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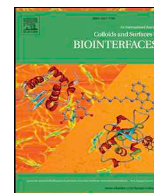
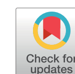


Selected aspects of the state of the art in biomaterials for cardiovascular applications



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ABSTRACT

A review of selected aspects of biomaterials used for cardiovascular applications is presented in honor of the long-term editorship of John Brash of the journal Colloids and Surfaces B: Biointerfaces. The topics to be discussed include the following: 1. Hemostasis, a high barrier to the use of biomaterials in the cardiovascular system; 2. Newer fundamental studies of protein interactions with surfaces; 3. Recent research on protein resistant materials; 4. Clinical application of nonfouling polymers; 5. A brief comment on "superhydrophobic" surfaces; 6. A short history of my many interactions with John Brash. The review topics were chosen on the basis of interest to the author as well as relevance to the research interests of John Brash, and on each topic chosen only a few representative articles are reviewed here.

1. Introduction

A short review of selected aspects of biomaterials used for cardiovascular applications is presented in honor of the long-term editorship of John Brash of the journal Colloids and Surfaces B: Biointerfaces. The topics I have chosen to include here are as follows: 1. Hemostasis, a high barrier to the use of biomaterials in the cardiovascular system. 2. Newer fundamental studies of protein interactions with surfaces. 3. Recent research on protein resistant materials. 4. Clinical application of nonfouling polymers. 5. A brief comment on "superhydrophobic" surfaces. 6. A short history of my many interactions with John Brash. The review topics were chosen on the basis of interest to the author as well as relevance to the research interests of John Brash, and on each topic chosen only a few representative articles are reviewed here.

2. Hemostasis, a high barrier to the use of biomaterials in the cardiovascular system

The many interacting molecular and cellular systems that underlie hemostasis in humans are highly effective in limiting blood loss even after large wounds occur and are fundamental to life. [1] One of the most important of these systems involves the platelet, aptly called the thrombocyte in the older literature. While the platelets normally circulate in the blood as individual cells in a resting state, they are readily activated by a variety of soluble and insoluble biochemical signals. So, for example, they are activated by interaction with von Willebrand's factor (vWf) bound to the subendothelial matrix, as well as ADP released from other platelets or lysed red cells. Exposure of subendothelial collagenous matrices due to injury and the interaction of platelets with vWf bound to the collagenous matrix cause platelet adhesion and activation even at high shear rates. [2] A major aspect of platelet activation is the conversion of the integrin receptor GP IIb/IIIa in the platelet membrane from a resting state to the active state, due to a large changes in the conformation of the receptor subunits that unmask the ligand binding site [3]. Once activated, platelets are able to bind to soluble fibrinogen. The fibrinogen molecule has platelet binding sites at each end of an elongated structure, so it can bind to two platelets simultaneously with little steric hindrance. The presence of multiple fibrinogen receptors on each platelet allows a platelet to bind to many other platelets via fibrinogen bridging, so platelet aggregates form readily. The activated platelets also release the soluble factors ADP and thromboxane to stimulate nearby suspended platelets that can then participate in the formation of even larger platelet aggregates, often called platelet plugs. The plugs seal off injured arteries and limit blood loss, even when large pressure gradients exist that would otherwise lead to rapid exsanguination and death. Fibrin fibers stabilize the ensuing aggregates at the wound site. Biochemical signal release by stimulated platelets is an amplification system to recruit more platelets to the forming plug. Activated platelet membranes are also powerful promoters of fibrin formation via their catalytic conversion of prothrombin to thrombin by formation of the Xa/Va prothrombinase complex with far higher ability to promote thrombin formation than the same factors in solution. Thus for example, the adhesion of platelets to biomaterials makes them highly procoagulant as they can form the prothrombinase complex [4]. Lastly, it should be noted that all of these hemostatic systems are continuously ready to go, so that there is a fine line between liquid blood and clot formation.

The introduction of a foreign material into contact with blood thus risks disturbing these finely controlled systems, and so it is not surprising that clotting is a major problem when devices are used in

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contact with blood, and that it is necessary to intervene with an anticoagulant to reduce the ability of blood to clot. Thus, for example, it is well known that even low levels of fibrinogen adsorption to biomaterials from blood plasma makes the biomaterial platelet adhesive [5] [6]. For a long time, use of permanent cardiovascular implants was largely impossible, but the advent of newer and safer antiplatelet drugs has allowed the use of permanent stents and vascular grafts for those with vessel disease, and even years long connection to left ventricular assist devices (LVADs) in people with failing hearts [7,8]. Finding blood compatible biomaterials was always a very difficult problem and currently there is a fair amount of pessimism about the prospects for more progress [9]. However, as discussed later, many polymeric biomaterials have been developed that display reduced interactions with blood. A few of the earlier types of those materials (poly 2-methoxyethyl acrylate (PMEA), and copolymers with poly methacryloyl phosphatidyl choline (PMPC)) are already used in devices that are widely used clinically, as reviewed later. Thus, some progress has occurred, and it seems very likely that new generations of biomaterials with greatly reduced interactions with blood will also eventually reach clinical practice and have major beneficial effects.

