Development of an in *vitro* novel device that simulates the real life of the biofilm formation on catheters under both static and continuous fluid flow systems

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Introduction;

Biofilm formation commonly associated with many infections and health problems are usually a serious concern for clinicians because of their resistance to a wide range of antibiotics [1,2] and to clearance by humoral or cellular host defense mechanisms [3]. It has been estimated that 65-80% of all human infections are biofilm-related [4]. The problem of treating biofilm-associated infections is not only attributed to the difficulty of eradicating the biofilm focus but also to the lack of susceptibility of cells disrupted from the biofilm to antimicrobial agents [2]. Disruption of the biofilm, which occurs during the removal of colonized catheters or infusing fluid through them, normally results in entrance of bacteria or groups of bacteria into circulation causing bloodstream infections [2].

Materials and Methods

Configuration of the device

The device can be set up flexibly in different configurations to suit the studied conditions as follows:

Static system

The device can be configured to test the biofilm under static condition by simply using it as a closed system (Fig. 1A). Ports 2 and 3 are blocked while port 1 is used to inoculate the chamber and provide the system with the culture medium.

Dynamic cell flow system

The biofilm can be kept under continuous flow of fresh medium provided from a reservoir via IV infusion pump and IV tubing set. The medium is allowed to flow through the lumen of the catheter before passing to the chamber. Port 1 is used as the inlet, port 2 is blocked while port 3 is used to collect the overflowed liquid which contain planktonic and cells dispersed from the biofilm .

The device could be configured to work as chemostat-like dynamic cell flow system by installing bacterial filter in port 3 . It could also be configured to mimic *in vivo* antimicrobial activity of a chemotherapeutic agent against the biofilm in pharmacokinetic biofilm model in a dynamic cell flow system (Fig.1B).

Validation of the device to support biofilm formation

The device was configured to test biofilm formation of *S. epidermidis* and *C. albicans* under static and dynamic conditions as mentioned above. For biofilm formation under static condition, 24 hours old culture of *S. epidermidis* or *C. albicans* were used to inoculate Tryptic Soya Broth (TSB) or Yeast Nitrogen Base (YNB) media supplemented with 2% dextrose respectively. The initial inoculum size was standardized in the media to give 1-5 X 10^6 CFU/ml. Peripheral venous catheter was placed in the sample insert. The culture medium, with either microorganism, was pumped into the catheter to fill the chamber. The device was then incubated at 37°C for 48 hours after which the catheter was washed by passing sterile phosphate buffered saline (PBS) (pH 7.3) at 30 ml/hour for 2 hours to remove the planktonic (free-floating) cells. The catheter was aseptically removed using sterile forceps and cut to 1 cm segments in microbiological safety cabinet. The biofilms on the catheters were washed and dislodged by sonication in 1 ml ice-cooled PBS at 30% cycle, 3.5 output for 30 seconds followed by vortexing for another 30 seconds. The number of sessile cells was determined by viable counts on SAB agar or TSA medium and calculated as colony forming unit (CFU)/$cm^2$of the catheter surface. In another set of experiments, the device was configured to test the biofilm formation under dynamic condition as mentioned above

Results

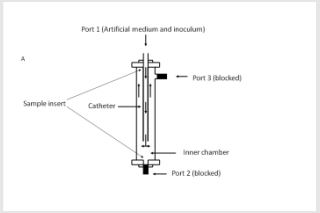

Fig 1 A & B: Configuration of the biofilm device under static and dynamic fluid flow systems The device is configured to create biofilm environment under static condition (A) or under continuous fluid flow system (B).

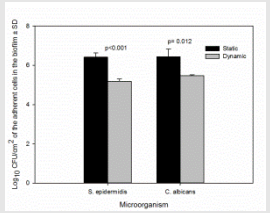

Fig 2:Validation of the device to support biofilm formation of *Streptococcus epidermidis* and *Candida albicans* on intravenous catheter under static and continuous fluid flow

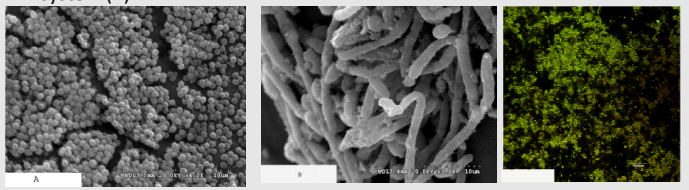

Fig. 3: SEM showing the biofilm architecture of *Streptococcus epidermidis* (A) and *C. albicans* (B) on vascular catheter segment.

Fig. 4: Visualization of the viability of *S. epidermidis* cells within the biofilm environment using Confocal Scanning Laser Microscopy (CSLM)

Conclusions

The presented device is simple and can be fabricated from cheap materials. It is not only simulating the real biofilm environments, but also could be modulated to contain most catheter and tubes and readily allows biofilm formation under different experimental conditions. The design permits low laminar flow system which is common in studying biofilms in flow cell systems. Accordingly, it is well suited to study the real life of the biofilm formation by bacteria and yeasts.

References

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