Plasma polymer films to regulate fibrinogen adsorption: Effect of pressure and competition with human serum albumin

Madhuwanthi Buddhadasa1 | Sophie Lerouge2 | Pierre-Luc Girard-Lauriault1

1 Plasma Processing Laboratory, Department of Chemical Engineering, McGill University, Montreal, Quebec, Canada
2 Laboratory of Endovascular Biomaterials (LBeV), CHUM Research Centre (CRCHUM) and Department of Mechanical Engineering, École de technologie supérieure, Montreal, Quebec, Canada

Correspondence
Madhuwanthi Buddhadasa and Pierre-Luc Girard-Lauriault, Plasma Processing Laboratory, Department of Chemical Engineering, McGill University, Montreal, Quebec, Canada. Email: madhuwanthi.buddhadasa@mail.mcgill.ca (M.B.); pierre-luc.girard-lauriault@mcgill.ca (P. L.G.-L.)

Funding information
Natural Sciences and Engineering Research Council of Canada (NSERC), Grant number: 418117-12; Fonds de Recherche du Québec–Nature et Technologies (FRQNT); Canada Foundation for Innovation (CFI), Grant number: 30264

Low-pressure plasma-deposited C2H4 and C4H6 films containing N- and O-groups, are used to regulate fibrinogen (Fg) adsorption in the presence of human serum albumin (HSA), with the long-term intention of achieving control over platelet activation. This work includes a study of the effect of pressure on the films’ surface chemistry, stability in phosphate buffer solution (PBS), and Fg adsorption. Tribometry tests against a polyethylene surface, in PBS, indicated that N-rich films were more susceptible to wear than the O-rich coating. Adsorption kinetics showed a distinct peak which suggested a multilayer formation of HSA owing to adsorption from a highly concentrated solution. Results conclude that Fg adsorption in the presence of a high concentration of HSA can still be regulated by the careful choice of film surface chemistry.

KEYWORDS
albunin, fibrinogen, plasma polymerization, surface plasmon resonance, wear resistance

1 INTRODUCTION

Thrombogenicity is a crucial aspect to be considered in the development of medical implants. It is broadly defined as the extent to which a device, intended for use in contact with biological tissues and fluids, causes a localized accumulation of fibrin and cellular blood elements leading to blood clot formation. While in most cases, thrombogenic devices are undesirable, in some cases, they are considered useful. This is the case in the endovascular treatment of intracranial aneurysms, a localized dilatation of a blood vessel wall in the brain. This minimally invasive treatment involves packing the aneurysm with small platinum coils using a catheter, to seal it and thereby separate it from the main blood flow, preventing it from further expansion and rupture. This method relies upon mechanical occlusion as well as on the thrombogenicity of the coils to induce thrombus formation in the aneurysm. The main concern with this treatment is the instability of the thrombus formed which leads to recanalization and re-growth of the aneurysm.[1] Research has been done in order to study the effects of less and more thrombogenic coils compared to platinum (Pt),[2,3] but the role of the extent of coil thrombogenicity on aneurysm healing remains unclear. In light of providing a platform to achieve the long-term goal of studying the effect of coil thrombogenicity on aneurysm healing, we here present the
design and characterization of a series of functional plasma polymer coatings that can render varying degrees of affinity for a blood clotting protein, fibrinogen (Fg).

Fg is a blood plasma protein that plays a vital role in the coagulation cascade and regulation of thrombosis. Fibrinogen adsorption on a biomaterial's surface is a main determinant of the surface thrombogenicity since it regulates platelet adhesion and activation.\[^{8}\] Various surface properties such as charge, morphology, and chemistry are known to influence protein adsorption, that may in turn cause structural and functional changes in the protein molecule. Recently, Zhang et al.\[^{6}\] investigated the effect of surface chemistry of various synthetic polymers on the extent, conformation, and orientation of adsorbed Fg, and how these influence platelet adhesion and fibrin fibre formation. Such a study shows that the extent and type of Fg adsorption and its propensity to bind platelets may be manipulated by the type of surface chemistry. This implies that by modulating the Fg-coil surface interactions, it could be possible to gain some control over the extent of thrombosis, thereby paving the way to comprehend and possibly improve aneurysm healing.

Past efforts to modify thrombogenicity of bare Pt coils involves enhancing the coil surface with bioactive components such as polyglycolic acid\[^{3,4}\] and hydrogel.\[^{7,8}\] Our approach is to use cold plasma processing, a dry (solvent-free) process, to develop functional polymeric coatings which can easily be deposited on to three-dimensional objects and whose surface chemistry can easily be varied and controlled, in order to regulate Fg adsorption as a first step toward studying the extent of thrombogenicity of the coils on aneurysm healing. Our main focus is on N-based plasma polymer films (PPFs) whose functional groups primarily include amines, imines, and nitriles. Primary amines and imines are considered of high interest since, when exposed to physiological (pH 7) aqueous environments, they acquire a positive charge that aids in attracting negatively charged biological molecules such as proteins. We also consider a high oxygen-based coating whose carboxylic acid groups induce a negative surface charge, when exposed to a physiological environment.

The affinity of PPFs to bind different plasma proteins such as Fg, immunoglobulin (IgG), and albumin has been investigated in literature.\[^{9-11}\] Lassen et al.\[^{11}\] showed that competitive protein adsorption from a mixture of Fg, human serum albumin (HSA), and IgG, on a hydrophobic plasma polymerized hexamethyldisiloxane (PP-HMDSO) surface, resulted in an adsorbed layer that mostly comprised of HSA and IgG, with Fg present to a smaller extent. In contrast, the adsorbed layers on positively and negatively charged hydrophilic plasma polymerized amino and carboxy functionalized surfaces, were completely dominated by Fg with almost no HSA and very low levels of IgG present. Individually, all proteins adsorbed to all surfaces showing that apart from electrostatic attractions, hydrophobic, vander Waals, and entropic interactions also contribute toward the adsorption process.