A final note in regard to hemostasis and the use of cardiovascular devices is that the tendency for clotting to be initiated by blood interactions with the biomaterial is not the only remaining challenge. Thus, while closing off of the flow through devices such as vascular grafts or stents due to clotting is a big problem, as is the shedding of thromboemboli from the device that can cause severe damage to downstream organs, including stroke from emboli accumulation in the brain [10], paradoxically, the use of devices is also accompanied by increased risk of bleeding events. Thus, for example, the anticoagulant therapy commonly used to prevent clotting in stents and other cardiovascular devices is accompanied by a risk of bleeding that requires careful management and has prompted extensive studies to determine the best anticoagulant regime [11]. In the case of large devices that actively pump the blood, such as left ventricular assist devices, gastrointestinal bleeding is a substantial risk, because the shear generated by the pumps destroys vWf multimers that are essential components of hemostasis in high shear regimes [12]. As reviewed recently, the shear stress in some LVADs may exceed physiologic values by one to two orders of magnitude, which is a source of blood trauma leading to bleeding including vWf degradation, and platelet activation and hemolysis that both contribute to thrombosis as reviewed recently [13]. This same review provides a good summary of the state of the art in devices, as well as a description of some next generation circulatory support devices that are still early in their development and evaluation. The new devices may prove to be more hemocompatible due to novel pumping systems that can greatly reduce exposure of the blood to high shear stresses.

3. Newer fundamental studies of protein interactions with surfaces

Protein adsorption to biomaterial surfaces occurs very rapidly after exposure to blood and is widely accepted to be the major mechanism by which surfaces become reactive to blood. Thus, for example, platelets are believed to interact with biomaterials because of adsorbed proteins, particularly fibrinogen and vWf. Even very small amounts of adsorption of these proteins is sufficient to trigger platelet adhesion and activation, as discussed in a recent review by the author. [14] For a good overview of the state of the art on protein adsorption and blood compatibility, I recommend the recent publication by Brash, myself, Latour, and Tengvall [6]. The importance of protein adsorption to blood clotting on biomaterials has thus prompted many studies of protein adsorption and its importance to blood clotting, far too many to review here. So, I have chosen to review a few recent proteins adsorption articles of a more fundamental type here, while also reviewing others more directly related to use of biomaterials in blood in several other sections in this minireview.

Wang et al. studied the mechanisms of resistance to protein adsorption using surfaces made from block copolymers that consisted of islands of protein adhesive nanostructures on a background of non-adhesive polymers. [15] For example, the authors spin coated surfaces with mixtures of polystyrene/polyHEMA copolymer (PS_{60}-b-$PHEMA_{150}$) with various amounts of polystyrene (PS), which resulted in islands of PS that were shown by AFM to increase from 43 to 280 nm in diameter as the fraction of PS was increased from 1 to 20 %. Surface plasmon resonance was used to measure adsorption kinetics, and fluorescence microscopy was used to track the movement of individual protein molecules of fluorescently tagged fibrinogen or albumin or myoglobin. It was found that proteins did not adsorb until the adhesive domain size was much larger than the protein (tens to hundreds of times the size of the protein). That is, the weakly adsorbed proteins continued to move along the surface, exhibiting two-dimensional diffusivity, because the residence time of the protein in the adhesive domains was too short to allow for multiple bond formation necessary for irreversible adsorption. The proteins land at the surface, exhibit surface diffusion, and either detach or become permanently parked on the surface. These authors created similar adhesive island/non-adhesive background structures using other polymer systems and found similar protein behavior. The other polymer systems were 1) multisegmented polyurethane (p(B-PEG-U), containing a hydrophobic betulinyl entity B, a hydrophilic PEG block, and urethane U mixed with polyurethane (PU) polymer; 2) poly vinyl pyridine mixtures with polystyrene; and 3) polydimethylsiloxane mixtures with polystyrene.

Another fundamental study of protein behavior on protein repellant surfaces was reported by Wu et al. [16] These workers studied the interactions of soluble PEG and zwitterionic polymers with model proteins (bovine serum albumin and lysozyme) to better understand the basic nature of protein interactions with these types of polymers. They used several spectral methods to determine whether the proteins were affected by the co-dissolved polymers. Intrinsic fluorescence measurements in a spectral region dominated by the protein's tryptophan residues was measured as a function of time and polymer concentration. The authors note that tryptophan fluorescence depends not only on the exposure or accessibility of the tryptophan residues in the proteins to the solvent, but also on the local protein environment immediately surrounding the tryptophan. Since the hydrophobic side chain of this amino acid is often buried in the protein interior, changes in its fluorescence are a signal of changes in protein structure that expose tryptophan to solvent. There was no significant perturbation of the tryptophan fluorescence in the presence of the zwitterionic polymer poly (sulfobetaine methacrylate) (pSBMA) whereas tryptophan fluorescence increased by up to 15 % when PEG was added to BSA solutions and up to 5% when PEG (up to 16 times the protein mass) was added to lysozyme solutions. Another probe was the binding of 1-anilino 8-naphthalene sulfonic acid (ANS), measured with fluorescence. The fluorescence of ANS is increased and its spectra changes when bound to protein hydrophobic surfaces. As with the tryptophan intrinsic fluorescence, the addition of pSBMA to BSA solutions had no effect on ANS fluorescence whereas PEG addition increased ANS fluorescence in proportion to the PEG/protein ratio. The authors also showed that chemical shifts measured with ^{1}H-NMR were caused by PEG addition to the protein solutions whereas pSBMA addition did not cause shifts. These various spectral methods thus showed the existence of weak hydrophobic interactions between PEG and proteins, while there were no detectable interactions between the zwitterionic material (poly (sulfobetaine methacrylate)) and proteins. These fundamental studies thus seem to explain the greater protein repellency observed for materials coated with zwitterionic polymers in comparison to PEG polymers, namely that the binding of water to the ether linkages in PEG only weakens but does not eliminate the ability of the protein to interact with alkyl groups between the ether linkages.

