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Comparison of fluorinated polymers against stainless steel, glass and polypropylene in microbial biofilm adherence and removal

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Biofilm formation is a long-standing problem in ultrapure water and bioprocess fluid transport lines. The standard materials used in these applications (316L stainless steel, polypropylene and glass) have long been known to be good surfaces for the attachment of bacteria and other biological materials. To compare the relative tenacity of biofilms grown on materials used in manufacturing processes, a model system for biofilm attachment was constructed that approximates the conditions in industrial process systems. New fluorinated polymers were compared to the above materials by evaluating the surface area coverage of bacterial populations on materials before and after mild chemical treatment. In addition, contact angle studies compared the relative hydrophobicity of surfaces to suspensions of bacteria in growth media, and scanning electron microscopy and atomic force microscopy studies were used to characterize surface smoothness and surface defects. Biofilm adherence to polymer-based substrata was determined to be a function of both surface finish and surface chemistry. Specifically, materials that are less chemically reactive, as indicated by higher contact angle, can have rougher surface finishes and still be amenable to biofilm removal.

Keywords: biofilm; fluoropolymer; adhesion; hydrophobicity; AFM; stainless steel

Introduction

Contamination of submerged surfaces by microorganisms is a well-known problem in water and chemical/biological process systems in which aqueous products are manufactured [3,6,15,20,21,23]. The presence of microorganisms in these fluid-handling systems results in product spoilage, contamination of water systems with pathogenic bacteria, and causes extreme maintenance problems in the manufacturing environment. These negative attributes have been under study for many years using a variety of methods to locate, characterize and eradicate the organisms in undesirable areas.

Biofilms have a number of positive attributes, particularly in the bioprocess area where attachment of bacterial cells to a number of different substrata is critical to their use as biocatalysts. However, microbial colonization of the substrata can also result in undesirable growth on liquid transport surfaces. Removal of such films is critical to the process quality of products such as pharmaceuticals, specialty chemicals, medical devices, and other products [7,10].

Biofilm removal is at best a challenging task and at worst, an impossible one. Microorganisms have many defense mechanisms at their disposal to resist antibiotics, chemical treatment, high temperatures and turbulent flow scrubbing actions [3,17]. Many liquid-handling systems are assembled from materials whose surfaces, due to their chemical composition and surface topography, foster the proliferation of biofilms. While new materials have been evaluated for critical fluid handling in the bioprocess industry, most of the key components currently being installed are constructed of high quality stainless steel (316L). However, stainless steel has its disadvantages: high maintenance costs, relatively frequent system downtime for repassivation and electropolishing of critical surfaces, and eventual degradation of the inner surface due to periodic sanitizing treatments [17,20,25]. Other, less critical liquid-handling applications utilize silicone, and medical or food-grade polypropylene.

To date little has been written about the use and application of fluorinated polymers in biological and pharmaceutical process manufacturing. Due to their intrinsic cleanliness, inert chemistry, and corrosion resistance, fluid-handling components made of fluorinated polymers have found favor in the semiconductor industry and in other applications where high purity and corrosive chemicals are used. Reportedly, due to the ease of constructing and maintaining fluoropolymer systems, pharmaceutical manufacturers have begun exploring the use of these components for high purity water and pharmaceutical products.

Components made from various derivatives of polytetrafluoroethylene (PTFE) possess well-known nonadherent qualities. However, PTFE must be compression-molded into block form and then machined into a finished product. This attribute limits its application due to cost and whether the products' dimensions can be machined. The development of injection-moldable grades of Teflon® materials has greatly increased the use of these materials in industry. A high-purity grade of injection-moldable fluoropolymer, perfluoroalkoxy tetrafluoroethylene (Teflon® 440HP PFA), was developed in the late 1980s for use in critical fluid-handling applications [13,14]. This material, unlike other

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injection-moldable fluoropolymers is fully fluorinated. This creates a polymer that will not hydrolyze over time, so surface reactivity and hydrophobicity remain stable.

Many variables related to the substratum including surface topography [1,2,12,16,19,21] and surface chemistry [4,7,11,19,21,24] have been studied to determine the rate of initial adhesion for a variety of biological systems. However, no references were found that included PFA, a material different from the typically used PTFE film by its surface topography. To study biofilms in industrial environments, we used biofilm management and surface analysis techniques to evaluate the biofilm removal characteristics of stainless steel, polypropylene, glass, silicone, PVDF and PFA under conditions more typical of the industrial environment.