This paper is organized in three sections. The effect of deposition pressure on PPFs properties such as surface chemistry, aqueous stability, and Fg adsorption was first studied. In the second part of this work, the influence of the type of PPF, defined by the HC precursor used for deposition, on Fg adsorption was investigated by surface plasmon resonance spectroscopy (SPRS). Friction and wear properties of the coatings were also tested using tribometry in the context of potential use as coatings for endovascular coils. Lastly, competitive adsorption of Fg in the presence of HSA was studied by surface plasmon fluorescence spectroscopy (SPFS) on a selected group of PPFs with varying surface chemistries. It is shown that the ability of the coatings to regulate Fg adsorption is successfully maintained despite the presence of a high concentration of the competing protein.

## 2 | EXPERIMENTAL SECTION

### 2.1 | Plasma polymer deposition

Films were deposited using a previously described\[^{12}\] Plasma Enhanced Chemical Vapor Deposition (PECVD) system, on 500 µm thick (100) p-type silicon wafers cut into appropriate sizes, except for SPR and SPFS studies where gold sensors were used as the substrates. The Si wafers were cleaned ultrasonically in isopropanol and deionized (DI) water for 5 min in each liquid and dried with a nitrogen flow prior to film deposition. The gold sensors were cleaned by immersing in isopropanol followed by DI water for 2 min in each liquid and drying using a nitrogen flow. Briefly, the PECVD setup consists of a cylindrical steel vacuum chamber connected to a turbo-molecular pump, backed by a two-stage rotary vane pump. The operating pressure, during plasma deposition was varied between 15 and 80 Pa. The low-pressure capacitively coupled r. f. (13.56 MHz) glow discharge was generated via an impedance matching network connected to a 10 cm diameter powered electrode in the center of the chamber, with the walls of the chamber acting as the grounded electrode.

PPFs were deposited using a mixture of a functional group source gas, such as ammonia (99.99%, MEGS), and a hydrocarbon (HC) gas, such as 1,3-butadiene (99.8%, MEGS), and/or ethylene (99.999%, MEGS). The films prepared include N-rich plasma polymerized ethylene (PPE:N) and butadiene (PPB:N) films, a co-polymerized ethylene and butadiene (PCEB:N) film, an O-rich plasma polymerized ethylene (PPE:O) film, deposited using carbon dioxide (99.99%, MEGS) as the functional group source gas, and a pure HC film (PPE) deposited using ethylene. HC flow rates for the PPB:N, PPE:N, PPE:O, and PPE films were 5, 10, 5, and 20 sccm, respectively. HC flow for PCEB:N film was
2 sccm of ethylene and 8 sccm of butadiene. These HC flow rates were maintained constant in all film depositions. Films were deposited under CW, at 10 W, except for PCEB:N and PPE:O which were deposited at 20 W. Pressure and gas flow ratio (R, functional group gas flow:total HC gas flow) used for each film are clearly defined in the results and discussion section.

2.2 Film characterization

X-ray Photoelectron Spectroscopy (XPS) analyses were performed soon after deposition, in a Thermo Scientific K-Alpha XPS instrument, using monochromated Al Kα X-rays, producing photons of 1486 eV. Wide scans with step size 1 eV, pass energy 160 eV, dwell time 200 ms, and in the range 1200 to −10 eV were acquired for each sample.

Derivatization with 4-Trifluoromethylbenzaldehyde (TFBA) (Sigma–Aldrich, purity 98%) followed by XPS analysis was performed on N-based PPFs according to the method described in our previous work.[12] Recent work[13,14] showed that the [NH₂] content calculated from this method includes all N functional groups that can react with aldehydes. Since the N source gas used here is ammonia which already contains the NH₂ component, it can be assumed that most N groups incorporated into the film are NH₂ species and that they have a good chance of survival under the mild plasma deposition conditions used. Nevertheless, it is important to keep in mind that the [Nu] symbol used here is a representation of any group that can react with aldehydes such as primary amines, imines, and also amides which are formed on the surface due to oxidation in air. The concentration of carboxylic acid groups in the PPE:O film was determined by derivatization with toluidine blue as described in ref.[15]

Film stability in phosphate buffered saline (PBS, Bio-Shop, Canada) solution of pH 7.4 for 1 and 24 h was measured using a Dektak profilometer and is presented as the percentage of film thickness loss after exposure to PBS. After immersion, samples were dried using a nitrogen flow and left outside for at least 10 min before measurement. The coating was deposited on a silicon wafer with a thin piece of Kapton tape, that is expected to leave no residue upon removal, to produce a step enabling thickness measurement. Approximately 100 nm thick coatings were used for the stability tests.

Dynamic water contact angle (CA) measurements were performed using a goniometer (Dataphysics OCA 15EC) with distilled water. Advancing and receding contact angles (ACA and RCA, respectively) were measured by pumping and withdrawing 8 μl of water, to and from a sessile drop of initial volume 2 μl at a rate of 0.1 μl s⁻¹. A wait time of 20 s was maintained between advancing and receding measurements to allow just enough time for the droplet to stabilize. All CA measurements were conducted at room temperature ranging from 20 to 25 °C and 30–50% relative humidity. At least three measurements were taken on each sample.

Friction and wear tests were performed using two instruments, depending on the normal load exerted. The first was a custom-built pin-on-flat reciprocating in situ tribometer, details of which are explained elsewhere.[16] The static counter-surface was a ball (1/4 in. dia.) made of high density polyethylene (HDPE), a typical material used in the fabrication of micro-catheters. Tests were performed at a constant 1 mm s⁻¹ sliding velocity of the flat surface, over a track length of 4 mm and for 500 cycles (total distance 4 m). The normal force applied was 0.6 N. From Hertzian contact mechanics,[17] this was expected to correspond to a maximum Hertzian contact pressure of 21.8 MPa and circular contact area diameter of 0.229 mm. Profilometry was conducted on these wear tracks after rinsing them with DI water to remove any residual salts from the PBS. In order to achieve a lower contact pressure, a nano-tribometer (NTR3, Anton Paar) was used which is also of a pin-on-flat configuration operated in linear reciprocating motion. A constant sliding velocity of the flat sample was maintained at 1 mm s⁻¹, over a track length of 2 mm for a total distance of 4 m (i.e., 1000 cycles). The diameter of the HDPE ball, the static counter-surface, was 6 mm. The normal force applied was 1 mN to yield a theoretical maximum Hertzian contact stress of 2.7 MPa and circular contact area diameter of 27 µm. Disregarding the end points of the wear track, an average coefficient of friction was calculated in real-time for each reciprocating cycle. Approximately 300 nm thick films were deposited on silicon wafer substrates. All experiments were done in PBS and at room temperature (~25 °C).

2.3 Protein adsorption by SPRS and SPFS

Fg (from bovine plasma, Sigma) adsorption was monitored using a commercial surface plasmon resonance (SPR) spectrometer (RES-TEC RT2005, Germany) where a thin noble metal layer (in this case gold), on the base of a prism is irradiated with a 632 nm He/Ne laser beam, while the reflectivity is measured, over a range of incident angles. A detailed description of the technique and its principles can be found in a contribution by Chu et al.[18] Substrates used were 25 mm × 25 mm × 1.5 mm LaSFN9 glass slides coated with ~2 nm of Cr and ~40 nm of Au using a NexDep e-beam evaporator (Angstrom Engineering Inc) and the test coatings (~15 nm) were deposited on the gold surface. Platinum coatings of ~7 nm thickness were deposited using a high vacuum sputter coater (Leica EM ACE600). Real-time kinetic measurements of protein adsorption were conducted using two methods. The first involved following the resonant angle with time and is called “minimum tracking.” In the second method, shift in the reflectivity at a fixed incident
angle, typically 1° below the resonant angle, was measured with time and is referred to as “time measurement.” A kinetic experiment involved gently flowing PBS into the 20 µl flow cell holding the sample-prism configuration, and leaving it for 2 min, followed by protein solution for 10 min (or until equilibrium is reached), and finally PBS for another 2 min to flush any loosely attached protein molecules. Kinetic measurements were conducted under both static and continuous flow conditions. The fluids were flown using a peristaltic pump at 0.7 ml min$^{-1}$, which corresponds to a venous shear rate of 100 s$^{-1}$. During a static experiment, 15 s of flow was sufficient to completely fill the flow cell. Angular scans of the reflectivity were measured over a range of incident angles between 42° and 70° in PBS before and after exposure to the protein mixture. Fg solution, prepared in PBS, was of concentration 2 mg ml$^{-1}$, which is equivalent to the average concentration of Fg in human blood. Similarly, individual adsorption of HSA (Sigma) to the films was also studied and the concentration of HSA in PBS was 40 mg ml$^{-1}$, equivalent to that in human plasma. All adsorption studies were performed within 20 min of exposure of the PPF to PBS and at room temperature (~25 °C). After each protein experiment, the flow system was cleaned by passing 10% bleach solution for 2 min, followed by a 2 min flow of 1% sodium dodecyl sulphate and finally rinsing with DI water for another 2 min.

Competitive Fg adsorption was measured using a combination of SPR and fluorescence spectroscopy, called SPFS (RES-TEC, Germany). The setup and the sample configuration is the same as that of the SPR spectrometer with the addition of a photomultiplier facing the base of the prism to detect the transmitted light. The binary mixture of proteins contained 2 mg ml$^{-1}$ of fluorescently labeled Fg from human plasma (Alexa Fluor 647 conjugate, Fisher Scientific) and 40 mg ml$^{-1}$ of HSA. The experiment was conducted under static flow and involved a 2 min flow of PBS, followed by a 10 min flow of protein mixture, and finally another 2 min flow of PBS to rinse off any loosely attached proteins. The intensities of the reflected light and transmitted light were measured over a range of incident angles between 42° and 70° in PBS before and after exposure to the protein mixture. Excitation of the fluorophores on the Fg molecules by the surface plasmon resonance results in a strong fluorescence peak about 1° below the resonant angle. The fluorescence signal was normalized by the reflectivity signal after subtracting the reflectivity curve from its maximum reflectivity and shifting it horizontally to match the angular position of the fluorescence peak. The reason for this angular difference between the resonant peak and fluorescence peak is related to the resonance character of the surface plasmon excitation and is explained in detail elsewhere.$^{[19]}$ Real-time kinetics of the total protein binding process were obtained by conducting a time measurement on the reflectivity curve.

The experimental reflectivity curves are simulated using the WinSpall data analysis software (Version 3.02) which uses an optical model based on solving Fresnel’s equations, to determine the optical thickness of the films and the adsorbed protein layer. If the refractive index of each layer is known, the geometric thickness of the layer can be calculated. The dielectric constants of adsorbed Fg and HSA layers used here are 1.93 and 1.968, respectively. These values were chosen based on literature$^{[20,21]}$ and the reflectivity curve fittings that assumed a monolayer packing of the protein molecules$^{[22]}$ with dimensions of HSA and Fg being in the ranges 2.7–12$^{[23]}$ and 6–45 nm$^{[24]}$, respectively. The dielectric constants of the plasma polymers used were 2.5 + 0.0022i in air and 2.4347 + 0.0012i in PBS. These were an average of values measured by depositing thick plasma polymers to provide waveguides in the reflectivity curve and allow the simulation of both refractive index and thickness, simultaneously.

In order to convert the fluorescence signal from the Fg molecules bound at the surface during a competitive adsorption experiment, single protein experiments with fluorescently labeled Fg molecules were first conducted on one of the PPFs and the thickness of the adsorbed Fg layer was determined by simulating the shift in the reflectivity curve. Thus, the fluorescence signal from a competitive adsorption experiment can be used to evaluate the amount of Fg in the protein mixture layer, assuming that the fluorescence signal is a linear function of the amount of Fg adsorbed. This assumption was made possible by manually reducing the intensity of the incident laser beam to avoid saturation of the photomultiplier (<1.5 × 10^6 cps). Owing to the Forster quenching of the emitted fluorescence that occurs near metal surfaces,$^{[19]}$ competitive protein experiments could not be successfully conducted on the Pt surface.

