloxy)ethyl]trimethylammonium (TMA), to form S_nA_m, S_nN_m, S_nD_m or S_nT_m hydrogels, respectively. An XPS analysis proved that the polymerization was well controlled from the initial monomer ratios. All gels present high surface hydrophilicity, but varying bulk hydration, depending on the nature/content of the comonomer, and on the immersion medium. The most negative interfaces (pure SA, S7A3, S5A5) showed significant fibrinogen adsorption, ascribed to the interactions of the αC domains of the protein with the gels, then correlated to considerable platelet adhesion; but low leukocyte/erythrocyte attachments were measured. Positive gels (excess of DMAEMA or TMA) are not hemocompatible. They mediate protein adsorption and the adhesion of human blood cells, through electrostatic attractive interactions. The neutral interfaces (zeta potential between −10 mV and +10 mV) are blood-inert only if they present a high surface and bulk hydrophilicity. Overall, this study presents a map of the hemocompatible behavior of hydrogels as a function of their surface charge-bias, essential to the design of blood-contacting devices.

1. Introduction

The design of bio-inert or blood-inert interfaces is one of the important keys to the development of biomaterials for medical applications, because interactions with proteins, cells or bacteria can readily lead to irreversible biofouling, a major obstacle to in-vivo applications. Hydrogels interfaces are one of the preferred biomaterials for the design of biofouling-resistant medical devices [1,2], because a key factor to their usage is their surface hydrophilicity. Therefore, literature on the design of biofouling-resistant hydrogels is rich [3–8].

It is well established that the hydrophilicity of the polymer network is an important parameter, and that it does not warrant the absence of interactions with biological fluids. The second important design parameter to consider is the electroneutrality of the material interface [9,10]. For these reasons, poly(ethylene glycol)-based materials, on the one hand, and zwitterionic materials, on the other hand, have long been at the center of molecular designs for nonfouling interfaces. Based on these principles, ideal gels for the design of hemocompatible devices could be poly(EGMA) [11–13] or poly(SBMA) derivatives [14–16]. Acrylamide [17], *N*-isopropylacrylamide (NIPAAm) [18], and 2-dimethylaminoethyl methacrylate (DMAEMA) [19] associated to other polymers in a single network are also interesting biomaterials for medical applications. These amine functional groups have been associated with other polymers or materials, and their versatility led to finding uses in tissue engineering [20,21], drug delivery [22,23], or the design of biosensors [24].

Ten years ago, some biomaterials have been presented, able to mimic zwitterionic systems: the mixed-charge polymers and networks [25–27]. These are interesting materials because a fine tuning of the monomer ratio in the initial polymerization mixture can enable the control of the surface charge, from negative bias to positive bias. However, very little is known on their blood compatibility. From the few reports of literature, it can be expected

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that if the surface charge is controlled around electroneutrality, they should resist blood cells adhesion, because they are able to efficiently resist fibrinogen through the formation of a dynamic hydrated surface [6], which, according to Andrade et al.'s study on poly(ethylene oxide) materials, is critical to blood compatibility [28]. But it has yet to be proven and supported by experimental data on such mixed-charge systems. Another striking gap in the current literature concerns the effect of the surface charge bias (especially negative-bias) on human blood compatibility. As fibrinogen is the protein mediating platelet adsorption and aggregation [29], and given its overall negative charge at physiological pH, positively charged surfaces should be logically avoided in view of improving the hemocompatibility of a material. However, the question of fibrinogen and cells behavior when contacted with negatively-charged surface remains unclear and much less investigated. In particular, one could expect a hydrophilic and negative interface to be bio-inert toward blood components, but reports on this aspect are rare.

The overall lack of knowledge of the charge bias of hydrogel interfaces on their blood compatibility was the starting point of the present study. In this work, we have combined negatively-charged material of 3-sulfopropyl methacrylate with different amide/amine functional groups. We used either acrylamide, NIPAAm, DMAEMA or [2-(methacryloyloxy)ethyl]trimethylammonium (TMA) to form poly(SA-*co*-acrylamide), poly(SA-*co*-NIPAAm), poly(SA-*co*-DMAEMA) or poly(SA-*co*-TMA) hydrogels (Scheme 1). We intended to control the surface charge and design negatively charged, neutral and positively charged biomaterials. Most of these polymer networks are unique, to the best of our knowledge, such that the first part of this study will be dedicated to their essential characterization. An important aspect on which we will focus is the surface charge, which we intend to modulate through the nature and the content of the amine/amide functional group in the material. The second major function of this study is to investigate the effect of charge bias, both positive and negative, on the blood compatibility of the 4 series of hydrogels, using fibrinogen adsorption tests, and human blood cells (thrombocytes, erythrocytes, leukocytes or whole blood) attachment tests. During this investigation, we will also evaluate the efficiency of mixed-charge materials as hemocompatible biomaterials, as there are few reports on this, as above mentioned.