Materials and methods

Materials tested

All microbial data collection was performed at the University of Minnesota's Bioprocess Technical Institute (BNPTI). Biofilms were evaluated on the surfaces of 316L stainless steel (SS, provided by DCI, St Cloud, MN, USA), polypropylene (PP, Himont, Wilmington, DE, USA), perfluoroalkoxy (Teflon® 440HP PFA, DuPont, Wilmington, DE, USA), polyvinylidene fluoride (Kynar® PVDF, Atochem, Paris, France), Silicone (coated onto borosilicate glass using Sigmacote, Sigma Chemical Company, St Louis, MO, USA), and borosilicate glass. The materials were evaluated in a circular plaque format, 75 mm diameter × 2 mm thick. Three PFA surface finishes were evaluated: an injection-molded plaque that was machine milled to a typical machined surface topography, an injection-molded plaque with a smooth surface topography determined by the surface finish of the polished mold, and a rotationally molded plaque whose surface finish had a somewhat rougher, 'pillowed' topography. This rougher topography was due to the plaque's surface being formed by the solidification of the PFA in air.

Biofilm generation: organisms, growth and fouling conditions

Development of biofilms on plaques composed of several materials was performed using a modified procedure [1]. Tap water was taken from a little used laboratory faucet that had not been flushed in over 2 weeks. The water was brown in color, slightly turbid from lack of flow through the tap, and contained a high level of particulates. Organisms used in this study were *Klebsiella pneumoniae* ATCC 12657, chosen for its production of a thick exopolysaccharide layer; *Escherichia coli* ATCC 8739, a strain commonly used in USDA, AOAC and FDA test protocols; and *Salmonella choleraisuis* biovar *typhimurium* ATCC 13311, chosen due to its importance in contamination of poultry and dairy product manufacturing systems. All organisms were grown in 0.1% Bacto-Peptone (BBL, Cockeysville, MD, USA) in 100-ml starter cultures, then transferred to 10-L glass carboy containers (Bellco Glass, Vineland, NJ, USA) and incubated at 37°C for 24 h with mild agitation to allow growth of the organisms in the batch vessel.

Biofilm incubation chamber

A fluid transport system capable of allowing the bacterial growth in the medium and for pumping the medium through a chamber containing test materials was designed using PFA and polypropylene fluid handling components, Masterflex tubing and a peristaltic sine-wave pumping system (Figure 1). The incubation chambers were constructed using a covered polypropylene tank containing two smaller chambers into which a plaque carrier was placed. The plaque carrier was composed of polypropylene and was able to hold 25 plaques at approximately 10 mm separation between plaque surfaces. The carrier contained two plaques of each material composition listed above.

Incubation of plaques

The bacterial suspensions were allowed to maintain contact with the plaques for 14 days in a ventilated 37°C warm room. The medium was recirculated twice weekly (by draining and refilling) to allow fresh microorganisms to attach to the plaques. Nonturbulent conditions were maintained to foster microbial attachment and biofilm formation. After 2 weeks, the plaque cassettes were removed from the process tanks and gently rinsed with deionized water and allowed to air dry.

Inactivation of biofilms on plaques

The inactivation of biofilms deposited on the surfaces of half of the plaques was accomplished by placing a loaded wafer cassette into a 3-L container filled with water supplemented with 50 ppm sodium hypochlorite. The container holding the plaques was covered and placed on an orbital shaker and allowed to incubate with mild shaking (10 rpm) at 22°C for 24 h, followed by a 30-min rinse with deionized water. Care was taken to isolate the plaques from turbulent water flow to maintain biofilm retention. The inactivation solution was decanted and the plaques were removed from the carrier, then blotted dry with a soft cloth. Prior to reuse, the biofilm incubation chambers were sanitized by circulating 10 L of 0.1% bleach in water through the test system for 24 h at 37°C after each series of experiments. Residual hypochlorite was removed by flushing the system with deionized water (10 L with three liquid changes). Tubing