The thickness of the protein layer can be related to the surface coverage of the protein by the Feijter equation$^{[25]}$ as shown below.

$$M = d_A \cdot \frac{n_A - n_{sol}}{dn/dc}$$

where $M$ is the surface density (mass per unit area) of proteins bound to the surface, $d_A$ is the average thickness of the adsorbed layer, $n_A$ is the average refractive index of the adsorbed layer, $n_{sol}$ is the refractive index of the cover media, and $dn/dc$ is the refractive index increment of adsorbed molecules which is 0.182 cm$^3$/g, a constant for proteins. This equation is valid as long as the refractive index is a linear function of the solute concentration which is the case for proteins. According to this equation the thickness of the protein layer is directly proportional to the mass of protein per unit surface area considering that $n_A$ is an average over all thicknesses.$^{[25]}$ Thus, the evaluated geometric thickness of the adsorbed layer can be interpreted as the orientation with
which the protein molecules are assembled in the layer as well as the amount of protein adsorbed per unit surface area. All error bars represent the standard error with experiments repeated at least three times.

3 | RESULTS AND DISCUSSION

3.1 | Effect of pressure on film properties

Pressure is a crucial parameter that influences the chemical and physical properties of the PPFs. In the area of plasma diagnostics, Saboohi et al.\cite{26} compared two pressure regimes namely, collision-less and collisional, where the transition from one regime to the other occurs around 6 Pa. While plasma diagnostics and polymerization mechanisms have been studied in a wide range of pressures, from <70 mTorr (9.33 Pa) to >350 mTorr (47 Pa),\cite{27,28} the influence of pressure, treated as a deposition variable, is less commonly studied in the analysis of low-pressure PPFs, especially in the high-pressure collisional regime. Therefore, we first studied the influence of pressure on film properties to optimize the PPF for further steps. The effect of deposition pressure and gas flow ratio, \( R = \frac{NH_3}{HC} \), on the deposition rate, surface chemistry, aqueous stability, and affinity for Fg adsorption on PPB:N films is presented in Figure 1. Figure 1(a) shows the deposition rates of the films at 15, 45, and 80 Pa and at \( R \) values from 1 to 4 for each pressure. As expected, deposition rates show an overall decreasing trend with increase in \( R \) owing to an increase in etching effect of ammonia, a known etchant for organic materials.\cite{29-31} As pressure is increased, we observe a reduction in the deposition rate at all \( R \) values. This may be explained by the lower energy per particle with increase in pressure or more specifically, the shorter average mean free path of electrons, leading to lower electron energies and thus, low plasma fragmentation resulting in less activated species that could potentially condense and contribute to film formation.

Figure 1(b) shows the films’ N content with the variation of pressure and \( R \). As expected, for all pressures, there is a clear increase in the amount of N incorporated in the films with higher values of \( R \) owing to the increase in the number of ammonia molecules in the plasma that are activated and participating in plasma polymerization. However, increase in pressure does not seem to have a consistent effect on the N content at all \( R \) values. At lower \( R \), we observe a slight increase in N content, despite the decrease in energy per molecule at increased pressure. This is attributed to the bond dissociation energy\cite{32} of H-NH\(_2\) (453 kJ mol\(^{-1}\)) being lower than that of C—C bond in butadiene (489 kJ mol\(^{-1}\)) and therefore, even at lower energies per particle, the increase in the number of molecular collisions with pressure offers a better chance of N species being activated and incorporated in the film than C—H species. At \( R = 3 \), the increase and decrease in N content with pressure (and vice versa for \( R = 4 \)) could be explained by the net polymerization effect of 1) increased formation of activated N species relative to C—H species due to increase in molecular collisions and 2) the decrease in energy per particle that leads to a decrease in activated species, with increase in pressure.

Figure 1(c) shows the content of nucleophiles [Nu], such as primary amines and imines as a function of pressure and \( R \). As expected, similar to [N], [Nu] also increase with \( R \). With increase in pressure, similar to [N] at low \( R \), we observe an increase in [Nu] due to an increase in the number of molecular collisions and thus, increase in the amount of activated NH\(_2\) that are incorporated in the film. The very low [Nu] values at 15 Pa compared with 45 and 80 Pa are likely due to the fact that under these conditions of pressure, the plasma is very close to a collision-less regime wherein the high energy electrons leading to increased fragmentation of the monomer molecules and increased dehydrogenation due to more pronounced ionic bombardment, results in more unsaturated N species in the film, such as nitriles.

The percentage of thickness loss after exposure to PBS for 1 h (Figure 1d) and 24 h (Figure 1e) was measured for the \( R = 1, 3 \), and 4 coatings at all three pressures. Negative values correspond to a net effect of swelling of the coating where the polymer network has expanded due to intake of water and remained expanded upon drying, whereas positive values denote a net effect of loss of film material due to dissolution. Thickness loss or gain within 10% is considered stable in the context of this work. The coatings are most stable at \( R = 1 \) and stability decreases as they lose more material at higher \( R \) values. This is to be expected since increased presence of polar nitrogen groups increases the films’ affinity toward polar solvents rendering them more soluble in aqueous media. Considering the effect of pressure, 15 Pa coatings seems to be the most stable and those deposited at 80 Pa seems the least stable. One of the reasons for this observation is the presence of very low [Nu] content in the 15 Pa coatings. It is also consistent with research where diamond-like carbon thin films, characteristic of increased hardness, are usually deposited at low pressures (~16 Pa)\cite{33,34} This was evident with the coatings prepared with 15 Pa as it was impossible to scratch them with a needle, whereas the 80 Pa coatings were scratched very easily. It is also interesting to note that there is only a slight difference in film's aqueous stability between 1 and 24 h measurements suggesting that the interactions between the film and the liquid leading to thickness changes occur mostly within the first hour of exposure and seem to have stabilized over the next 24 h.

Taking into account the above observations, we chose a low and high N-containing coating with acceptable [Nu] content and PBS stability, \( R = 1 \) and \( R = 3 \) films, to investigate Fg adsorption as a function of pressure (Figure 1f). Higher Fg adsorption is detected on \( R = 3 \)
coatings. This is expected owing to the higher [Nu] of these films that make them more positively charged, attracting the net negative charge and/or local negatively charged domains of more protein molecules in physiological pH media.\textsuperscript{[35]}

There is also an increase in Fg adsorption on films with increase in deposition pressure, however, this increase is small compared with the distinct increase in the films’ [Nu] content with pressure (Figure 1c). This clearly shows that there are other factors encouraging Fg adsorption apart from the positively charged surface functional groups which may include hydrophilic, vander Waals, and entropic interactions.

