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Materials and surface properties optimization to prevent biofouling of a novel bacterial concentrator

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Abstract

Traditionally, bacterial pathogens in the blood have been identified using culture-based methods that can take several days to obtain results. This can lead to physicians making treatment decisions based on an incomplete diagnosis, which can increase the patient’s risk of death. One major limitation to the development of faster diagnostic methods is that bacteria are found in very low concentrations in the blood, thus to detect them one either needs to increase their numbers by culturing the bacteria or effectively isolate and concentrate them from the blood. We are developing a device that purifies as few as 10 bacteria from 10 milliliters of whole blood, and concentrates the bacteria into a ~30 microliter volume through a combination of preferential cell lysis and centrifugation steps. To enable the efficient capture of so few bacteria in a sea of billions of blood cells, the material properties of the device were optimized. Critical parameters include material selection, manufacturing method, surface roughness, and surface chemistry. In this paper, we present the design of this novel concentrator, optimization of these parameters, and the achieved performance.

1. Introduction

Bacteremia refers to the condition when viable bacteria are present in the circulating blood. As blood is normally sterile, any live bacteria in the blood is abnormal. Bacteremia can occur as a consequence of a surgical wound infection, a contaminated prosthetic devices implanted in the host, or a severe infection at another location in the host (meningitis, pneumonia). Sepsis is the result of an overwhelming systemic inflammatory response caused by an infection [1]. It is a cascade of events that are initiated with an infection and can end in the dysfunction of microcirculation that result in organ failure and/or death. Approximately 2–11% of patients in hospital intensive care units become septic [2]. Sepsis is 13th in overall causes of death in the United States (9% of all deaths) with annual estimates of 751,000 cases of severe sepsis [1-3]. It is predicted that cases of sepsis will continue to escalate because of an aging American population, a rise in antimicrobial resistance, a growing immuno-suppressed population, and an increase in the use of invasive catheters and prosthetic materials [4].

The recovery of the infecting microorganisms from the blood specimen is crucial for proper diagnosis and treatment of infection. To obtain accurate results, it is necessary to maximize the number of organisms collected from a given sample. This can be challenging...
due to the fact that the concentrations of pathogenic organisms in the blood are often quite low [5, 6]. In bacteremic patients, the concentrations of bacteria can range from 1–100 cfu/mL (colony forming units per milliliter), but can be up to $10^3$ cfu/mL in severe cases [7-9]. In contrast, the concentration of red blood cells is on the order of $10^5$ cells/mL.

When developing bacteremic diagnostics, bacterial adhesion to the surface is an important factor to consider. Bacterial adhesion is a multifaceted process that is dependent on the organism and its environment. Unfortunately, the molecular and physical interactions that govern adhesion are not yet understood in detail [10]. Nonetheless, it is known that specific and non-specific interactions affect the bacteria’s ability to attach to a surface [11-14]. It follows that the properties of the surface, specifically the chemical composition and roughness, play an integral role in determining the bacteria’s attachment to a material.

Bacterial adhesion is influenced by the chemical properties of the material’s surface. Materials have a diverse range of functional groups that can change bacterial adhesion depending on their hydrophobicity and charge. For example, stainless steel, titanium, and titanium alloys are all frequently employed for osteosynthesis implants; however, stainless steel implants are known to have greater bacterial adhesion (infection rates) than titanium implants [15, 16]. In work by Speranza et al., Escherichia coli’s preference to adhere to polyvinyl chloride (PVC), low density polyethylene (LDPE), and polymethylmethacrylate (PMMA) was explored. E. coli, a Gram negative rod, has a negative charge at neutral pH and a hydrophilic membrane. Due to these characteristics, E. coli’s partiality to adhere to PVC, LDPE, and PMMA decreased, respectively, as each of these polymers have decreasing acidic character [17]. However, for many materials, the surface chemistry is quite complex since many commercially available materials have trace impurities and surface-active additives. These constituents can complicate the interaction of bacteria to the surface and can result in greater ambiguity concerning the types of functional groups present at the surface and how bacteria will react to them [18, 19].

The material’s surface roughness plays a large role in bacterial adhesion. Generally, surface irregularities foster bacterial adhesion whereas smooth surfaces discourage adhesion because irregular surfaces have a greater surface area and the depressions in the roughened surfaces provide an advantageous location for colonization [20-23]. In an experiment by Taylor et al., bacterial adhesion was tested over a wide range of roughnesses on PMMA. Large augmentations in roughness produced by silicon carbide paper (grades P400 and P120) had no significant effect in adhesion compared to the smooth surface. But, with a slight increase in surface roughness using silicon carbide paper P1200, a significant increase in bacterial adhesion was observed [24]. In another study, when a material’s surface roughness had sub-bacterial dimensions, it reduced the material’s surface area accessible to bacteria. The researchers concluded that these features decreased the probability of bacteria interacting with the surface and/or with adhesive proteins adsorbed on the material. In other words, a properly structured surface could resist bacterial adhesion and biofilm formation such that when fluid flowed over the surface, it removed bacteria more efficiently from a nano-textured surface than a smooth surface [25]. Consequently, it is necessary to investigate surface chemistry and surface roughness as factors for bacterial adhesion when developing diagnostics for bacteremia.