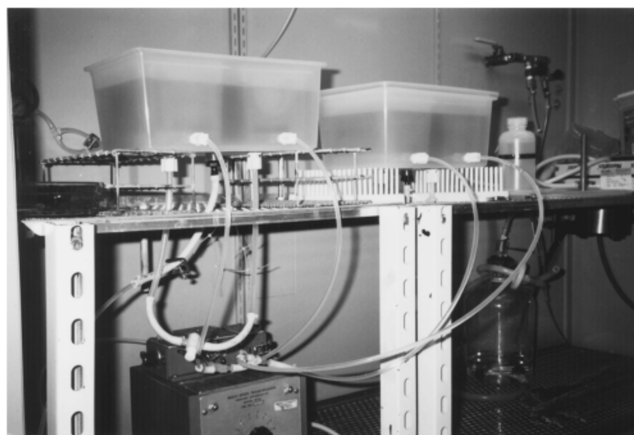

Figure 1 Photograph of the biofilm chamber. The plaques placed in carriers were incubated for 14 days at 37°C to allow biofilm deposition and then removed, treated and analyzed.

sets were sterilized by autoclaving at 121°C for 1 h and allowed to cool prior to equipment reassembly. In this report, biofilms on hypochlorite-treated plaques are labeled 'treated' while those subjected only to water rinsing are labeled 'untreated.'

Fluorescent staining of biofilms on plaque materials

Untreated and treated biofilms on all plaques were visualized by staining with a 10 ppm solution of acridine orange. The plaques and carriers were incubated in the dye for 1 h at room temperature, and rinsed for 30 min in deionized water to remove excess acridine dye. The plaques were carefully blotted dry with a soft cloth and visually inspected using scanning confocal laser microscopy and fluorescence microscopy. At least three areas were examined on each plaque.

Fluorescence microscopy

The acridine-stained plaques were placed on the stage of an Olympus reflected fluorescence microscope and illuminated with 488 nm wavelength light with a fluorescein filter cube using a method adapted from [25] and [26]. Biofilm images were recorded digitally using video micrography, and saved as RGB tag-image files (TIF). Images were viewed at 200× with no digital magnification increase. Surface coverage of plaques was rated on a scale of 0 (no residual biofilms) to 4 (surface completely covered by microorganisms).

Scanning confocal laser microscopy (SCLM)

Scanning confocal laser microscopy was used as an alternative to fluorescence microscopy and was performed using a Bio-Rad SCL microscope as described [5]. Depending on the biofilm thickness up to 15 image planes were collected, each being 0.2 μm thick. Images were edited to a uniform number of planes (six) by eliminating the planes that contained extraneous information. The images were taken at a magnification of either 200× or 400×. Fluorescence and SCLM images were qualitatively assessed for biofilm presence by approximating coverage of the surface by microorganisms. Biofilms were rated identically to those observed using fluorescence microscopy.

Atomic Force Microscopy (AFM)

AFM was used to measure surface roughness of plaques for each material. This technique involves moving a sensitive stylus over the plaque surface and measuring the vertical height changes. The technique yielded parameters of R_a, R_{ms}, R_{max} and Z range. The R_a and R_{ms} values are the averages of all the deviations of the traced line from the center line along some predetermined length. The R_a value is an arithmetic average, while the R_{ms} value is a geometric average. The Z value is the largest perpendicular distance measured along the trace line. The samples were analyzed using a Digital Instruments Nanoscope® AFM in tapping mode and scanning 256 areas on each sample.

Tapping mode was used to increase image resolution without damaging the polymer surfaces. In conventional contact mode AFM, the probe tip is dragged along the surface of the sample and the resulting image is a topographical mapping of the sample. Conventional AFM is not suitable for analysis of soft-surface samples due to the potential for sample surface and probe damage, which may result in inaccuracies in the measurement data. Tapping mode overcomes this problem by alternately placing the tip of the probe on the surface and then lifting the tip off the surface to avoid damaging the sample.

Scanning Electron Microscopy (SEM)

A JEOL JSM-5800LV SEM was used to provide 200× photographic details of the surfaces that were evaluated in the biofilm test. The stainless steel images were obtained using the secondary electron imaging technique (SEI). Alternatively, the nonconductive samples were imaged using the back scatter electron imaging technique (BSEI). The information revealed in these images included surface topography and identification of surface defect types.

Contact angle measurements

Contact angle measurements were performed using a Rame Hart contact angle profilometer, using the procedure outlined [8,9,16]. The medium used to evaluate the contact angle consisted of: (1) 0.1% peptone water, identical to that used to grow the microorganisms in the biofilm incubation study; (2) medium containing dead *Klebsiella pneumoniae* or *E. coli* cells at a concentration of 1×10^6 cells ml^{-1}; and (3) 18 MΩ deionized water. Measurements were taken by placing 2 μl of solution onto the surface of each test plaque. Advancing contact angle was measured 1 min after the liquid had been placed onto the plaque's surface. Measurement error is less than 0.5 degrees.