3.2 | Influence of film type on Fg adsorption

We then compared Fg adsorption behavior on PPFs that differ based on their HC precursor and surface functionality. All
PPFs studied here were tested for stability and showed a thickness loss of <20% after 24 h of exposure to DI water (results not shown). A platinum surface and the bare gold surface of a SPR sensor are used as controls. Deposition conditions and surface chemistry of the PPFs are summarized in Table 1. Figure 2(a) shows the thickness of the adsorbed Fg layer on all films. Results from static and continuous flow (0.7 ml min\(^{-1}\)) experiments are also reported and under these flow conditions, there seems to be no significant difference between them, especially in the case of the N-based films. PCEB:N film gives the highest Fg adsorption, likely due to it being higher [N] and [Nu] content. With the increase in R, we can observe an increasing trend in Fg adsorption in the case of PPB:N and PPE:N films. This is to be expected, since the electrostatic attraction between the negatively charged Fg molecules and the positively charged surfaces is strengthened owing to the increase in [Nu] in the films with increase in R. Comparing the PPB:N R = 1 and R = 3 coatings with the PPE:N coatings, despite the higher [Nu] and selectivity, [Nu]/[N], of PPB:Ns (Table 1), Fg adsorption between the PPB:N and PPE:N coatings is similar. For PPB:Ns, as R is increased from 1 to 4, there is a net decrease in the water contact angle (Figure 3), particularly the RCA, which is more sensitive to changes in the high energy component of the surface (N groups in this case), denoting the increase in hydrophilicity of the coatings with increase in the films’ N content, which also correlates with the increase in Fg adsorption. However, for PPE:N, while the increase in the N content from R = 0.5 to 0.75 resulted in a clear increase in Fg adsorption, no significant change in its hydrophilicity was observed.

The non-N based films, namely PPE:O, PPE, Pt and Au, show relatively lower Fg adsorption. The PPE:O, owing to its strongly negative surface charge in aqueous media (−28 mV\(^{[15]}\)), shows the lowest affinity toward the protein. However, its low RCA value, making it the most hydrophilic surface of all, is thought to support some degree of adsorption via hydrophilic interactions. Furthermore, although the overall net charge of the Fg molecule is negative, the molecule possesses an anisotropic charge distribution,\(^{[35]}\) suggesting that the local positive charges on the molecule may also contribute to binding with the negatively charged PPE:O surface through some degree of electrostatic attraction. PPE, despite the absence of hetero-atomic functional groups, is considered to react with water or dissolved oxygen in aqueous media to form negatively charged functional groups such as alcohols and aldehydes,\(^{[36]}\) resulting in a similar electrostatic interaction to that of PPE:O with Fg. It can be noted that PPE is the most hydrophobic surface of all and Fg adsorption on it is higher than that on the most hydrophilic PPE:O surface. Fg adsorption on uncharged Pt and Au is likely due to Van der Waals and hydrophobic/hydrophilic interactions.

Figure 2(b,c) show the kinetics of Fg adsorption on all films, under continuous flow. The kinetic curves are characterized by an initial fast-adsorption stage followed by a second slow stage where the curves tend more and more toward a plateau. The rate of adsorption of Fg to the surface is determined by the maximum slope of the adsorption profile which is dependent on mass transport and intrinsic kinetics. Since diffusion of the protein through the boundary layer to the surface is often slower than the intrinsic binding of the protein and is therefore the rate-determining step, the initial rate of protein adsorption is often dependent upon the mass transfer behavior under the working flow conditions.\(^{[24]}\) In this case, since there is no change in the protein concentration or the flow rate, there is no change in the mass transfer and hence, no difference in the initial gradients of all the adsorption curves is observed and the initial Fg layer is formed within less than 1 min for all samples. In the second slow stage of the kinetic curves, a dependency on R can be observed. In the case of PPB:Ns (Figure 2b), it can be seen that the time taken to reach a plateau increases from R = 1 to 4. This could either be due to further protein adsorption with time and reorientation of the adsorbed layer or possible swelling of the plasma polymer with the increase in N content and hydrophilicity. In Figure 2(c), a similar observation can be made for the N-based films, where the PCEB:N with the highest N-group content takes a longer time to reach a plateau. It has been found that swelling of the plasma polymer results in an increase in its surface roughness,\(^{[22]}\) which may favor further Fg adsorption, thereby contributing to a longer time to reach a plateau.

Longer adsorption kinetics are observed for PPE:O and PPE, as seen in Figure 2(c). This may be due to electrostatic repulsion with the net negatively charged Fg molecules, considering that PPE could also acquire a negative charge as discussed earlier. A similar observation was made in the work of Lassen et al.,\(^{[11]}\) where Fg and IgG adsorption on negatively charged carboxyl surface showed slower adsorption kinetics compared with that of positively charged amino acids.

**Table 1** Surface chemistry of PPFs

<table>
<thead>
<tr>
<th>Plasma polymer</th>
<th>[N] (at%)</th>
<th>[Nu] (at%)</th>
<th>[Nu]/[N] (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPB:N R = 1</td>
<td>7.4</td>
<td>4.4</td>
<td>59.5</td>
</tr>
<tr>
<td>PPB:N R = 2</td>
<td>10.2</td>
<td>5.7</td>
<td>55.9</td>
</tr>
<tr>
<td>PPB:N R = 3</td>
<td>12.9</td>
<td>6.5</td>
<td>50.4</td>
</tr>
<tr>
<td>PPB:N R = 4</td>
<td>14.2</td>
<td>7.3</td>
<td>51.4</td>
</tr>
<tr>
<td>PPE:N R = 0.5</td>
<td>8.8</td>
<td>3.2</td>
<td>36.4</td>
</tr>
<tr>
<td>PPE:N R = 0.75</td>
<td>12.5</td>
<td>4.2</td>
<td>33.6</td>
</tr>
<tr>
<td>PCEB:N R = 4</td>
<td>16.1</td>
<td>5.6</td>
<td>34.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plasma polymer</th>
<th>[O] (at%)</th>
<th>[COOH] (nmol cm(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPE:O R = 8</td>
<td>22.5</td>
<td>–</td>
</tr>
<tr>
<td>PPE</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Films were deposited at 80 Pa.
and hydrophobic PP-HMDSO surfaces. Pt and Au seem to follow similar adsorption kinetics, although Pt is more attractive to Fg than Au. These observations show that, electrostatic attractions, when present, result in faster Fg adsorption kinetics allowing for a steady state to be reached more quickly.

