Neutrophil Chemotaxis on Silicone and Polyurethane Surfaces

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Silicone vascular catheters have a greater risk of infection and produce greater inflammation in vivo and greater complement activation in vitro than other vascular catheter polymer materials. This study investigated whether polymorphonuclear leukocyte (PMNL) chemotaxis under agarose on silicone surfaces is different than on polyurethane (PU). Glass slides were coated with silicone and PU by use of a constant-speed dipping apparatus. Chemotaxis (3 h) in response to (10^{-7} M) FMLP, zymosan-activated serum, and fresh serum (100%) was greater on silicone than on PU (P < 0.05). Polyclonal antibody to C5a blocked >50% of the movement toward serum (P < 0.05). Serum in the PMNL well significantly decreased chemotaxis toward FMLP on silicone (P < 0.05) but not on PU. These findings suggest that excessive complement activation by silicone may interfere with chemotaxis, but further work is necessary to determine whether this is relevant to an increased risk of catheter-related infection.

Materials and Methods

Preparation of polymer-coated slides. Silicone was prepared by dissolving dimethyl silicone elastomer dispersion (FGB001; Admiral Materials, Santa Barbara, CA) in toluene to a 10% total solids content. Silicone cross-linker was added in a 1:1000 ratio to the 10% base solids solution. PU was prepared by diluting PU base (Becton Dickinson Polymer Research, Sandy, UT) with an equal mixture of dimethylacetamide and methyl ethyl ketone to a 4% total solids content. Standard 25 x 76 mm glass microscope slides were coated with either silicone or PU with a constant speed (0.2 cm/s) vertical-dipping apparatus. Residual solvent was removed with a 186.68°C heat gun, and the slides were vacuum dried for 48 h. Scanning electron microscopy (×5000) demonstrated uniform smooth coats of slides and coverslips with both polymers.

Preparation of agarose. Agarose (Indubiose A37; Gallard-Schlesinger Chemical Manufacturing, Carle Place, NY) was dissolved at a concentration of 1.8% in sterile distilled water by boiling and then placed in a 47°C water bath. Heat-inactivated human serum (HIS; 56°C for 30 min) and 2× MEM with Hanks’ salts and 1-glutamine (Grand Island Biologicals, Grand Island, NY) were combined in a 21%/79% MEM ratio and placed in a 37°C water bath. After equilibrating to their water bath temperatures, equal volumes of the agarose and HIS/MEM preparations were mixed. The agarose mixture was immediately poured into 35 x 10 mm polystyrene plates (Falcon 1008; Becton Dickinson, Lincoln Park, NJ) and over polymer-coated and uncoated glass slides to a thickness of 3–4 mm; plates and slides were refrigerated at 4°C for 30–60 min so that the agarose could solidify.

Sets of 3 wells, each 4 mm in diameter and 4 mm apart, were cut into the agarose on slides by use of a template and stainless steel punch (figure 1). Similarly, with the petri dishes a different template was used to cut sets of 3 wells, each 2.4 mm in diameter and 2.4 mm apart, as described elsewhere [9]. Agar plugs were removed from the wells by use of a pipet suction device, carefully avoiding distortion of the wells or scratching the surface below the agarose.

Preparation of neutrophils. Blood was drawn by venipuncture from human volunteers into sterile plastic syringes with 20 U of...
heparin sodium per milliliter of blood. An equal volume of 3% Dextran was drawn into the syringe, which was inverted several times and allowed to sediment at room temperature for 30 min. The supernatant was removed and centrifuged at 150 g for 10 min. The supernatant was discarded, and the pellet was suspended in Hanks’ balanced salt solution (HBSS) without calcium or magnesium (Grand Island Biologicals). This was layered over Isolymph (Gallard-Schlesinger) and centrifuged at 1400 g for 40 min. The pellet, containing residual red blood cells and neutrophils, was mixed sequentially with equal volumes of 0.2% and 1.6% sodium chloride for red blood cell lysis. After centrifugation at 150 g for 10 min, the supernatant was discarded and the step repeated if a visible red blood cell layer was still present. The pellet was suspended in HBSS, and an aliquot was counted by Coulter counter. The neutrophils were resuspended in HBSS containing gelatin, calcium, and magnesium (gHBSS) to a final concentration of 5 x 10^7 cells/mL. Trypan blue staining of cells revealed 98%–99% viability.