4. Recent research on protein resistant materials

The development of biomaterials that are resistant to protein adsorption has been a major research focus of many biomaterial scientists for some time now and as a result several reviews of this topic have appeared previously [6,14,17,18]. Thus, I chose to review only a few more recent publications on protein resistant materials here.

The interaction of proteins and platelets with poly(ω-methoxyalkyl acrylates), including poly 2-methoxyethyl acrylate or PMEA, has been extensively studied by Tanaka and his coworkers. [19] Their interest stems partly from the clinical usage of PMEA which "has been approved by the FDA for use in medical devices, and thus, currently, PMEA has the largest market share in the world as a nonthrombogenic coating agent for artificial oxygenators." [19] The interactions of proteins and platelets with a series of polyacrylates with different n-alkyl side chain lengths (1–6, and 12 carbons) and a ω-methoxy terminal group (poly (ω-methoxyalkyl acrylate): PMCxA) were characterized. It had been previously shown that the polymer with x = 2, i.e. poly 2-methoxyethyl acrylate or PMEA, is highly resistant to platelet adhesion. The authors reported that platelet adhesion was very low on PMCxA with x = 1,2,3,4, and 5, but was elevated on surface coatings made with polymers where x = 6 or 12. Fibrinogen adsorption was also substantially higher on PMCxA with x = 4, 5, 6, or 12. The authors probed the availability of platelet binding epitopes in adsorbed fibrinogen using an antibody to the C-terminal dodecapeptide of the γ chain of fibrinogen, but found this epitope was expressed at very low levels in fibrinogen adsorbed on all members of the PMCxA polymers they studied so there did not appear to be a good correlation with epitope exposure and platelet adhesion. The authors also studied hydration water in this series with differential scanning calorimetry and found that the so-called intermediate water (IW) was maximal on PMC2A/PMEA but decreased by about 5-fold in the series as the alkyl chain length increased. IW is a state of water exhibiting a different thermal transition than normal water due to its interaction with the polymers. Previous studies of water structure in PMEA and other polymers using differential scanning calorimetry (DSC) had established the importance of IW [20]. Tanaka and coworkers have studied platelet and protein interactions as well as water state on a large number of other polymers with generally similar results that show that strong interactions with water is a key attribute in reducing platelet and protein interactions. [19–26].

A particularly informative study compared platelet adhesion, total protein adsorption from plasma, and exposure of platelet binding sites in adsorbed fibrinogen on a wide series of existing polymers as well as the new polymer poly(3-methoxypropionic acid vinyl ester) (PMePVE). [25] The existing polymers included poly(vinyl acetate) (PVAc), poly (methylacrylate) (PMA), poly(n-butylacrylate) (PBuA), poly(n-butyl methacrylate70-co-2-methacryloyloxyethyl phosphorylcholine30) (P (BMa-co-MPC), as well as poly(propylene) (PP) and poly(ethylene terephthalate) (PET) surfaces that the other polymers were coated onto. The adsorption amount on PMePVE was reduced to the same extent as that of PMEA and was much less than the amount of adsorbed proteins on PVAc. The adsorption to the PMPC and PBuA samples were higher than the PMePVE and PMEA samples. Fibrinogen gamma chain antibody binding, a measure of platelet epitope availability, was lowest and about the same on PMePVE and PMEA, slightly higher on PMPC, and much higher for the PVAC, PBuA, and PMA samples. Platelet adhesion was lowest on PMePVE, about 2-fold higher on PMEA and PMPC but roughly 10X higher on all the other polymers. Thus, these results provide good support for the idea that PMEA and PMPC copolymers used in current cardiovascular devices (see below) should be more blood compatible, and that the new polymer PMePVE should be even more blood compatible when used in a device.

A more detailed study of fibrinogen interactions with PMEA and poly(butylacrylate) (PBA) was carried out using atomic force microscopy. [26] PMEA has nanometer-scale protrusions thought to be caused by a phase separation of polymer and water in the interface region, i.e. polymer rich and water rich domains exist. A fibrinogen coated cantilever was used in force measurements, which showed little or no attractive force as it approached water rich regions, but significant force as it approached the polymer rich domains. The attractive forces were far greater when the fibrinogen coated cantilever approached the PBA surface. Adhesion force to the water rich domain of PMEA was less than the detection limit, and even for the polymer rich domain, the adhesion was within 30–90 pN. For PBA, the adhesion force was strong and converged at around 210 pN. These results agree well with the macroscopic adsorption behavior of fibrinogen to PMEA and PBA, i.e. fibrinogen adsorption to PMEA was less than half that on PBA.

Copolymers of the zwitterionic polymer poly(methacryloyl phosphatidyl choline) (PMPC) have been applied to improve the biocompatibility of a number of cardiovascular devices, including guide wires, stents, left ventricular assist devices, as well as in devices used outside the cardiovascular system including contact lenses and artificial hip joints, as nicely summarized in a recent review by Ishihara. [27] Ishihara and his coworkers have shown coatings with these types of materials are resistant to the adhesion of many cell types, including platelets, most likely because they reduce protein adsorption. Ishihara provides a nice summary of the protein adsorption properties of PMPC and other polymers as a function of their free water in the hydrated polymer (Figure 3 in reference [28]), showing for example that fibrinogen adsorption to MPC polymers with 60–80% free water is about 2/3 less than to conventional hydrated polymers with about 40 % water. Ishihara explains the cell and protein resistant properties of PMPC as being due to the way it interacts with water: "In MPC polymers, the three hydrophobic methyl groups bonded to nitrogen in phosphorylcholine are surface exposed because the phosphate anion and trimethylammonium cation are closely linked by electrostatics. Accordingly, the methyl groups induce hydrophobic hydration, in which water molecules aggregate into bulk-like structures. In other words, MPC polymers are covered with a layer of bulklike water. Thus, protein molecules do not accept significant molecular interaction such as electrostatic interaction and hydrophobic interaction on the surface." A more complete discussion of the hydration state of PMPC polymers has been given by Ishihara and his colleagues [29].

A recent example of the use of PMPC to reduce interactions with proteins and cells was the use of gas plasma treatment of PTFE to allow the coupling of PMPC to this otherwise inert substrate. [30] After plasma treatment of the PTFE to introduce OH and COOH groups, dopamine (3,4-dihydroxyphenethylamine) reaction resulted in the formation of a polydopamine-PTFE intermediary that was treated with ethylene sulfide to form free SH groups that when exposed to monomeric MPC under UV irradiation allowed the coupling of PMPC. The coupling was confirmed when infrared spectroscopy revealed the reduction of peaks due to CF_2 and the appearance of new peaks due to C═C and C═N bonds. XPS studies also confirmed the PMPC coatings. As expected, the water contact angles of the PMPC coated PTFE substrates were much lower than for PTFE (ca. 10° compared to ca. 110° for PTFE). Protein adsorption after exposure to fetal bovine serum (detected with a colorimetric method) was much lower on the PMPC coated surface, although adsorption values reported to PTFE were far beyond monolayer values. This study also included the measurement of adsorption from blood plasma using antibody coated colloidal particles to probe for the presence of specific proteins, namely albumin and fibrinogen, using SEM to measure the number of bound colloidal particles. These studies showed that binding of anti-albumin or anti-fibrinogen coated particles was reduced by approximately 10-fold on PMPC-PTFE compared to PTFE. Although it was not reported, it seems likely that these novel PMPC coated PTFE surfaces will prove resistant to platelet adhesion as has been observed in many previous studies by Ishihara and his coworkers.

Another very good study of protein interactions with PMPC coated surfaces was done in a collaboration between the Ishihara and Brash

labs, who found greatly reduced fibrinogen adsorption from plasma. [31] These studies were done using ^{125}I-radiolabeled fibrinogen added to plasma, a technique that allows quantitative measurement of protein adsorption. In these studies, fibrinogen adsorption was measured from a series of plasma dilutions to assess the Vroman effect, which is the peak in adsorption at an intermediate concentration of plasma. Poly(MPC)- and poly(OEGMA)- grafted silicon, with varying graft densities and chain lengths, were prepared by combining self-assembly of initiator monolayers and surface-initiated atom transfer rapid polymerization (ATRP). The control silicon surfaces adsorbed 106.2 ± 9.1 ng/cm^2 of fibrinogen at the Vroman peak that occurred for adsorption from approximately 1% plasma, whereas the most repellant MPC coating had a maximum of only 1.5 ± 0.4 ng/cm^2, and no clear peak in adsorption was observed. As expected, these highly protein repellent coatings also had extremely low platelet adhesion compared to the control. The platelet studies were done by exposing the surfaces to reconstituted whole blood containing ^{51}Cr labeled platelets and ^{125}I-radiolabeled fibrinogen under shear flow using a cone and plate controlled shear device, thus better simulating the adhesion of platelets from flowing blood that occurs in clinical use of devices. By comparing platelet adhesion and fibrinogen adsorption to the surfaces made with each polymer and at varying graft densities and chain lengths, the authors found that there was a threshold of fibrinogen adsorption required for platelet adhesion, in the vicinity of about 20–30 ng/cm^2, below which platelet adhesion was very low. Previous studies from my lab found a much lower threshold of 5 ng/cm^2 fibrinogen needed to support platelet adhesion to polystyrene surfaces. [5] Different experimental conditions in the two studies likely account for the difference, namely my lab studied platelet adhesion from suspensions without flow and also the platelets suspensions used in my lab contained physiological levels of free calcium ion, the latter being a requirement for the activity of the fibrinogen receptor GPIIb/IIIa.