As mentioned previously, the layer thickness can be attributed to the orientation of the Fg molecules with which they are attached to the surface. The hydrated Fg molecule has been found to have a characteristic trinodular structure which is about 45 nm in length and 6–10 nm in width.[24] The charge distribution on the Fg molecule under physiological pH conditions is described as, the central nodule carrying a charge of $-6e$, the end nodules each carrying a charge of $-4e$, and the two polar appendages extending from either end of the molecule, each carrying a charge of $+3e$.[35] Thus, the thinner layers observed with the non-N based films, can be a result of side-on adsorption of the protein molecule to the surface via the polar appendages. Deformation or spreading of the molecule, sometimes leading to denaturation, may occur depending on the hydrophobicity of the surface, resulting in thicknesses of up to $\sim 15$ nm.[5] The thicker layers observed with the increase in N content in the N-based films, can be attributed to an end-on adsorption of the Fg molecule via the end nodules, allowing for layer thickness values ranging from $\sim 15$ to 45 nm.

**FIGURE 2** Fg adsorption on PPFs, platinum, and gold determined by SPRS. (a) Thickness of the Fg layer adsorbed on all films ($n = 3$). The $R$ values of the PPFs are, from left to right, 1–4 for the four PPB:N films, 0.5 and 0.75 for the PPE:Ns, 4 for the PCEB:N and 8 for the PPE:O. Adsorption kinetics measured by minimum tracking, under continuous flow, are presented for (b) the four PPB:N films and (c) PPE:N, PCEB:N, PPEO, PPE, Pt, and Au films. Each curve represents an individual experiment which was reproducible. Arrows indicate the approximate times at which PBS or Fg was introduced. All PPFs are deposited at 80 Pa
2.7 ellipsoidal in shape with a length of 12− and a charged maleic anhydride film. A HSA molecule is usually charged plasma polymerized allylamine and a negatively showed no clear difference in adsorption between a positively observation was made with bovine serum albumin where it shown no particular relationship with film type. A similar shown in Figure 4. HSA adsorption on all four films seems to pure HSA solution is studied using SPRS and results are film is the same PPE:O coating discussed previously. 

3.3 Influence of HSA on Fg adsorption 

Competitive protein adsorption on a surface depends strongly upon the bulk concentration of the proteins and the surface affinity of each protein which varies with the surface chemistry. Proteins upon adsorption can go through conformational changes due to their low structural stability. This leads to molecular spreading, causing a larger molecular footprint, thereby allowing for further bond formation with the surface. These conformational changes can affect the biological activity of the adsorbed proteins and thus, cell responses. It is well known that, by Vroman effect, surface adsorbed Fg is displaced by high molecular weight kininogen and factor XII, which are also human clotting factors as Fg. The goal here is to investigate the influence of another protein on Fg adsorption. Since it is not directly involved in the coagulation cascade nor platelet adhesion and activation, and being the most abundant protein in blood plasma, we have chosen HSA as the competing protein to study Fg adsorption from a simple binary mixture of Fg and HSA. Surfaces studied include a low-N, high-N, O-rich, and a Pt surface. PPB:N R = 1 and R = 3 coatings deposited at 45 Pa were chosen as the low- and high-N films since they demonstrated the optimum compromise between stability in PBS, wear resistance, and nucleophile content. The O-rich film is the same PPE:O coating discussed previously.

As the first step, HSA adsorption on these films from a pure HSA solution is studied using SPRS and results are shown in Figure 4. HSA adsorption on all four films seems to show no particular relationship with film type. A similar observation was made with bovine serum albumin where it showed no clear difference in adsorption between a positively charged plasma polymerized allylamine and a negatively charged maleic anhydride film. A HSA molecule is usually ellipsoidal in shape with a length of 12−14.1 nm and width of 2.7−4.1 nm. It carries a −9e and +2e charge on each end and a −8e charge at the center, thus bearing an overall net −15e charge. Therefore, its interaction with the positively charged PPB:N films is probably mainly due to electrostatic attractions. It may also adsorb on the negatively charged PPE:O film via the +2e charge present at one of its ends. The fact that HSA adsorption on the PPE:O coating is higher than that on the PPB:N R = 1 coating could be due to the protein molecule being attached in a more upright orientation on the PPE:O surface, via the positive end of the molecule, allowing for a slightly thicker protein layer. The more hydrophilic nature of the PPE:O coating may also lead to stronger hydrophilic interactions with the protein.

Figure 4(b) shows the adsorption kinetics of HSA on the four types of films. Unlike with Fg, we observe a peak in the kinetic curve of HSA, soon after protein injection. This is related to the high HSA bulk concentration used here, unlike in other studies concerning HSA adsorption where the concentrations used were two to three orders of magnitude smaller. An experiment repeated (results not shown) with a lower HSA concentration (4 mg ml\(^{-1}\)) also showed no such peak. Due to the high bulk concentration, 40 mg ml\(^{-1}\), used in the current experiments, a large quantity of protein is driven to the surface by mass transfer laws and it is possible that albumin gets adsorbed in multilayers as was observed on positively charged surfaces terminating with polyallylamine. The authors proposed two mechanisms one of which is the formation of an organized layer of end-on adsorbed HSA molecules, exposing their positive ends on the top, that would in turn attract more proteins, thereby leading to the building up of a multilayer. As the thickness of the multilayer increases, the less organization of the protein layers at the top would cause this building-up process to stop.

