Consumables for custom material - biofilm interactions studies under flow

New BioFlux plate types allow users to control physiological parameters and observe bacterial attachment and biofilm growth over a variety of substrates.

The challenge of biofilm-substrate interactions

Biofilms are a resilient pathogen source for infection-related failure in medical devices. For example, for implants, infection-related failure rates range from 0-1% for dental and orthopedic implants to as much as 20% for transcutaneous pins (Yue et al, Fig 1). Another example are indwelling devices such as catheters, which are often associated with microbial biofilms and eventually lead to catherer-related bloodstream infections (CLABSIs). An estimated 250,000-400,000 CLABSIs occur every year in the United States, at a rate of 1.5 per 1,000 central venous catheter days and a mortality rate of 12-25%. In environmental applications the material choice, finish and coating options need to be optimized to inhibit biofouling. The need to grow biofilms onto non-standard surfaces also applies to anti-biofouling applications.

In addition to the dispersal of mature biofilms, a promising approach is the prevention of resilient biofilm formation on various surfaces, staring with bacterial attachment and continuing with matrix growth. 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. Previous studies have used coupons placed in macro-scale bioreactors to achieve substrate testing. Here we present a novel micro-scale system based on the BioFlux that can look at virtually any material but preserve the advantages of high parallelism, shear force, temperature and gas control.

Biofilm applications in the BioFlux

Bacterial biofilm experiments were among the first applications of the BioFlux system due to the need for flow / shear control and parallelism for experiments.

![Figure 1: Biofilms on implant surfaces are a serious threat in the development of infection post surgery. The development of materials and material coatings to reduce encrustation and biofilm formation on these surfaces is an active area of research. Key examples are (a) dental implants (b) hip arthroplasties and (c) external fixators. (Reproduced from Yue et al.).](image1)

![Figure 2: BioFlux plates were developed to enable physiological biofilms grown using physiological media (human saliva) under flow. Confocal 3D imaging of the entire channel cross section was shown in the publication above. This enables a determination of the prevalence of live and dead bacteria throughout an oral biofilm in response to various treatments. Reproduced from Kolkerlerman et al. 2015.](image2)
that take a long time to complete. Up to 24 biofilms can be established per traditional BioFlux plate, at the same time and under controlled shear conditions. When coupled to the BioFlux, the BioFlux Quattro module further increases the capacity of the BioFlux from 24 flow cells to 96 flow cells, thus providing the system with high throughput capabilities. This is an unmatched throughput level for high complexity flow experiments.

Another key advantage is the ability to use 1ml or below of media per experiment – this enables the use of ex-vivo bodily fluids such as saliva or blood as a physiological media, and the deployment of murine models. For animal models in general, BioFlux adoption aids adherence to the 3R principle through the dramatic reduction of the material required (reducing the number of animals required) and improved physiological relevance of ex vivo experiments.

Key applications have been orthopedic and dental implants. An example experiment in Figure 2, is reproduced from Kolderman et al. looking at dental biofilms with human saliva being used as media source. Note that increasing compound concentrations and combinations of compounds lead to increasing bacterial death as observed by a live/dead stain. IC50 curves can be assembled from the data via image analysis. Thus far, materials tested have been limited to glass and (more recently) silicone, a common catheter and medical device material. Unlike volume displacement devices (syringe pumps, peristaltic pumps), the BioFlux uses controlled gas pressure differentials to drive flow. This gives users the ability to change the shear rate very quickly and accurately study adhesion as a function of the shear force applied.

**Custom bottom plate architecture and feasibility studies**

Our aim was to provide a primary attachment and growth surface with a user defined material, finish and coating to mimic any medical device surface. For BioFlux plates currently in use, the primary growth surfaces are either a glass (borosilicate) coverslip or a PDMS layer. In order to provide access to a custom material surface, the PDMS bottom device (Fig 3A) is modified thought the addition of a port approximately 3mm in diameter, and surrounded by a pressure sensitive adhesive surface. Any custom substrate can then be adhered to close the bottom of the chamber (Fig 3B) and create a leak-proof flow conduit.

![Figure 3](image-url)

**Figure 3:** Schematic comparison of the traditional BioFlux plates (A) and the new custom substrate plate (B). While the traditional plates were developed to ensure immediate compatibility with a majority of imaging systems, the new consumables maintain optical access while providing the user with a fully customizable growth surface.
Such devices are assembled by using each pair of flow channels as inputs. For example, in Fig 4A, you can see the position of one access port in the top down view with respect to the inlet/outlet wells of channels number 3 and 4 in the over 48-well pattern. The devices are delivered with a protective layer covering the PSA (pressure sensitive adhesive area). This layer is removed, and custom substrates can be sealed to the underside by the user (Fig 4B).

Feasibility data was generated by adhering several glass slide substrates (25x25x0.75mm) to the underside of custom substrate BioFlux plates. After device priming, growth chambers were inoculated with E. coli (strain K-12 BW25113). Overnight E. coli biofilm growth was readily observed in both the feeder channels and on top of the glass substrate. The ability to establish mature biofilms over the entire area of the substrate (about 3mm diameter) with a high success rate was demonstrated by this feasibility data set.

Conclusions

• The formation of bacterial biofilms on various surfaces is an active area of research, due to the high importance of biofilm control in a number of settings
• Newly enabled research includes medical device infections, in particular infections of dental implant, orthopedic implant and in-dwelling device surfaces and environmental biofilms in industrial settings
• The recently introduced custom substrate plates feature the ability to add any material to the bottom of microfluidic flow chambers, and enable a full range of experimental protocols utilizing custom substrates

Bibliography


Ordering Information

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<th>Product</th>
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Figure 5: In this example experiment, a glass slide substrate was attached as shown in Fig. 4. Bacteria (E. coli strain k-12BW24113) was introduced from left to right (orange arrows), with media flow in the opposite direction (right to left). Biofilm images were acquired after 24 hrs. at a low flow rate. Biofilm is shown to cover the full substrate region (yellow dashed line circle). In addition, biofilms have established in seeding channels as well.