A study that is important because of its attempt to extend the use of nonfouling zwitterionic coating technology to fibrous structures was reported by Xu et al. [32] The authors used a fairly simple method to coat the fibers, namely swelling polystyrene fibers in ethanol and then in monomer (carboxybetaine acrylamide) solution to get the monomer into the fibers, followed by removing the extraneous solution and polymerizing the monomer by exposure to UV light. The hydrogel fibrous structures remained stable in a physiological condition for at least 3 months and exhibited good resistance to protein and cell uptake.

Another novel study in the field of nonfouling materials was to develop a way to apply highly protein resistant polycarboxybetaine (pCB) coatings to artificial lungs. [33] Three different pCB grafting approaches to artificial lung surfaces were investigated: 1) graft-to approach using 3,4- dihydroxyphenylalanine conjugated with pCB (DOPA-pCB); 2) graft-from approach using the Activators ReGenerated by Electron Transfer method of atom transfer radical polymerization (ARGETATRP); and 3) graft-to approach using pCB randomly copolymerized with hydrophobic moieties. (DOPA, or dihydroxyphenylalanine, should not to be confused with dopamine or 3,4-dihydroxyphenethylamine used to form a polydopamine coating as an intermediary to coupling PMPC to PTFE that was discussed above). The coated and uncoated devices were evaluated in parallel within a veno-venous sheep extracorporeal circuit with no continuous anticoagulation. The DOPA-pCB approach showed the least increase in blood flow resistance and the lowest incidence of device failure over 36 hours. In a separate study using rabbits, blood was circulated through a veno-venous micro-artificial lung circuit. The level of circulating fibrin degradation products, a measure of clotting, was much less (ca. 0.1 ng/mL) in animals exposed to DOPA-pcB coated oxygenators than in the uncoated version of same device (ca. 0.3 ng/mL). The device was weighed, and the change in device weight between the beginning and end of the experiment was recorded as the clot weight. The coating group had 59 % less clot weight than the uncoated after the 4 -h study.

Li et al. observed that the protein resistant zwitterionic polymers based on poly(phosphorylcholine), poly(sulfobetaine), and poly (carboxybetaine) currently available have the opposite charges that make them zwitterionic separated by several carbon atoms, perhaps limiting their ability to bind water. [34] So, these investigators developed polymers based on trimethylamine N-oxide (TMAO), in which the opposite charges are closer together, as a new class of ultralow fouling biomaterials [34]. Of particular note in this work is that not only did the polymeric TMAO materials (PTMAO) strongly resist protein adsorption and cell adhesion when used as coatings, but they proved to be non-immunogenic and did not activate complement, while PVA and PEG are both complement activators. Molecular dynamics simulation results showed that the TMAO oxygen accepts an average of 2.5 hydrogen bonds from water, while oxygen atoms in the ether linkages of PEG typically accept only one hydrogen bond from water. Thus, the superhydrophilicity of TMAO stems from fundamental differences in how it interacts with water, supporting the original hypothesis that a structure in which the counterions were more closely spaced would be better at binding water. These materials appear to be promising candidates for application to the next generation of LVADs and other cardiovascular devices, although it remains to be seen how they could be applied as stable coatings on the surfaces of these devices.

Zwitterionic hydrogels have also recently been shown to be useful for stem cell culture by reducing differentiation and promoting self-renewal. [35] Biodegradable zwitterionic hydrogels were formed from star-shaped poly(carboxybetaine acrylamide) and polypeptide cross-linkers containing a metalloproteinase-degradable motif through a step-growth polymerization mechanism. Stem cells encapsulated in these hydrogels had a 73-fold increase in long-term hematopoietic stem cell frequency. The basis for this effect could be damping down of the strong signals that adhesion sends to cells, e.g. many cells are adhesion dependent for growth ("anchorage dependent") and undergo a special form of apoptotic cell death called anoikis if deprived of a suitable substrate to adhere to [36]. Thus, inhibition of cell attachment is likely to reduce the tendency to differentiate. However, the authors did not discuss this idea and instead proposed another mechanism: the inhibition of excessive reactive oxygen species (ROS) production via suppression of O_2-related metabolism.

A comparative study of the effect of various types of polymer brushes on protein adsorption from whole blood plasma and on platelet adhesion from whole blood under shear flow showed that all the polymer brushes greatly reduced protein and platelet interactions compared to uncoated polycarbonate control surfaces. [37] However, the zwitterionic polymer tested ("poly(CBAA)", made from the monomer (3-acryloylamino-propyl)-(2-carboxyethyl)-dimethyl-ammonium monomer (CBAA)) was superior to the neutral polymers tested (polyHEMA, polyHPMA(made with the monomer *N*-(2-hydroxypropyl) methacrylamide (HPMA)), poly(MeOEGMA) (made with the monomer oligo(ethylene glycol) methyl ether methacrylate (MeOEGMA)). Thus, no protein adsorption from plasma was detected on the poly(CBAA) polymer coatings, while some protein adsorption was detectable on the other polymers. In a subsequent publication, this group showed that application of these polymeric brushes to poly(4-methyl-1-pentene) ("TPX") polymers used in extracorporeal membrane oxygenators (ECMOs) greatly reduced platelet adhesion from platelet rich plasma. [38]