A similar mechanism is proposed here. The fact that the final thickness of the HSA layers on all films are thin, shows that despite the differences in the surface charge of the films, the protein has undergone a more side-on adsorption, unlike in the mechanism proposed in ref. This causes the molecules to be less regularly packed due to their shape and uneven charge distribution. The resonant angle shift at the initial peak after protein injection, corresponds to a layer thickness of about 10 nm which we propose to be a bilayer, instead of a closely packed monolayer of end-on adsorbed proteins. This supports the fact that half the thickness is lost upon flushing with PBS, as the irregularly and loosely bound proteins to each other on the top layer are detached, leaving behind just the layer of proteins that are attached to the surface. The reason for this kind of adsorption is most likely due to the high concentration of HSA in the bulk and the fact that HSA is already a low affinity protein, compared to others such as Fg and haemoglobin, resulting in a less organized protein layer on the surface.

Having studied the adsorption behavior of HSA molecules, the next step is to investigate whether Fg adsorption on these surfaces would be affected by the presence of HSA.
Figure 5(a) shows the thickness of the Fg layers adsorbed from a pure solution and the Fg-HSA mixture. It must be noted that, due to the nature of the measurement, the thickness of the Fg layer adsorbed under the competitive environment is not the actual Fg layer thickness present in the layer, but rather the equivalent thickness of a closely packed monolayer of Fg. This is because the fluorescence signal measured is directly proportional to the amount of Fg molecules present in the layer, which can be converted to a monolayer thickness via the Feijter equation, however, this does not give information about the true orientation with which the Fg molecules are attached in the layer. Consequently, results in Figure 5(a) are interpreted on a comparative basis for the amount of Fg adsorbed with and without the presence of HSA.

It can be seen from Figure 5(a) that there is an enhancement of Fg adsorption to PPB:N R = 1 and PPE:O and no change in Fg adsorption to PPB:N R = 3. A similar observation was made by Lassen et al. where an enhancement of Fg adsorption on positively charged amino-based surface was seen in the presence of HSA. Studies conducted with fibronectin showed that the ability of the protein to promote cell spreading on the protein-adsorbed surfaces was enhanced by the addition of albumin to the fibronectin solution. This was thought to be due to the adsorption of albumin alongside fibronectin, occupying surface sites near fibronectin, and preventing it from undergoing molecular spreading (thereby structural changes) over the adjacent surface sites, that would otherwise be empty in the absence of albumin. Likewise, it is possible that limited molecular spreading may have occurred in the adsorbed Fg molecules in the presence of HSA, leading to an increase in Fg adsorbed on some of the films. Further investigation is required to fully explain the observations made, in particular the absence of change in Fg adsorption observed on PPB:N R = 3.

Figure 5(b,c) show the kinetics of the total protein adsorption on the three PPFs and Pt. Kinetics of Fg adsorption alone is also shown for PPB:N R = 3 and Pt surfaces. The same initial peak, soon after protein injection, that appears in the case of pure HSA adsorption, is also observed with the Fg-HSA experiments. Moreover, this initial peak is not seen in the case of pure Fg adsorption on either PPB:N R = 3 or Pt, confirming that this behavior in the kinetic curves is related to the adsorption of HSA. Another interesting point to note is the sharp drop in the signal upon flushing with PBS, in the case of Fg-HSA, as opposed to the slight drop in the signal with the pure Fg experiments. For PPE:O and Pt, this drop is almost half of that of the plateau, similar to that observed with the pure HSA experiments. These curves were obtained by a time measurement of the reflectivity signal and it is important to mention that information on the total adsorbed protein layer cannot be gained by fitting the reflectivity curve after protein adsorption, because, the refractive index, \( n_{\text{mix}} \), of the protein mixture layer, which considers the relative proportions of the two proteins, is unknown. However, by repeating similar experiments with fluorescently labeled HSA, the total and individual surface densities of Fg and HSA in the adsorbed layer can be determined, from which \( n_{\text{mix}} \) can be calculated and consequently, by fitting the reflectivity curve, the total average thickness of the protein mixture layer can also be evaluated.

### 3.4 Friction and wear testing

In addition to Fg adsorption, another important criteria for the development of a coating for aneurysm coils would be to test for its friction and wear properties. We have measured, using a tribometer, the wear resistance of some of the above coatings and the test conditions used here are motivated by foreseeing a plasma polymer coated platinum coil being used.
in the endovascular treatment of brain aneurysms. Thus, good wear resistance against the inner-wall of a micro-catheter used for coil delivery is indispensable. Tests were conducted in PBS to simulate physiological conditions. First, measurements were done by applying a normal load of 0.6 N, corresponding to a Hertzian contact stress of about 22 MPa. The coefficients of friction (\(\mu\)) are given in Table 2. These values of \(\mu\) show no particular relationship with the gas flow rate or the type of precursor gases used. Images of the wear tracks taken during profilometry measurements of all samples are shown in Figure 6. Figure 6(a–c), corresponding to PPB:N \(R = 1, 3,\) and 4, show that, with increase in the film’s N content, there is a clear reduction in the wear resistance of the film. These coatings were approximately 300 nm thick. The PPB:N \(R = 1\) coating is lightly scratched by the counter-surface, whereas for PPB:N \(R = 3,\) the contact profile from the profilometer (not shown) indicated considerable wear of the film that reached roughly half-way into the coating. In the case of PPB:N \(R = 4,\) at certain regions of the wear track, the coating is completely delaminated, as shown by the clear areas in the wear track of Figure 6(c). Similar to PPB:N \(R = 4,\) the high N PPE:N film also showed complete delamination in certain regions of its wear track (Figure 6d). Furthermore, the PCEB:N coating is completely delaminated by the motion of

FIGURE 5 Competitive Fg adsorption from a Fg-HSA mixture, on PPB:N \(R = 1,\) PPB:N \(R = 3,\) and PPE:O coatings determined by SPFS. (a) Thickness of the Fg layer adsorbed from a pure Fg solution and the Fg-HSA mixture \((n = 3).\) (b) Adsorption kinetics of Fg-HSA for the PPFs. Kinetics of labeled Fg from a pure solution on PPB:N \(R = 3\) is also shown (gray line). (c) Adsorption kinetics of Fg-HSA and labeled Fg from a pure solution on the Pt coating. Kinetic experiments were done by time measurement under static flow. Each curve represents an individual experiment which was reproducible. Arrows indicate the approximate times at which PBS or Fg was introduced. The PPB:N films were deposited at 45 Pa and PPE:O at 80 Pa.