2. Results and discussion

2.1. Material Selection

A bacteria concentration device must be made of a material that minimizes bacterial adhesion so that the low numbers of microorganisms in the bacteremic blood are not lost to device surfaces. The following experiment investigated simple conical devices made of various types of materials to assess the affinity of bacteria to the surfaces. The conical devices were all machined in-house. After a bacteria solution was added to the devices, they were centrifuged to concentrate the microorganisms to the apical end of the conical devices. This procedure drove the bacteria into the surfaces of the devices with a force of 3200 x g, presumably promoting adhesion. Next, the bacteria were removed from the bottoms of the devices and quantified. The empty devices were also vigorously rinsed with water and this liquid was analyzed to quantify the number of bacteria loosely adhered to the material’s surface. This quantification made it possible to know what materials were most resistant to bacterial adhesion; the highest recoveries corresponded to the least adhesive materials.

Figure 1 shows the results of this experiment. The lower portions of the bars represent the percentage of bacteria removed with a single aspiration while the upper portions show the percentage of bacteria that required an additional rinse to be removed. The total recoveries from most materials hovered around 80%, however, acrylic devices gave a total recovery greater than 100%. This is possible because bacteria can grow over the course of the experiment and all bacterial counts contain a certain amount of error. Some materials, like Delrin, had relatively high recoveries, but with the majority of that quantity coming from the rinse.
This suggests that the bacteria adhered more tightly to Delrin and required additional effort to be removed.

Similar to the previous experiment, bacteria were added to the devices and centrifuged. However, in this experiment, the bacteria were spiked into 100 µL of whole blood and 900 µL of the detergent Tween-20 (0.005% [v/v] concentration). Tween-20 was chosen to lyse blood components while leaving bacteria intact. By combining blood, bacteria, and a lysing agent, the experimental system more closely resembled an actual bacterial concentration device. Additionally, the use of a removable collection drawer underneath the device chamber provided for more targeted sample collection. After the device was centrifuged, bacteria were recovered and quantified from the collection drawer (10 µL), the chamber (100 µL volume directly above the drawer), and the chamber rinse (100 µL). The goal was to determine if machined or molded chambers maximized the bacteria recoveries.

2.2. Material Processing

Machining processes, such as boring and drilling, are relatively easy ways to shape a material into a desired geometry. However, even with sanding and polishing, it is difficult to make the surface of a machined polymer as smooth as a molded polymer’s surface. This is especially true for a soft plastic like polypropylene. In the following experiment, machined and molded devices were compared to determine which type of surface trapped the least bacteria. Unlike in the previous experiment, where the devices were simple cones, this experiment used open-ended funnels that fed into collection drawers. Figure 2 shows that both devices had nearly identical geometries, but the device in Figure 2a was machined out of polypropylene (R_a 0.63 μm, R_z 2.1 μm), while the device in Figure 2b used a molded polypropylene insert as the chamber (R_a 0.39 μm, R_z 1.3 μm). The surfaces of these devices are also shown at 10X magnification.

Figure 3 displays the results from the comparison of machined and molded parts. It shows that recoveries in both the chambers and collection drawers were higher when the molded chambers were used. For example, the total recovery from the chambers, rinses and drawers was 93% when the molded chambers were used, compared to 55% when the machined chambers were used. One reason for this difference may be that the roughness values for the machined chambers were nearly twice as large as those obtained for the molded chambers. The machined surfaces had larger microscopic irregularities, which could have promoted bacterial adhesion than the smoother molded surfaces. Also, it is interesting to note that a greater total number of bacteria were recovered from the chambers of the molded devices than the machined devices. One possible
interpretation could be that in the machined chambers the bacteria were so strongly attached to the rougher surfaces that they could not be recovered. These devices were intended to concentrate the bacteria and the molded chambers accomplished this better than the machined chambers.

Fig. 3. Bacteria recovered from various areas of molded and machined polypropylene concentrator devices.

3. Conclusions

By testing variations of our devices, we were able to optimize the material and surface properties necessary for maximizing bacterial recovery under centrifugation. We concluded that polypropylene was the easiest material to sterilize and the most cost effective material for our purposes. Using polypropylene in the next set of experiments, we were able to ascertain that smooth, molded surfaces were preferable over machined surfaces for the best bacterial recovery when centrifuged. Consequently, when designing a device that concentrates bacteria under centrifugation, one composed of molded polypropylene will generate the best outcome.

4. References