A very novel type of surface that exhibits much lower interactions with blood is the "tethered-liquid perfluorocarbon" (TLP) coating. [39] The TLP surfaces was inspired by the "slippery, liquid-infused, porous surface (SLIPS) approach" that creates non-adhesive surfaces by infiltrating porous or roughened substrates with liquid perfluorocarbons [40]. The TLP coatings were made by oxygen plasma activation of a variety of substrates (including polycarbonate, PVC, polysulfone, polyethylene, polypropylene, polyethylene terephthalate PET, polyimide, polystyrene, polydimethylsiloxane, fluorinated ethylene propylene and polytetrafluoroethylene) followed by incubation with tridecafluoro-1,1,2,2-tetrahydrooctyl trichlorosilane to form a highly fluorinated coating. These coatings were then exposed to the liquid

perfluorcarbon perfluorodecalin. The liquid perfluorcarbon is believed to infiltrate the chains of the covalently coupled perfluorcarbon polymer. These materials exhibited much lower fibrin formation and platelet adhesion when exposed to heparinized whole blood in vitro as well as improved in vivo blood compatibility in a porcine arteriovenous shunt model for periods up to 6 h. More recently, the TLP approach was evaluated for use in extracorporeal life support system for up to 6 h in pigs, where it prevented thrombus deposition even in the absence of heparin, unlike the control immobilized heparin circuits. [41]

Another unique approach to reducing blood interactions with surfaces is based on making coatings that mimic the endothelial glycocalyx. [42] In a rather complex scheme, polyelectrolyte multilayers (PEMs) were formed from hyaluronan (a polyanionic GAG) and chitosan (CHI) (a polycationic polysaccharide), using the layer-by-layer approach. Secondly, they formed polyelectrolyte complex nanoparticles (PCNs) containing heparin (HEP) or chondroitin sulfate (CS) as the polyanion and chitosan (CHI) as the polycation, by mixing the oppositely charged molecules in solution. The CS-CHI and HEP-CHI PCNs were then adsorbed to the PEMs to form PEM + PCN surfaces, resulting in 100 − 200 nm hemispherical bushlike domains presenting a high density of HEP or CS glycosaminoglycan chains, mimicking nanostructures in the endothelial glycocalyx. Many of the complex surfaces produced in this way displayed much reduced adsorption of albumin and fibrinogen from buffer solutions. In addition, some of the surfaces resisted the binding of fibrin in the presence of fibrinogen and thrombin. Whether any of these materials will prove resistant to platelet adhesion or clot formation when exposed to whole blood remains to be seen.

While PEG coatings have long been known to reduce protein interactions with surfaces, it has been recently proposed that a better coating would consist of poly(2-alkyl-2-oxazoline) (PAOXA) or poly(2-methyl-2-oxazine) (PMOZI) brushes. [43] It was proposed that these materials would have better hydration and main-chain flexibility than PEG, leading to complete bioinertness that could surpass the bioinert and lubricious properties of PEG analogues. It was thought that these coatings might be more resistant to oxidation that occurs with PEG coatings, and perhaps also avoid anti-PEG antibody formation. These investigators demonstrated low protein adsorption to the oxazoline and oxazine based brushes, even lower than on the PEG surfaces they studied, but no studies on chemical stability or resistance to platelet adhesion or blood clotting were reported.

5. Clinical application of nonfouling polymers

The protein resistant polymer PMEA studied in the publications from Tanaka's group are used for coatings in clinical blood oxygenators and other devices made by the Terumo Cardiovascular Group, who designate it as "Xcoating". [44] The Terumo oxygenators and other Xcoated devices are in widespread clinical use. The PMPC protein resistant polymer studied by Ishihara's group is used as a coating in clinical oxygenators and other devices made by the Sorin cardiovascular group of the Liva Nova Corporation, who designate it "Ph.I.S.I.O" [45] and in left ventricular assist devices made by Sun Medical Technology Research Corporation.

Left ventricular assist devices (LVAD) were originally intended as a bridge to transplant but because newer versions have proved capable of long-term usage in humans, they can now be thought of as pseudo total artificial hearts with the main difference being that the patient's heart is not removed as is the case for a total artificial heart. A good summary of the state of the art was given recently. [13] (The abbreviation LVAS (left ventricular assist system) is used instead of LVAD by some companies, e.g. Evaheart, Inc or EVI). EVI bills itself as the "Leading LVAD company in the State of Texas" and notes that a total of 183 EVAHEART® LVAS have been implanted to-date [46]. The 183 implants referred to are those that were done in Japan. In the USA, the EVI website discloses that "Under an FDA-approved IDE, a bridge-to-transplant (BTT) clinical trial of the EVAHEART®2 LVAS is currently ongoing in US" [46] but the number of USA patients enrolled so far is not stated.

The EVAHEART LVAS is made by the Sun Medical Technology Research Corporation, Suwa City, Japan. The history of the development of EVAHEART, the technology underlying the EVAHEART devices, and the relationship of EVI and Sun Medical is summarized in a brochure available online. [47] An important innovation in the most recent version of the EVAHEART LVAD, EVAHEART 2, in comparison to predecessor devices is the way it connects to the patient's heart, namely using a cuff to attach the inflow catheter to the surface of the left ventricle, so that the cannula cannot contact the interior of the ventricle or the heart valves [48]. In older LVADs, including EVAHEART 1, the cannula went through the ventricle wall and the tip was free to move in the interior of the heart, a situation that caused problems due to injury to the ventricle wall or the valves.