<table>
<thead>
<tr>
<th>Plasma polymer</th>
<th>(\mu) at 21.8 MPa</th>
<th>(\mu) at 2.7 MPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPB:N (R = 1)</td>
<td>0.16 ± 0.04</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td>PPB:N (R = 3)</td>
<td>0.17 ± 0.05</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>PPB:N (R = 4)</td>
<td>0.19 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>PPE:N (R = 0.75)</td>
<td>0.17 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>PCEB:N</td>
<td>0.15 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>PPE:O</td>
<td>0.13 ± 0.04</td>
<td>0.20 ± 0.02</td>
</tr>
</tbody>
</table>

Films deposited at 80 Pa. The standard deviation values shown are the average values over 500 and 1000 cycles for 21.8 and 2.7 MPa, respectively.
the counterface, leaving almost no film residue in the wear track (Figure 6e). This is attributed to the high N content and thus, hydrophilicity of the film that causes it to undergo considerable swelling, weakening the polymer structure, allowing it to be easily removed by an external force. On the contrary, no visible wear track is observed on the PPE:O coating indicating good wear resistance to HDPE, under these test conditions (Figure 6f). Hence, it can be presumed that, the PPE:O coating, relative to the N-based films, is more strongly cross-linked and compact which renders it more resilient to shear stresses.

Out of the N-based coatings, PPB:N \( R = 1 \) and \( R = 3 \) (80 Pa) were chosen as a low and high N coating, to test for wear resistance against a reduced normal load of 1 mN, corresponding to a Hertzian contact stress of 2.7 MPa. No visible wear tracks were observed on either sample, indicating good wear resistance, under these test conditions. The coefficients of friction are given in Table 2. PPB:N \( R = 1 \) and \( R = 3 \) coatings deposited at 45 Pa also showed similarly good wear resistance under the same test conditions (results not shown).

4 CONCLUSIONS

In this work, we have characterized plasma polymer films with N- and O-groups, deposited using PECVD, to study adsorption of \( \text{Fg} \), a vital plasma protein responsible for platelet adhesion and activation, as a first step toward thrombogenicity testing of PPFs, for the application of
endovascular coiling for aneurysm treatment. The goal of this study was to be able to regulate the extent of Fg adsorption in the presence of a competing protein such as HSA, with the intention to later provide some control over thrombosis.

As a part of initial film characterization, we investigated the effect of pressure on the properties of N-based PPFs, an area that has not been studied widely. At the low pressure 15 Pa, the nucleophile content in the films is noticeably lower than that observed at higher pressures. Nevertheless, the increase in Fg adsorption to films deposited at increased pressures was comparatively small, implying that protein adsorption is not only a result of electrostatic attraction between the negatively charged proteins and positively charged films, but also significantly influenced by other factors such as hydrophobic and Van der Waals interactions.

Study of Fg adsorption on film types that differed based on the HC precursor used implied that PPE:N films exhibit higher affinity for Fg adsorption than the PPB:Ns with similar surface chemistry. Fg adsorption was observed on both N- and O-rich surfaces, but to a much lower extent on the O-rich coating owing to its strongly negative surface charge. Fastest adsorption kinetics were observed with the N-based positively charged coatings and slowest kinetics were seen with the films that carried a negative charge, that is, PPE:O and PPE. Study of wear resistance of the PPFs against HDPE, in PBS, showed a decrease in resilience of N-based films with an increase in the N content, whereas, the O-rich coating showed no visible wear at the higher contact pressure exerted, suggesting a strong cross-linking and compact nature of the O-rich film that makes it more tolerant toward shearing forces.

Pure HSA adsorption on low-N, high-N, high-O PPFs, and Pt surfaces showed no particular relationship between the film types. A thin layer, corresponding to more side-on adsorbed HSA molecules was detected on all surfaces. HSA adsorption kinetics show a rather distinct peak soon after protein injection. This is likely due to a bilayer formation of HSA molecules owing to the large concentration used for injection. The same behavior was observed with kinetics of the protein mixture but not with pure Fg adsorption, confirming that this behavior of the kinetic curve is related to binding of HSA molecules to the surface. This may suggest that the absolute concentrations used in competitive protein adsorption studies could influence the results obtained at different levels of dilution.

Lastly, presence of HSA caused an enhancement of Fg adsorption on both low-N and O-coatings, with a more pronounced increase on the O-rich surface. No change was observed for the high-N coating. Results suggest that regulation of Fg adsorption can be achieved by the careful choice of surface chemistry of the PPFs even in the presence of an abundance of competing HSA. The successful regulation of Fg by the present coatings suggests the possibility of triggering thrombosis to different extents, thereby providing a basis for studying the effect of the degree of coil thrombogenicity on aneurysm healing. In the present context, Fg being the protein of interest, the goal has been to study Fg adsorption regulation in the presence of HSA. Carrying out a similar set of experiments to monitor HSA adsorption under the influence of Fg, would certainly be a complement since it can give information on the amount of HSA present in the adsorbed layers. Thus, this would be the next step of this study, followed by platelet adhesion experiments and blood clot formation tests, to gain insight into the control of thrombosis and blood coagulation using various plasma polymer coatings, thereby helping to improve aneurysm healing following an endovascular coiling procedure.

ACKNOWLEDGMENTS
The authors are thankful to Yinyin Zhang, PhD, Richard Chromik, PhD, and Anton Paar for their kind support with the tribometry measurements. This work was funded by the Natural Sciences and Engineering Research Council of Canada (NSERC) (418117-12), Canada Foundation for Innovation (CFI) (30264), and the Fonds de Recherche du Québec — Nature et Technologies (FRQNT).

ORCID
Madhuwanthi Buddhadasa [10] http://orcid.org/0000-0002-6573-7017

REFERENCES