Results

Biofilm formation

The data from the biofilm retention, atomic force microscopy and advancing contact angle experiments are summarized in Table 1. A scale was developed for comparing the degree of biofilm surface coverage on the plaques. Percent biofilm coverage was divided into five categories as shown below: biofilm retention rating scale 0 = no biofilm coverage; 1 = 1–10% coverage by biofilm; 2 = 10–30% coverage by biofilm; 3 = 30–60% coverage by biofilm; 4 = >60% coverage by biofilm. (+) or (–) symbols following the biofilm rating indicate coverage at the upper or lower end of the rating.

Stainless steel

Untreated and treated plaque surfaces infected with *Klebsiella pneumoniae* are shown in Figure 2(a) and (b). Microscopy yielded variable data on the untreated plaques, ranging from small clumps of cells in thin films established in water to thick, resilient films established under exposure to microbial cultures. Inactivation of the biofilms on stainless steel plaques in 50 ppm bleach using mild agitation action was more effective on the noncapsule-producing bacteria (*Salmonella* spp and *E. coli*, data not shown) than on *Klebsiella* spp. The images of the *Klebsiella* spp biofilms show considerable numbers of organisms and debris from the incubation tank remain on treated plaques.

Table 1 Results summary: surface roughness, contact angle and biofilm retention indices

Material	AFM surface analysis (nm)				Contact angle (degrees)			Biofilm ratings (untreated/treated)[a]			
	R_a	R_{ms}	R_{max}	Z range	Water	Medium	Culture	*K. pneumonia*	*S. choleraisuis*	*E. coli*	Tap water
Stainless steel	26.64	41.74	272.84	293.09	41.5	32.5	36.3	4/2	4/3	3/2	3/3
Polypropylene	16.19	21.2	165.40	165.4	101.3	85.5	87.3	4/2	4/2	4/2	2/2
PFA (injection-molded)	17.17	24.35	438.09	438.85	98.5	94.6	85.3	4/1−	4/1−	3/1−	1/1
PFA (machine-cut)	36.83	47.47	310.93	310.99	99.5	87.8	93.8	4/1	4/1	4/1	2−/1
PFA (rotationally molded)	147.84	190.21	1425	1402	101.8	96.5	91.5	4/1	4/1+	4/1+	1/1
Glass	1.11	7.42	78.42	78.41	38.5	29.5	26.8	3/1	1/1	1/1	2/2
Silicone-coated glass	0.84	1.56	35.06	35.14	50.2	48.5	43.2	4/1	3/1	3/1+	2/1
PVDF	28.48	35.09	250.11	244.24	71.8	68.0	61.0	3/1	3/1	3/1	2/1

[a]Rating system described in text.

Teflon® Perfluoroalkoxy (PFA)
Images of untreated and treated *Klebsiella* spp biofilms on PFA injection-molded plaques are shown in Figure 2(c) and (d). The plaques of PFA used in the testing were of three surface types: (1) injection molded; (2) machined; and (3) rotationally molded. All of the PFA plaques allowed deposition of biofilm during the 14-day incubation period with some variability in percentage of the surface coverage. The films exhibited less tenacious attachment as indicated by the ease of biofilm removal from the machined and injection-molded surfaces with chemical treatment. The rougher topography of the rotationally molded plaque was more difficult to clean due to the depth of the pores. The machined plaque surface also showed residual debris along some of the cut lines observed in the surface. The most graphic example of biofilm removal from the injection-molded PFA is shown in the *Salmonella* spp images in Figure 2(e) and (f). The untreated plaques show considerable fluorescence from cells and debris from the biofilm deposited on the plaque surface, and the treated plaques appear to remove all but a few residual cells and debris. These images indicate that substantial film removal did occur, although occasional large debris is noted. Despite the rougher surface of the rotationally molded PFA plaques, film removal was still accomplished relatively easily. The biofilm is very evident in the untreated images, while only a few cell clumps and debris particles remain after being treated. The smooth surfaces of the injection-molded PFA plaques appeared resistant to retention of all tested microorganisms.

