"Fluorinated biomaterials: a tale of two cell types"

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Statement of Purpose: Fluoropolymers have widespread technological, biological and biomedical applications as clinical biomaterial implants biotechnology components (protein blotting and filtration membranes) and anti-fouling marine coatings. Fluoropolymer surface properties exploited in these applications appear to be divided into two distinct behaviors, specifically the desired capability of fluoropolymer surfaces to (1) resist microbial colonization and cell attachment, and (2) readily transfer and filter proteins from aqueous gels and milieu. These properties result from common inseparable consequences of the strong interactions of low-energy fluoropolymer surfaces with soluble globular proteins in complex physiological and marine environments. While fluoropolymers are often considered chemically inert, their surfaces are not inert to protein adsorption, either in vitro or in vivo. Tight binding of serum albumin, fibronectin and hemoglobin on PVDF, and various serum proteins and significant fibrinogen on PTFE have been detected in vitro. Protein adsorption to fluoropolymers is also observed in vivo, correlated with platelet activation often observed on expanded PTFE (e-PTFE) vascular graft materials.1

While activating to platelets, the protein conditioning film adsorbed on fluoropolymers does not support attachment of most cell types,1,2 mandating preclotting or treatment with adhesive proteins to promote cell adhesion. Serum albumin, the largest protein mass in blood, blocks cell attachment and non-specific binding while fibronectin, collagens, and other subtle trace proteins (osteopontin, laminin, vitronectin) act as adhesive proteins for integrin receptor-based cell attachment and proliferation on surfaces, important components of extracellular matrix (ECM). Hence, observed fluoropolymer inability to support cell attachment for many cell types is plausibly related to ratios of non-adhesive (e.g., albumin) to adhesive (e.g., fibronectin) proteins selectively adsorbed to fluoropolymer surfaces from multi-component solutions (e.g., serum).1

We have attempted to distinguish different types of cell-surfaces interactions on fluorocarbon surfaces exposed to serum under in vitro culture conditions.

Methods: Human umbilical endothelial cells (HUVECs, ATCC CRL-1730) and murine 3T3 fibroblasts (ATCC) were cultured to confluency in commercial culture plates and relevant media. Bone marrow cells (BMC) were harvested from murine tibias and femurs of specific-pathogen-free female C57BL/6 mice, 6 to 8 weeks old (Jackson Labs, Bar Harbor, ME) and differentiated into macrophage cells using previously described methods.3 Murine cell lines IC-21, J774A.1 and RAW 264.7 were purchased (ATCC, Manassas, VA) and cultured in per ATCC recommendations on tissue culture polystyrene (TCPS) flasks prior to seeding on experimental surfaces (at or below passage 30 as received from ATCC).

Fibronectin and albumin labeled with 125iiodine were pre-adsorbed to fluorinated surfaces as described.1 Fluorinated polymer surfaces (PTFE and TeflonAF™) were exposed to 125I-human fibronectin (Fn), fibronectin:serum albumin (BSA) binary mixtures of various ratios or human plasma dilutions for 1 hr. Total adsorbed Fn and albumin was quantified on this surface. 125I-labeled monoclonal antibodies against either the 10th type III Fn repeat unit (containing the cell-binding RGDS integrin binding motif) or the fibronectin amino terminal domain proved the accessibility of these respective Fn regions post-adsorption. Cells were adhered to these different surfaces in static cultures.

Results: Fibronectin adsorption to fluoropolymers is dependent upon the concentration of albumin co-adsorbing from solution: albumin out-competes Fn for PTFE surface sites even at non-physiological Fn:HSA ratios 10-100 fold biased in Fn. Antibodies against Fn do not readily recognize Fn adsorbed on PTFE as the HSA co-adsorption concentration in either binary mixtures or in plasma increases, indicating albumin masking of adsorbed Fn. At Fn:HSA ratios rich in Fn (1:1, 1:100), albumin co-adsorption actually improves anti-Fn antibody recognition of adsorbed Fn. HUVEC and 3T3 cell attachment to PTFE after protein adsorption correlates with amounts of Fn adsorbed and levels of anti-Fn antibody recognition of Fn on PTFE, linking cell attachment to integrin recognition of both adsorbed Fn density and Fn adsorbed conformation on PTFE surfaces. By contrast, murine macrophages cell lines (IC-21, J774A.1 and RAW 264.7) and primary murine bone marrow macrophage cells adhere readily and proliferate steadily on Teflon-AF™ in serum conditions.

Conclusions: Monocyte and/or macrophage cell types are distinct from many other attachment-dependent cell types in their ready adhesion to fluorinated surfaces highly enriched in adsorbed albumin and depleted in Fn.

Acknowledgements: NIH grant R01 EB 00894 and longstanding support from D.G. Castner and NESAC/Bio.

References: