Plasma-treated substrates reduces protein adsorption as studied by electron spectroscopy for chemical analysis

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Statement of Purpose: Protein-based pharmaceuticals present unique challenges in processing, packaging and delivery. All proteins rapidly adsorb to solid surfaces. The adsorption is essentially irreversible and frequently leads to a denaturation or aggregation of the protein. Also, there are concerns with substances from the packaging (glass, plastic) leaching out and affecting the proteins. This research examines fundamental aspects of protein interactions with surfaces, particularly using glow discharge plasma-treated surfaces. Such surfaces are readily applied to delivery devices, packaging and processing equipment [1-3] and may lead to a new generation of surfaces particularly effective for protein manufacture, storage and delivery. This research particularly focuses on tetraglyme and acrylic acid treated glass and stainless steel substrates and its effect on protein adsorption using electron spectroscopy for chemical analysis (ESCA).

Methods: *Cleaning protocol:* Glass (8mm φ discs) and 316L stainless steel (7 x 7 mm^2) substrates were sequentially cleaned with hexanes, dichlormomethane, acetone, and methanol in a sonication bath for ten minutes. Substrates were allowed to air dry in a chemical hood before plasma deposition. *Glow discharge Plasma deposition:* Plasma deposition was done using protocols as described previously by Shen et al. [3]. Briefly, the monomer of interest (either tetraglyme or acrylic acid) was degassed. The chamber was oxygen etched and then vented. Samples (glass and stainless steel) were loaded into the reactor, and then argon etched (40W for 3 minutes). Using a mass flow controller the monomer was introduced into the chamber and was plasma deposited: 80W for 1 minute and 10W for 20 minutes for tetraglyme; 80W for 1 minute and 10W for 10 minutes for acrylic acid. The plasma generator was turned off and samples were quenched for 5 minutes before venting the chamber and retrieving samples. Plasma-treated samples (n=3/treatment group) were washed using deionized water three times over a 24 hour period to assess whether the coatings would delaminate. Analyses of plasma-treated substrates were done using an S-Probe ESCA. *IgG adsorption study:* Plasma-treated samples (n=3/treatment group) were immersed in 0.2mg/mL bovine IgG solution in 1x PBS for 2 hours. After the adsorption period, samples were rinsed with 1% SDS solution three times, and then eluted in 1x PBS solution at 37^0C for 1 hour. At each time point, samples were rinsed with 1% SDS solution three times and allowed to air dry before analyzing the surface using ESCA. *ESCA analysis:* An S-Probe ESCA with monochromatic Al K-alpha X-rays focused to 800μm spot size was used for all ESCA analyses. 4 scans per spot were used for the survey of the surface, and 32 scans were used for detailed scans of nitrogen element. Data was analyzed using ESCA analysis software.

Results: To test whether the plasma coatings delaminate, plasma-treated samples were washed with deionized water and the surface was analyzed using ESCA. The carbon, oxygen and nitrogen content of the plasma-treated substrates as surveyed by ESCA can be seen in Table 1. To test whether the plasma-treated samples had an effect on IgG protein adsorption, samples were soaked in bovine IgG solution. The carbon, oxygen and nitrogen content of bovine IgG adsorbed plasma-treated substrates as surveyed by ESCA is shown in Table 2.

Conclusions: The survey scan from ESCA analysis on the plasma-treated substrates showed only peaks for carbon and oxygen and no silicon nor iron peaks (scans not shown). This suggests that neither the tetraglyme nor acrylic acid coatings delaminated after 24 hours. Furthermore, this would also suggest that the coatings on the substrates were at least 100nm thick (future studies will determine coating thickness using ellipsometry).

Table 1: Summary of ESCA analysis of plasma treated substrates

Plasma Treatment	Glass		Stainless Steel	
	C %	0%	C %	0%
Tetraglyme	67.3 ± 1.7	32.7 ± 1.7	68.7 ± 3.6	31.3 ± 3.6
Acrylic Acid	75.1 ± 1.3	24.9 ± 1.3	72.9 ± 1.3	27.1 ± 1.6

Table 2: Summary of ESCA analysis of bovine IgG adsorbed plasma treated substrates

Plasma Treatment	Glass			Stainless Steel		
	C %	0%	N%	C %	0%	N%
Tetraglyme	73.4 ± 1.6	26.4 ± 1.6	0.2 ± 0.2	47.5 ± 2.2	51.8 ± 2.4	0.7 ± 0.3
Acrylic Acid	74.1 ± 1.1	23.8 ± 1.5	2.1 ± 0.7	77.0 ± 1.5	21.9 ± 1.6	1.1 ± 0.5
Uncoated	44.2 ± 1.8	53.5 ± 2.2	2.3 ± 0.4	51.9 ± 4.1	44.9 ± 4.4	3.2 ± 0.4

Bovine IgG was adsorbed onto plasma-treated substrates and were analyzed using ESCA. We see that tetraglyme treated substrates had no nitrogen content indicating minimal IgG protein adsorption. However, for the tetraglyme-treated stainless steel samples, we noticed iron peaks which indicated that the coatings were damaged during the washing process (peaks not shown). For the acrylic acid treated substrates, we see some nitrogen content on the surface suggesting IgG protein adsorption. However, these values are smaller compared to the nitrogen content of the untreated substrates. In conclusion, these results demonstrate that plasma-treated samples reduces protein adsorption compared to untreated substrates. Future studies will be aimed at investigating the interaction between plasma deposited films protein IgG using quantitative radioimmunolabeling with I-125. Moreover, cell culture will be performed *in vitro* on these surfaces to assess basic toxicology of RF plasma deposited films. Additionally, other polyethers as well as fluoropolymers and fluoropolyethers will also be studied to create a new generation of materials that can be used for the manufacture, packaging and delivery of proteins.

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