Kynar® polyvinylidene fluoride (PVDF)
The PVDF material used in the testing was observed to behave similarly to the PFA in its resistance to biofilm adherence (Table 1). The PVDF exhibited a moderate level of background fluorescence that may have been the result of some nonspecific dye binding to residual media protein, polysaccharide film or cells (apparently reduced or eliminated in the washing step to remove excess dye after treatment), or excitation of the material by UV light. The images of the treated and untreated biofilms indicate that PVDF resists long-term biofilm attachment. The *Klebsiella pneumoniae* biofilms, with their attendant exopolysaccharide layer, left little residue after being treated. In the case of *E. coli*, little difference is seen between the untreated and treated, as little initial organism deposition is observed. These organisms do not secrete a polysaccharide capsule, thus do not adhere as well to the plaque surface. The organisms deposited on the plaques by the tap water likely did not adhere past the washing step in which excess medium was rinsed off prior to staining.

Borosilicate glass
The borosilicate glass plaques showed minor differences in biofilm formation between the pure culture organisms and between the treated and untreated surfaces (Table 1). Apparently, few detectable organisms were retained by the glass initially and those that remained after washing and staining were likely removed by water rinses. However, the biofilm growth resulting from tap water exposure was quite tenacious, resulting in a lower ranking for glass.

Silicone-coated glass
The silicone-coated borosilicate glass experiments revealed few microorganisms are retained by the silicone surface (Table 1). While biofilms do form on the plaques, as exemplified by the *Klebsiella* spp images, the film appears to be readily removed. Biofilms of lesser thickness and thus lower fluorescent intensity were formed on the silicone surface by *Salmonella* spp and *E. coli*. The images indicate that they were removed by the hypochlorite treatment; a few residual cells or debris from the tanks remain on the plaque.

Polypropylene
The *Klebsiella* spp bacteria left a film residue on the plaque surface that was not effectively removed by hypochlorite exposure, though many of the microbes initially on the surface appear to have been removed, Figure 2(g) and (h). While some film removal is observed in the *Salmonella* sp and *E. coli*-infected plaques after hypochlorite treatment, many residual cells were visible, indicating incomplete removal of the organisms. The biofilm deposited by the tap water bath also left some residual cells, indicating incomplete removal of bacteria by bleach treatment.