Chemotaxis assay. The center (neutrophil) well of each set of 3 wells received a 10-μL volume of neutrophil suspension (i.e., 5 x 10^6 cells/well); 2 experiments also included the addition of 10 μL of undiluted fresh human serum to the neutrophils in this well. One outer (chemotactic) well received a 10-μL volume of one of the factors being studied: FMLP, zymosan-activated human serum (ZAS), fresh human serum, fresh human serum diluted 1:1 with either rabbit serum containing polyclonal antibodies to C5a (Sigma, St. Louis), nonimmune rabbit serum, or HIS. ZAS and HIS were prepared as described elsewhere [2]. One experiment also included the addition of equal volumes of undiluted fresh human serum and FMLP to this well. The other outer well received a 10-μL volume of gHBSS to determine random migration. The plates and slides were incubated for 3 h at 37°C in a 5% CO2 environment.

Cellular movement was stopped, and the cells were fixed by addition of 2.5% glutaraldehyde for ≥45 min. The agarose was then carefully removed, and the plates and slides were stained with Wright’s stain, rinsed with distilled water, and air dried. Neutrophil migration distances were measured by ×40 magnification (light microscope with ×4 objective and ×10 ocular lens micrometer). Results are expressed as a chemotactic differential (CD; mm × 10) measured as follows: distance that neutrophils migrated toward the chemotactic stimulus − random migration. Each experiment used at least 3 different slides of each polymer type for each condition and was repeated at least twice. Chemotaxis on polystyrene was done as described elsewhere [10].

Data analysis. Statistical analysis of the data (expressed as mean ± SD) was done by analysis of variance, Student’s t test, and Mann-Whitney rank sum (Minitab; State College, PA). P < .05 was considered significant.

Results

Chemotactic activity of FMLP on PU and silicone surfaces. We used 4 molar concentrations (10^-9, 10^-8, 10^-7, and 10^-6 M) of FMLP as chemotactic stimuli to determine the concentration

![Figure 2](image)

Figure 2. FMLP dose-response curves for neutrophil chemotaxis under agarose on surface of glass slides coated with silicone and polyurethane. Bars represent SE.
that produced maximal directed cellular movement (CD) on both a PU and a silicone surface (figure 2). The maximum CD for both PU (16.3 ± 3.1) and silicone (30.0 ± 2.7) occurred at a 10^{-7} M concentration of FMLP. This concentration was used for all subsequent experiments in which FMLP was used as a chemotactic stimulus.

Chemotaxis to standard chemotactic stimuli on PU and silicone surfaces. The chemotactic response to ZAS and FMLP stimuli on PU and silicone surfaces is shown in figure 3. The CD with a ZAS stimulus was greater (P = .018) on silicone (13.3 ± 3.4, n = 6) than on PU (8.0 ± 2.6, n = 5). Similarly, the CD with an FMLP stimulus was greater (P = .0002) on silicone (33.0 ± 4.2, n = 6) than on PU (18.33 ± 1.5, n = 6). When serum plus polyclonal rabbit antibody to C5a (1:1 ratio) was used as the stimulus, chemotaxis on silicone was reduced compared with serum alone (CD for serum, 19.6 ± 19.7 vs. CD for serum + antibody, 8.7 ± 16.5; P = .03, Mann-Whitney). Non-immune rabbit serum had no effect.

Chemotactic response on four surfaces. We measured the chemotactic response to ZAS, serum (100%), and HIS (100%) on 4 surfaces—polystyrene, glass, PU, and silicone (figure 4). With a ZAS stimulus, the CD was greater on silicone than on polystyrene (P = .002), glass (P < .0001), and PU (P = .0003). Similarly, with a serum stimulus, the CD was greater on silicone than on polystyrene (P = .004), glass (P = .001), and PU (P = .038).

On silicone, the CD with a ZAS stimulus (16.7 ± 4.4) was greater than with a serum (100%) stimulus (13.6 ± 4.9; P = .03). The CD was significantly less (P < .001) on all 4 surfaces when HIS was the substance used in the chemotactic well compared with ZAS and serum (n = 8 for all conditions).

