All-Silicone Consumables For the Study of Medical Device Biofilm Colonization

New plate type with flow channels constructed entirely from PDMS silicones.

Silicones in medical device applications

Since the 1960s, silicone rubber has found widespread use in medical, aerospace, electrical, construction, and industrial applications. Flexibility over wide temperature ranges, good resistance to compression, a wide range of durometers, and inert and stable compounds are among the reasons for its popularity. Common silicone medical components and assemblies include airways; balloon catheters; tubing for feeding, drainage, and use with peristaltic pumps; compression bars; electrosurgical handpieces; infusion sleeves and test chambers; introducer tips and flexible sheaths; wire/fluid-path coextrusions; ear plugs and hearing aids; shunts and septa; and a variety of seals, stoppers, valves, and clips.

Silicone rubbers are synthetic polymers with an unusual molecular structure and a giant backbone of alternating silicon and oxygen atoms. This structural linkage is similar to that found, for example, in a mineral such as quartz, and silicones have superior heat resistance compared with other elastomers.

The challenge of catheter biofilm infections

When catheter materials are colonized by bacteria in the clinical setting, serious complications can arise. For example, different types of central venous catheters (CVCs) are used in clinical practice to improve the quality of life of chronically and critically ill patients. Unfortunately, indwelling devices are usually associated with microbial biofilms and eventually lead to catheter-related bloodstream infections (CLABSIIs). An estimated 250,000-400,000 CLABSIIs occur every year in the United States, at a rate of 1.5 per 1,000 CVC days and a mortality rate of 12-25%. The annual cost of caring for patients with CLABSIIs ranges from 296 million to 2.3 billion dollars. Biofilm formation occurs on biotic and abiotic surfaces in the clinical setting (Figure 1). Extensive studies have been conducted to understand biofilm formation, including different biofilm developmental stages, biofilm matrix compositions, quorum-sensing regulated biofilm formation, biofilm dispersal (and its clinical implications), and multi-species biofilms that are relevant to polymicrobial infections. The proper in vitro modelling of such biofilms needs devices that can simulate the in vivo conditions in terms of shear forces, nutrient delivery, and substrate material.

Figure 1-Silicones (PDMS) are commonly used as a medical device material in catheters, shunts, pacemakers and other medical devices (A). The development of material coatings and treatments to reduce encrustation and biofilm formation on these surfaces is an active area of research.

Biofilm applications in the BioFlux System

Bacterial biofilm experiments were some of the first applications for the BioFlux System due to the need for...
flow and shear control in biofilm studies. A big advantage of this highly parallel flow system is the fact that 24 independent biofilms can be established per plate, simultaneously and under controlled shear conditions. With the BioFlux Quattro add-on, 4 plates, or 96 independent biofilms can be inoculated and established at the same time. This is especially useful when multiple conditions or compounds are to be tested, as forming the biofilm can take a few days. An example is shown in Figure 2, from one of our customer’s publications (Nance et al, 2013). Note that increasing compound concentrations lead to increasing bacterial death as observed by a live/dead stain using Syto 9 and propidium iodide (green are alive and red are dead bacteria). IC50 curves can be assembled from these data via image analysis.

In addition to looking at compound efficacy for the destruction of established biofilms, a number of researchers study both surface coatings (Li et al, 2013) and compounds (Singh et al, 2012) intended to prevent the establishment of biofilms in the first place. Here, the most important phase is bacterial adhesion, and that can be studied as a function of applied shear. Unlike volume displacement devices (syringe pumps, peristaltic pumps), BioFlux uses controlled gas pressure differentials to drive flow. This gives users the ability to change the shear rate very quickly and accurately to study adhesion as a function of the shear force applied.

**Silicone bottom plate architecture**

Our aim was to provide a primary attachment and growth surface with silicone chemistry (PDMS) to mimic catheter and other silicone medical device surfaces. For BioFlux plates currently in use, the primary growth surface is a glass (borosilicate) coverslip, 170μm thick. This provides excellent compatibility with all microscopy imaging modalities. In order to convert to silicone, a PDMS layer is molded against highly polish surfaces to create a flat substrate that is 250um thick across the full device bottom and secures the channels (Figure 3). The increased thickness is necessary in order to maintain mechanical stability and is within the compatibility zone for commonly used objectives. Another advantage of this new plate type is the resistance to mechanical stress during transport and experimentation. These stressors can lead to cracking of the cover slip glass in traditional BioFlux devices but will only result in temporary elastic deformation in the silicone version of the device.

![Image of plates](image_url)

Figure 2- BioFlux plates were developed to ensure excellent image quality, including the possibility of confocal imaging of the entire channel volume as seen the image above (Nance et al, 2013). This enables a determination of the prevalence of the live and dead bacteria throughout the biofilm in response to various compound concentrations. All-Silicone plates enable similar imaging modalities, albeit through a PDMS silicone bottom layer as opposed to glass layers.

**Modeling catheter infections using primary media**

PDMS bottom 48-well 0-20dyn plates (910-0115) were used to model a catheter-affiliated biofilm. Primary human urine was collected and sterilized using a 0.2 μm filter. Urine was used to prime the BioFlux plate, cultures were seeded into the viewing channel from the outlet well, cells were incubated to allow adhesion, and urine was later perfused from the outlet (should be inlet?) well at 0.15 dyn. Images were taken in brightfield every 20 minutes at 100x magnification using the BioFlux 1000z imaging station. Mixed cultures of Pseudomonas aeruginosa and E. coli had robust biofilm growth and a dynamic phenotype. Staphylococcus aureus was also able to adhere and colonize the PDMS surface (Figure 4). Videos of this and other biofilms growing can be viewed at:
Conclusions

- Silicones, of which polydimethylsiloxane (PDMS) is the most commonly used, are a key component of many medical devices due to superior biocompatibility and material stability.
- The formation of bacterial biofilms on these surfaces is an active area of research, due to the high importance of biofilms in medical device infections.
- The recently introduced silicone bottom plates feature all-PDMS flow channels to address this need, and enable a full range of experimental protocols utilizing silicone substrates.
- Catheter infections can be modeled using primary media including human urine with BioFlux PDMS bottom plates.
- The BioFlux microfluidic platform allows for reduced sample volume enabling the use of primary media including: saliva, plasma, urine, and tears.

References


Ordering Information

<table>
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<th>Product</th>
<th>Catalog No.</th>
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<td>BioFlux 200 48 well silicone bottom plates 0-20 Dynes - Qty 1</td>
<td>910-0115</td>
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<tr>
<td>BioFlux 200 24 well silicone bottom plates 0-20 Dynes - Qty 1</td>
<td>910-0116</td>
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Figure 4- BioFlux all PDMS (silicone) plates were used with primary human urine to model catheter affiliated infections. Show above: negative control (A), *Pseudomonas aeruginosa* and *E. coli* co-culture (B), and *Staphylococcus aureus* grown over 13 hours.