Based on the biofilm adhesion results, the ability of

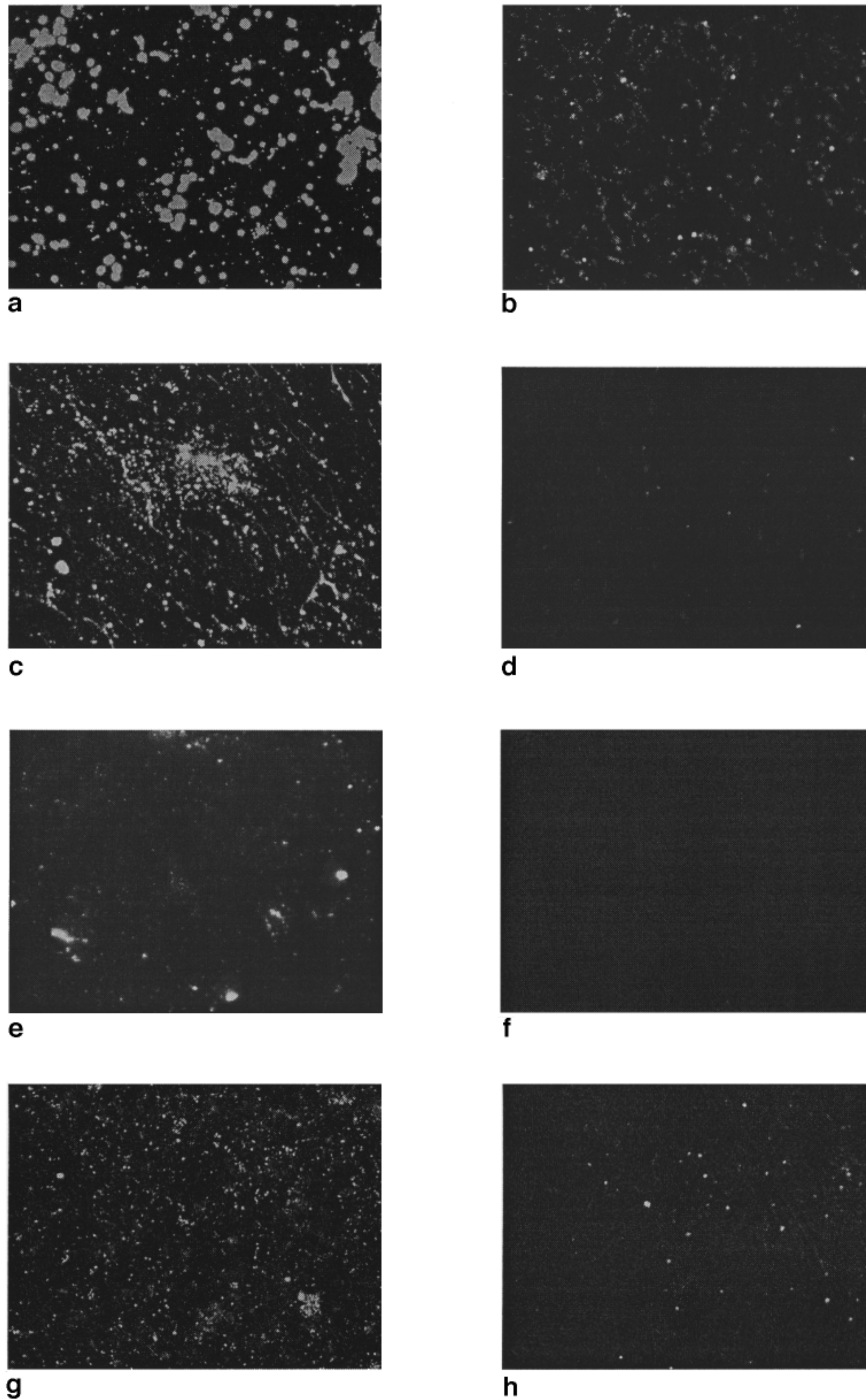

Figure 2 Fluorescent microscopy images at 200× of biofilms on various materials were rinsed with tap water (untreated) or with 50 ppm NaOCl (treated). Untreated (a) and treated (b) *Klebsiella pneumoniae* biofilms on stainless steel. Untreated (c) and treated (d) *Klebsiella pneumoniae* biofilms on injection-molded PFA. Untreated (e) and treated (f) *Salmonella choleraisius* sv *typhimurium* biofilms on PFA. Untreated (g) and treated (h) *Klebsiella pneumoniae* biofilms on polypropylene.

microorganisms to adhere to these surfaces (from highest to lowest) is stainless steel > polypropylene > glass, silicone, machined PFA, rotationally molded PFA > PVDF, injection-molded PFA.

Surface topography and contact angle

Atomic Force Microscopy

The results of the AFM measurements for the various surfaces are shown in Table 1. Two of the most common surface measurements are average surface roughness R_a and R_{ms}. Both of these measurements quantify the same feature. The results of this analysis indicate that the surface smoothness of the samples varies greatly depending on composition. The roughness order from highest to lowest is: rotationally molded PFA > machined PFA > PVDF > stainess steel > injection-molded PFA > polypropylene > borosilicate glass, silicone-coated glass.

These surface roughness measurements indicate that the samples' surface finishes can be considered to be very smooth (silicone-coated glass and borosilicate glass), smooth (polypropylene and injection-molded PFA), moderately smooth (stainless steel, PVDF, machined PFA) and rough (rotationally molded PFA).

Scanning Electron Microscopy

Figure 3(a), (b) and (c) shows the 200× photomicrographs of the PFA, stainless steel and polypropylene plaques, respectively. Based upon visual inspection of 200× images, the surface finishes were rated from roughest to smoothest as follows: rotationally molded PFA > machined PFA > injection-molded PFA > stainless steel > polypropylene > PVDF > silicone-coated glass, borosilicate glass. The qualitative results of the SEM analysis approximate the quantitative results of the AFM analysis.

Contact angle

The results of the advancing angle measurements for the samples are shown in Table 1. Based on the contact angle measurements, the following observations can be made:

(1) Stainless steel and borosilicate glass allow solutions (water or protein-based) to wet their surfaces better than do the polymeric materials.
(2) The silicone-coated glass surface has higher contact angle than does the uncoated glass samples. However, it has lower contact angle than the other polymer surfaces. PVDF, characterized by its vinyl surface chemistry, is the most hydrophilic of the remaining polymers that were tested. Polypropylene, with its polyolefinic surface chemistry, repels water to the same extent as the PFA materials. The PFA surfaces are very hydrophobic with only small differences seen between the various surfaces' topographies.
(3) The presence of protein and microorganisms causes the solution to wet more efficiently on all surfaces. However, these effects are slightly more noticeable in the peptone solution containing the *Klebsiella* bacteria. Both solutions appeared to change contact angle by 15–20 degrees on the polymer surfaces but to a lesser extent on the glass and stainless steel samples. The PFA plaques were the least affected of the polymer surfaces.