From the point of view of this article, where I have mostly concentrated on biomaterials used in devices, the most important fact about the EVAHEART device is that it uses poly(MPC-*co*-*n*-butyl methacrylate) as coating on all its internal surfaces. Recent review articles by Ishihara are helpful to understand why PMPC has been adopted clinically (its high hydration and consequent resistance to protein and platelet adhesion), and in what form it is used (as a copolymer with poly butyl methacrylate, as reviewed above) [27,28].

Ishihara points out that polymers made with MPC alone are water soluble and thus would not form stable coatings on devices, so in practice copolymers containing hydrophobic segments such as butyl methacrylate must be used to improve polymer adhesivity to the hydrophobic surfaces used to form the bulk structure of LVADs and other cardiovascular devices. The inclusion of hydrophobic copolymers to MPC polymers is likely to reduce the protein repellency of the resulting polymer. It seems likely that the polymers used in coating the EVAHEART and Liva Nova/Sorin devices reflect a balance between good stability of the coating under physiologic conditions (due to the hydrophobic copolymer) while retaining good protein and platelet repellency (due to pMPC), and that careful evaluations were performed in choosing the best copolymer to use.

It should be noted that exposure to blood at physiologic temperature and pH is a challenging environment for any non-covalent polymeric coating because the proteins and lipids in blood act as good surfactants that have a strong tendency to adsorb to hydrophobic polymers. Any weakness in the binding of a polymeric overcoat would allow the wetting and potential stripping away of the overcoat by the blood borne surfactants. Presumably this issue has been carefully studied by the developers of the PMPC coatings, and it thus seems likely that the clinically used coatings resist delamination despite the big challenge from the surfactants in the blood.

A final comment on the EVAHEART and Liva Nova/Sorin devices is that extremely good protein repellency for the MPC based polymers in the collaborative studies between Ishihara and Brash discussed above [31] seems unlikely to be achieved for the these devices because the types of MPC polymers used in the two situations was quite different. While the copolymer poly(MPC-*co*-*n*-butyl methacrylate) is used to coat EVAHEART and Liva Nova/Sorin devices, in the collaborative studies of fibrinogen adsorption, the ATRP method and the monomer 2-methacryloyloxyethyl phosphorylcholine (MPC) was used to covalently form MPC rich polymers on a silicon surface. Thus, the MPC surface concentration was probably much higher on the coated silicon surfaces than on the EVAHEART and Liva Nova/Sorin device coatings.

In support of the expectations that MPC polymers containing hydrophobic copolymers would probably adsorb considerably more protein than a polymer containing MPC only, we can cite the work of Ishihara and his collaborators who studied the adsorption of albumin and fibrinogen from single protein solutions in buffer to poly(MPC-*co*-*n*-butyl methacrylate) polymers containing 10 or 30 mol per cent MPC [49]. While the reported values were less than to polyHEMA surfaces used as controls, the adsorption was still quite high (1–2 $\mu g/cm^2$, the

lower values for the surfaces with 30 mol per cent MPC). On the other hand, this report did show that the proteins adsorbed to the MPC surfaces retained their native structure as measured with circular dichroism, and the retention of native structure was greater for proteins adsorbed to surfaces with higher MPC content. In summary, while there is good evidence that pure MPC polymers are highly non-fouling, there is also evidence that MPC polymers copolymerized with hydrophobic copolymers seem to be only moderately protein repellant.

The performance of Terumo and other oxygenators was studied by Stanzel et al. [50] Oxygenators assessed included the Terumo models FX15® and FX25®, Sorin company's Synthesis®, Inspire 6F® and Inspire 8F® versions, the Maquet Quadrox-i®, and the Medtronic Fusion®. Various measures of performance were measured, including prime volumes, gas exchange, pressure gradients and the effects on patient hematology. The pressure drops, which are indicative of clotting events in the devices that restrict flow rate, varied by almost a factor of 4, with the best performing device being the Quadrox device. Platelet counts post treatment were about 20 % decreased but there was not much difference in this parameter among the 7 devices tested. The author's concluded that "The data demonstrate that no single product is superior in all aspects."

6. A brief comment on "superhydrophobic" surfaces

Silicone rubber sheets stored at room temperature and then immersed in buffer solutions and brought to 37 °C develop many adherent air bubbles at the surface, due to the reduced solubility of the air dissolved in silicone rubber at 37 °C . To ensure uniform contact of silicone rubber with protein solutions in protein adsorption studies in my lab, air bubble formation was eliminated by exposing the silicone rubber (while submerged in buffer) to a temperature above 37° and to a water aspirator vacuum source for ca. 20 min. before transfer of the films to 37° buffer and addition of protein solution to start the adsorption experiment with bubble free surfaces. Similar observations were made by other investigators, who also showed that removal of the air from silicone rubber reduced complement activation and platelet aggregation. [51]