Presence of serum in the neutrophil well: effect on chemotaxis toward different stimuli on PU and silicone surfaces. Because in vivo polymorphonuclear leukocyte (PMNL) chemotaxis occurs in a serum environment and because we have shown that silicone activates complement in serum 10-fold greater than PU, the effect of serum in the PMNL well on chemotaxis was determined (figure 5). With an FMLP stimulus (n = 7 or 8 for each condition), serum in the PMNL well significantly decreased the CD on a silicone surface (P = .017) but not on a PU surface (P = .32). By use of a serum stimulus (n = 8 for each condition), serum in the PMNL well produced a marked reduction in the CD on both PU (P = .0005) and silicone (P < .0001) compared with no serum in the PMNL well. When we used FMLP plus serum as the stimulus (n = 8 for each variable), serum in the PMNL well produced a significant reduction in the CD on both PU (P = .0002) and silicone (P = .0009). Notably, with FMLP plus serum as the stimulus, the CD was greater than when the stimulus was FMLP or serum alone on both PU and silicone surfaces (P < .05).

Discussion

In 2 previous studies, greater inflammation around silicone catheters [1] and greater complement activation by silicone catheters in vitro [2] in comparison with other catheter materials caused us to speculate that silicone surfaces might generate a greater chemotactic gradient. Indeed, in this study, we found that neutrophils had greater chemotaxis on a silicone surface toward a serum or ZAS stimulus than on PU, polystyrene, or glass surfaces. This difference could be abolished by heat-inactivation of the serum and significantly inhibited by pretreatment of the serum with polyclonal antibodies to C5a (P < .05), suggesting strongly that the source of the chemotactic gradient was C5a. Thus, greater C5a production by silicone surfaces in vivo is the likely explanation for the greater inflammation seen around silicone catheters [1].
Because PMNL chemotaxis toward bacteria on polymer surfaces in vivo is likely to occur in a serum environment, we attempted to simulate those conditions in vitro. FMLP was used to represent a bacterial chemotactic gradient, because neither washed bacteria nor overnight bacterial supernatant produced adequate PMNL chemotaxis in our system (data not shown). With serum in the PMNL well and FMLP as the stimulus, chemotaxis was significantly reduced on silicone in comparison with no serum; this did not happen on PU. This suggested that the greater complement activation by silicone surfaces might actually interfere with PMNL chemotaxis. It can be postulated that, although greater C5a production by silicone surfaces attracts large numbers of neutrophils, as the neutrophils get near the source of complement activation (that is, silicone surfaces and very highly localized concentration of C5a), their motility is ultimately inhibited.

There is clinical precedent for excess complement activation by a synthetic material having an adverse in vivo effect on PMNL. Early hemodialysis membranes produced acute pulmonary edema shortly after dialysis initiation [11]. Additional investigations showed that the mechanism for this phenomenon was excess complement activation with C5a formation and resultant intravascular agglutination of PMNL [12]. Fernandez et al. [13] found that high concentrations of C5a could inhibit chemotaxis, suggesting that PMNL surface killing on silicone might be inhibited by high C5a concentrations. Inhibition of chemotaxis by high concentrations of the chemotactic stimulus correlates with loss of the normal bipolar morphology of PMNL undergoing chemotaxis [14, 15], increased PMNL adhesiveness [16], and the presence of an extremely flattened appearance with prominent ruffled membranes [16]. Because microorganisms can activate complement by both the classical and alternate pathways [17–21], their presence on the surface of silicone catheters may augment baseline complement activation.

An alternative explanation for the effect that localized, excessive complement activation by silicone might have on the risk of infection is that it might locally deplete the ability to opsonize microorganisms. While opsonization is not necessary for phagocytosis to occur on surfaces, lack of complement will significantly interfere with bacterial killing [22–25]. Significant complement depletion has been demonstrated in vivo both in the pleural space [26–28] and adjacent to a foreign body [29]. Further work will be necessary to determine whether excessive complement activation by silicone is the explanation for the increased risk of infection previously demonstrated.

In summary, we developed a new method that allows for the evaluation of chemotaxis on polymer surfaces. It incorporates a method of measuring chemotaxis under agarose utilizing polymer-coated slides rather than a polystyrene dish as the surface on which chemotaxis occurs. Our findings suggest that neutrophils can move well on silicone surfaces in response to a variety of stimuli and do not appear to be inhibited by any mechanical properties of the silicone surface. They also suggest that complement activation on silicone surfaces contributes to differences in chemotaxis on these surfaces and thus may play a role in the differences in neutrophil killing and incidence of infection observed in previous studies.

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References