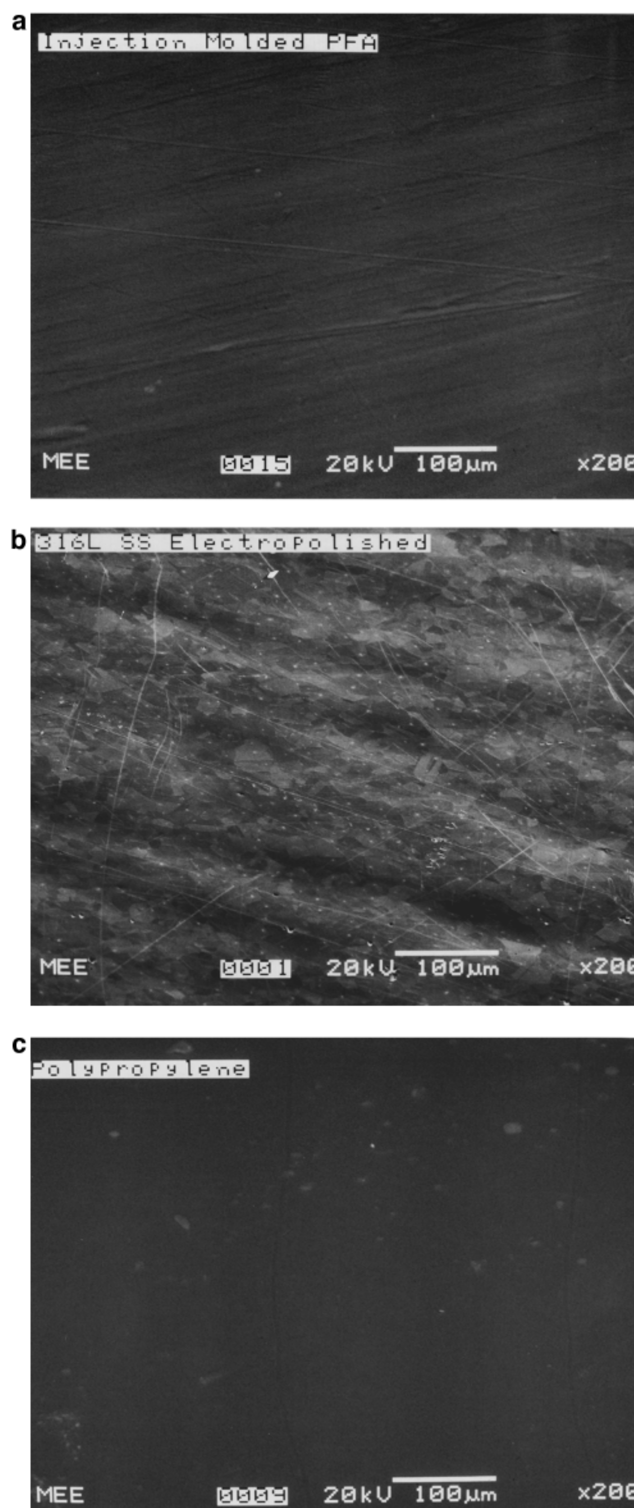

Figure 3 Scanning electron micrographs at 200× magnification of surfaces of (a) injection-molded PFA, (b) electropolished stainless steel and (c) polypropylene.

Discussion and conclusion

Previous studies have shown that both physical and chemical interactions with the substratum affect biofilm adhesion. The literature generally agrees that increasing substratum surface roughness will enhance the rate of biofilm adhesion [1,2,20,21]. However, the chemical mechanisms of the adhesion process seem to be much less universal. Some studies suggest that substratum surface tension and hydrophobicity, both derived from contact angle measurements, enhance adhesion [1,10,11,18,20] and other literature suggest the opposite [1,2,10,12,21]. Focusing on substrata influences on adhesion and considering this background, it is difficult to predict whether the chemical or physical characteristics of a particular substratum will predominate during the adhesion process. With the recent changes to both the chemistry and surface finish of fluoropolymers, it was necessary to experimentally determine their performance with common microorganisms. This study approximates the anticipated behavior of several materials in an industrial environment where the development of a biofilm may be intentional, or it may be the result of incomplete cleaning or accidental contamination. Whether the biofilm is desired or not, there is a need to efficiently and intermittently remove the biofilm.