It is believed that "superhydrophobic" surfaces with surface asperities that resist protein adsorption do so because of their ability to hold onto air bubbles, as noted in a recent review of the role of protein adsorption in blood compatibility partly written by myself [6]. It was noted in that review that protein adsorption is usually higher on hydrophobic surfaces than hydrophilic surfaces, while the so called superhydrophobic surfaces (water contact angle > 100°) have been reported to show very low adsorption [52]. However, the superhydrophobic surfaces appear to require the presence of trapped air in surface irregularities. Any proteins that try to reach the surface are blocked by the air bubbles, and if any adsorb to the air-plasma interface itself, it is likely that the protein easily washes off under flow, with the result that the remaining surface has very low protein adsorption. However, the air-plasma interface would likely denature proteins that come in contact with it and are likely to encourage blood clotting as well. Jokinen et al. [53] discuss the possible role of trapped air in activating platelets and the likely instability of the trapped air pockets because the proteins and lipids at the bubble interface would likely adsorb to the interface under the bubble and thus promote its detachment. Another comprehensive review of biological interactions with superhydrophobic materials was provided by Falde et al., who also thought air bubbles would be unstable in contact with protein containing media such as blood plasma [54].

7. A brief history of my interactions with John Brash

I was invited to make this contribution to this special issue in honor of John Brash's long term editorship of this journal partly because I have known and worked with Brash in several different ways for much of my career. So here I would like to communicate a brief history of my interactions with Brash as a small tribute to honor his many contributions to the field of biomaterials.

As I recall, when I began my work in the field of biomaterials, one of the first articles I ever read about protein adsorption was published in 1969 and written by Brash, who worked together with Don Lyman at that time at the Stanford Research Institute. It was a very thorough study of adsorption of albumin, γ-globulin, and fibrinogen from buffer solutions to several hydrophobic polymers (polystyrene, polyethylene. Silastic, and Teflon FEP) using the then relatively new technique of infrared internal reflection spectra taken of proteins adsorbed to the polymer films placed against the reflector plate. [55] I remember being pleased to learn that a nice, easily understood result was obtained, namely the protein adsorption increased to a well-defined maximum or saturation level as bulk concentration was increased, and that the maximal level was in the range to be expected if a fully packed monolayer of adsorbed protein were formed. I liked the principles of protein adsorption that I learned in this article, and they provided a solid basis for my many subsequent studies of protein adsorption.

Brash and I subsequently worked together on organizing three large symposia on protein adsorption (held in 1986, 1994, and 2012) that were sponsored by the American Chemical Society (ACS). The earliest of those symposia was invited by Robert Baier, who at the time was in charge of organizing symposia supported by the Colloids and Surface Science Division of the ACS. Baier knew of the work of both myself and Brash on protein adsorption and was very supportive of our efforts to facilitate international exchange on the topic. Willem Norde joined Brash and myself in organizing the 2012 symposia. All three symposia resulted in books with contributions from many of those who attended the symposia as well as introductory chapters by the symposia organizers summarizing the state of the art at the time. [56–58]

All of these interactions with Brash were productive and positive and in the process, we ended up not only colleagues but also pretty good friends, especially when we discovered our mutual interest in the game of golf. Brash and I had other overlap in our careers as we both avidly pursued our own research on protein and blood platelet interactions with biomaterials, an area we both published heavily in over the years. We also were simultaneously active in working out the mechanisms of the Vroman effect and published the first detailed adsorption studies of it in the same journal. [59,60] Notably, both Brash's lab and mine soon adopted the ^{125}I-radiolabeled protein method that enabled quantitative measurement of the amount of an individual protein from complex mixtures such as blood plasma, an investment in equipment and learning that very few other labs made. This technique was a key one in determining what the Vroman effect was, i.e. both labs showed that it involved actual decreases in fibrinogen adsorption at higher plasma concentrations or longer exposure times. Vroman had used anti-fibrinogen antibodies and referred to the decrease of anti-fibrinogen binding as "conversion" since he did not know if the loss in anti-fibrinogen binding meant the binding epitopes for the antibodies were hidden or lost with adsorption time, or if there was an actual decline in adsorbed fibrinogen. The reader should remember that at the time this work was being done, the idea that the adsorption of a protein would decrease when its bulk concentration increased or after longer adsorption times were both quite unexpected because they are just the opposite of what happens when the adsorption of a single protein from buffer is measured.

I also had another very beneficial result of my interactions with Brash. Probably the single most important event of my scientific career was a visit to the McMaster University laboratories of Professors J. Fraser Mustard and Railene L. Kinlough-Rathbone to learn about preparing stable, non-activated platelet suspensions using the enzyme apyrase to prevent activation by catalyzing the breakdown of ADP. My visit was made possible because of the help of John Brash and Irwin Feuerstein. Many of my lab's subsequent studies involved use of platelet suspensions made with that approach. Notably, washed platelet suspensions made that way can be suspended in media containing

physiologic calcium and magnesium concentrations, both necessary to assure full activity of the platelet GP IIb/IIIa receptors that are key to mediating interactions with fibrinogen. I always recall that visit with fondness because I learned a lot in a one week visit that I was able to use through much of the rest of my career.

Finally, while Brash was editor of this journal, he called on me at times to review articles for him, and I was happy to do so. In this and all the many other interactions I have had with Brash, I am happy to say I found him to be highly professional and consistent in his dedication to the tasks we shared, so it was always a pleasure.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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