Glow-discharge RF plasma polyether and fluoropolyether coated substrates prevent protein adsorption

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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 study 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 and may lead to a new generation of surfaces effective for protein manufacture, storage and delivery. This research particularly focuses on plasma tetraglyme (TG), acrylic acid (AA), perfluoropropylene (C3F6), and perfluoromethyl vinyl ether (C3F6O) treated glass, stainless steel, and cyclic olefin polymer (COP) substrates and their interaction with IgG.

Methods: Cleaning protocol: Glass and COP (8mm φ discs), and 316L stainless steel (7 x 7 mm^2) substrates were sequentially cleaned with DCM, acetone, and methanol in a sonication bath for 10 mins x 2. Substrates were allowed to air dry in a chemical hood before plasma deposition. RF-Plasma deposition and delamination: Plasma chamber was oxygen etched and then vented. Substrates were loaded into the reactor, and argon etched (40W for 5 min). Using a mass flow controller, the monomer of choice was introduced into the chamber and were plasma deposited (Table 1). Substrates

Table 1. Summary of plasma treatment parameters

Parameters	Acrylic Acid	Tetraglyme	C3F6	C3F6O
Flow Rate (SCCM)	4	1.33	10.2	10.3
Pressure (mT)	250	350	150	250
Treatment	80 W/1.5 mins 20 W/30 secs 5W/5 mins 1W/5 mins	80 W/1 min 10 W/20 mins	60 W/1 min 20 W/20 mins	60 W/1 min 20 W/15 mins

were quenched for 5 mins before venting the chamber and retrieving coated samples. Plasma-treated samples (n=3/group) were washed using DI water x 3 over 24 hours to assess whether coatings would delaminate. Analyses of plasma-treated substrates were done using an S-Probe ESCA using survey and detailed C1s scans. An S-Probe ESCA with monochromatic Al K-alpha X-rays focused to 800µm spot size was used for all ESCA analyses. Data was analyzed using ESCA analysis software. IgG adsorption studies: Bovine IgG will be tagged using an ICl method. Plasma-treated samples (n=3/treatment group) were immersed in 0.1mg/mL I-125 tagged bovine IgG solution in 1x cPBS with sodium iodide (cPBSI) for 1 hr. Samples were rinsed with cPBS x 3 and counted for 10 mins using a gamma counter. Cytotoxicity studies: Plasma-coated samples were eluted in complete growth medium for 24 hrs (37^0C, 5% CO_2 incubator). ~ 80% confluent NIH-3T3 fibroblasts were exposed to eluates from the plasma coated substrates, and observed over 48 hrs using an inverted microscope. ISO 10993-5 guidelines were used for cytotoxic evaluation.

Results: The carbon, oxygen and fluorine elemental composition of washed plasma-treated substrate can be seen in Fig 1. The amount of protein adsorbed on the plasma-treated substrates can be seen in Fig 2.

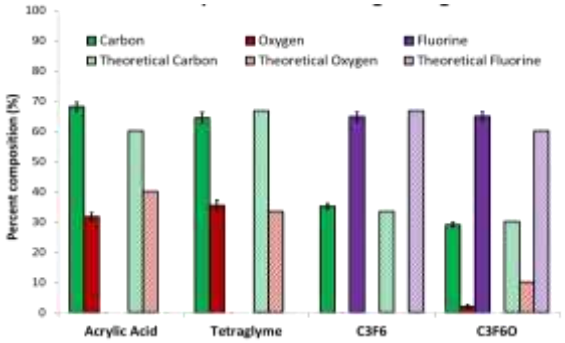

Figure 2. Elemental composition of plasma coatings using ESCA

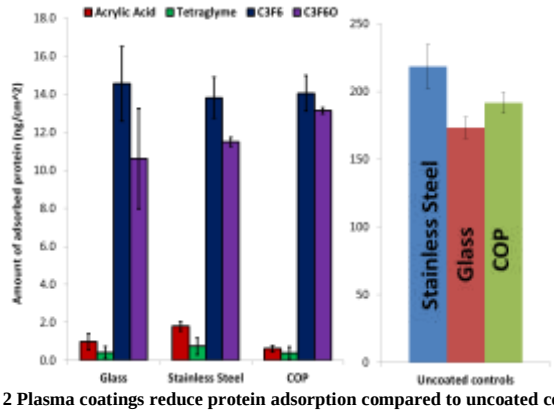

Figure 2 Plasma coatings reduce protein adsorption compared to uncoated controls

Conclusions: ESCA scans of plasma-treated substrates showed absence of substrate associated peaks (such as Fe or Si) (scans not shown), suggesting that coatings did not delaminate; also implies that plasma coatings on substrates are at least 10 nm thick—ESCA resolution is 100Å (coating thickness will be measured using ellipsometry). Furthermore, experimental and theoretical elemental compositions of the surfaces align well (Fig 1). Protein adsorption studies show that tetraglyme and acrylic acid plasma coatings are essentially non-fouling (< 2 ng/cm^2) as compared to C3F6 and C3F6O plasma coatings which adsorbed proteins in the range of 10-14 ng/cm^2. Furthermore, all plasma treated substrates show reduced protein adsorption compared to untreated controls (Fig 2). Moreover, fibroblasts exposed to all plasma coating eluates remained viable after 48 hours (images not shown). In conclusion, these data demonstrate that plasma-treated substrates can create a new generation of surfaces that can be used for contact with protein therapeutic agents. Future studies will be aimed at investigating the strength of bonding of IgG to these coated surfaces, protein aggregation studies, as well as determine the amount of protein that detaches from the surface. Additionally, other polyethers as well as fluoropolymers and fluoropolyethers will also be studied to create a new generation of materials that perform efficaciously for the manufacture, packaging and delivery of proteins.

References: Shen, M.O; Martinson, L.; Wagner, M. S.; Castner, D.G.; Ratner, B. D.; Horbett, T. A.; J. Biomat Sci, Polymer Edition 13(4), 367-390,(2002)

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