Based on the ease of biofilm removal, this study determined the following propensity for substrata to develop tenacious biofilms that are not easily removed: stainless steel > polypropylene > glass > machined PFA, rotationally molded PFA, silicone-coated glass > PVDF, injection-molded PFA. The various substrata showed the same relative adhesion and removal characteristics for the four microorganisms examined. The differences in physical and chemical characteristics of the substrata are considered in the following discussion of these results.

Relative biofilm removal from stainless steel, glass and silicone is consistent with previous studies and industrial experience. The ease of biofilm removal from glass and silicone can be partially attributed to the very smooth surfaces. It also seems likely that chemical adhesion between microorganisms and glass is reversible when the polar surface is exposed to the ionic hypochorite treatment [7]. For glass and silicone, the biofilm development and removal process appears to be dominated by a reversible chemical process, with minimal mechanical adhesion on these consistently very smooth surfaces. The tenacity of the biofilm developed on glass exposed to tap water requires further study. For stainless steel, adhesion has been shown to be dependent on surface topography [21]. A partially reversible ionic chemical bonding similar to glass is also suspected. For stainless steel, the biofilm development and removal process appears to be dominated by mechanical adhesion, with the chemical processes taking a secondary role.

The three PFA substrata are chemically similar at the surface, as seen by the consistently high contact angles. However, the surface topography among the PFA samples was quite different, resulting in removal ease consistent with the expectation that a rougher surface enhances the entrapment of microorganisms. The conundrum arises in understanding the relative adhesion and removal behavior of PFA and those studies that showed high adhesion to PTFE.

There are two factors to consider regarding this apparent discrepancy; the focus of earlier studies and the physical differences between PFA and PTFE surfaces. First, this study shows mature biofilm development in virtually every situation, confirming the concept that biofilms, given time, are ubiquitous [6,19]. In addition, it focused on the ease of removal of the biofilm after intentionally growing substantial films. Other studies focus on the kinetics of initial film development [4,7,12,20] and note the adsorption/desorption of microorganisms on thin biofilms at equilibrium [1]. While initial kinetics and film removal are both adhesion phenomena, no references could be found showing a correlation. Second, commercially available PTFE films typically have rough, porous surfaces. The porosity is the result of shrinkage and void formation during crystallization when PTFE is compression molded. The rough surface finish is the result of skiving the solid block to form sheets. In one study the porosity of PTFE was shown to influence adhesion [19] and roughness has generally been shown to increase adhesion.

Therefore, mechanical mechanisms were more likely to play a role in initial adhesion to PTFE than to molded PFA. In conclusion, it is proposed that the smooth surface of the PFA and a tightly bonded C–F surface allowed the bacteria to land and build a biofilm in a nonturbulent environment with little mechanical or chemical adhesion to the substrata, thus allowing the biofilm to slough off.

This study shows that PVDF responds to adhesion and removal similarly to PFA. It is speculated that the mechanism for weak mechanical and chemical adhesion of PVDF to biofilms is similar to PFA.

Polypropylene, in spite of a smooth surface finish, maintained a significant biofilm even after chemical treatment. The adhesion mechanism appears to lie in a chemical attraction between the microorganism and the substratum. The surface of polypropylene in water is altered through oxidation, resulting in carbonyl and hydroxyl groups along the polymer chain [22]. These active sites offer the opportunity for ionic bonding and/or van der Waals attraction with the bacterial surface chemistry [19]. In conclusion, the biofilm development and removal process for polypropylene appears to be dominated by chemical processes with mechanical mechanisms taking a secondary role.

It is clear that biofilm adhesion and removal are not controlled by a single variable. When choosing a material for industrial processing, several factors must be considered including but not limited to: (1) the intended and incidental microorganisms involved; (2) potential for chemical interaction between the biological components and the substratum; (3) surface finish of the material; and (4) the chemical and physical capability of the material to handle all aspects of the manufacturing process. Many different materials are becoming available for use with critical fluid handling components in pharmaceutical and bioprocessing applications. Industry concerns about cleanability, as well as physical durability have kept some of these materials from serious consideration in critical manufacturing applications. However, based on the results of this analysis, the fluoropolymer materials with their corrosion resistance and

relatively high melting points, can be considered for use in transport systems where their resistance to tenacious biofilm adhesion, demonstrated by the ease of removal, outperform the more traditional materials such as stainless steel.

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