2. Materials and methods

2.1. Materials

Acrylamide (Mw: 71.08 g/mol) and diiodomethane 99% (Mw: 257.84 g/mol) were purchased from Alfa Aesar Co. *N*-isopropylacrylamide (NIPAAm. Mw: 113.16 g/mol), 2-(dimethylaminoethyl) methacrylate (DMAEMA, Mw: 157.21 g/mol) and glutaraldehyde (25%, solution in water) were bought from ACROS Co. 3-sulfopropyl methacrylate potassium salt (SA, Mw: 246.32 g/mol),[2-(methacryloyloxy)ethyl]trimethylammonium chloride and phosphate- buffered saline (PBS buffer), bovine serum albumin (BSA), lysozyme from chicken egg white (LY) and fibrinogen (FN) were purchased from Sigma Chemical Co. *N*,*N*'-methylene bisacrylamide (NMBA, Mw: 154.17 g/mol), ammonium peroxodisulfate (APS, Mw: 228.2 g/mol) and *N*,*N*,*N*',*N*' tetramethylethylenediamine (TEMED, Mw: 116.21) were bought from Aldrich Co. Blood, stored in blood bags using citrate phosphate dextrose adenine (CPDA-1), was obtained from a pool of healthy donors and provided by the Taipei blood center (Beitou dist., Taipei City 112, Taiwan). Deionized water (minimum resistivity of 18 MΩ cm) was obtained from a Millipore purification system.

2.2. Preparation of hydrogels

For all gels, the total solid content – monomer, initiator (APS), cross-linker (NMBA) and catalyst (TEMED) – was 20 wt%. The monomer mixture:APS:TEMED:NMBA wt% ratio was set to 90:1:1:10. S_mA_n and S_mD_n gels were prepared as follows. The monomer mixture (which molar ratio is given in Table 1) was first dissolved in DI water. After 10 min stirring at 300 rpm and 25 °C, NMBA was added to the mixture. The blend was stirred for another 5 min, and the reaction vessel immediately transferred into an ice bath. After 5 min, APS was added, followed by TEMED, 3 min later. 10 s after adding TEMED, a given amount of polymeric solution was poured within two microscope slides separated by a silicon spacer such that the final thickness of hydrogels was 1 mm. The polymeric system was allowed to rest at 25 °C for 1 h to complete gelation. Concerning S_mN_n and S_mT_n gels, a similar procedure was followed except that all steps were performed at 25 °C. Thus, APS was added to the monomer mixture containing NMBA after 10 min stirring at 25 °C. In addition, TEMED was added after another 10 min. The final hydrogel disks were punched from the rectangular shape hydrogel obtained, and stored in DI water at 4 °C until use.

2.3. Characterization of hydrogels

The surface and bulk hydrophilicity of the 4 series of hydrogels were characterized by determining their diiodomethane contact angle and swelling ratio, respectively. For the measurement of diiodomethane contact angle, hydrogel disks (1-cm-diameter) were immersed in a bath of DI water, and a 4-μL-diiodomethane droplet was dropped on the surface of the hydrogels. The contact angle at equilibrium was measured using an automatic contact angle meter (Kyowa Interface Science Co.). In this work, the values reported for contact angle correspond to the average of 7 independent measurements. The swelling ratio was evaluated in DI water or in PBS. Regardless the nature of the immersion bath, gels (1-cm-diameter) were weighed and then immersed in the bath for 24 h. Subsequently, superficial liquid was wiped out with tissue and gels weighed again. The swelling ratio was evaluated from the knowledge of the wet weight and the dry wet. For each type of gel, 5 independent measurements were done, and the average obtained correspond to the swelling ratio value reported in this study. The surface chemistry of hydrogels was analyzed by two techniques, either Fourier infrared spectroscopy (FTIR) or X-ray photoelectron spectroscopy. These are two standard techniques for analyzing the surface chemistry of polymer interfaces, and detailed protocols have been reported elsewhere [12]. The zeta potential of the gels was measured by a zeta-potential meter. Measurements were performed at pH 7.4, which corresponds to physiological pH. The hydrogel disks were positioned in the adjustable cell of the zeta-potential meter instrument (SurPASS^{TM} instrument). Both the instrument and the hydrogels were then infiltrated with a PBS solution (0.162 M) freshly prepared. Then the zeta potential of the materials considered could be measured.

2.4. Biofouling tests

The adsorption of three different proteins, BSA, LY and FN was studied. The method used for both BSA and LY is as follows. Hydrogels were first punched into disks of 1-cm-diameter. 3 replicates of each hydrogel of the 4 series were placed in 24-well plates, and incubated with ethanol for 30 min. Then, the alcohol was replaced by PBS. After a 2-h-incubation in PBS at room temperature (about 25 °C), PBS was removed and replaced by a BSA or LY aqueous solution (1 mg mL^{-1}). Incubation of the hydrogels with the protein solution at play lasted for 2 h and was also performed at room temperature. Following this step, the absorbance of the protein solution