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DEPARTMENT OF IMMUNOLOGY AND MICROBIOLOGY

A simple method for size fractioning of biofilm aggregates for better control of experiments

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Purpose:

The role of biofilm aggregates on the outcome and aetiology of chronic infections is continuously being investigated. However, *in vitro* aggregate models often also contain planktonic bacteria. Vice versa, presumably planktonic cultures often contain many aggregates. We aimed to develop a method to separate planktonic cells and aggregates and further to separate aggregates by size for better control of *in vitro* and *in vivo* experiments on the role of aggregates.

Method:

We approached this by filtration using cell strainers with varying mesh sizes. Week old shaken culture of *Pseudomonas aeruginosa* was passed through the filters and aggregates and single cells were collected either in the filtrated liquid or by backwashing the collected fractions from the filters. Aggregate fraction were then washed in saline to reduce the number of remnant single cells and small aggregates.

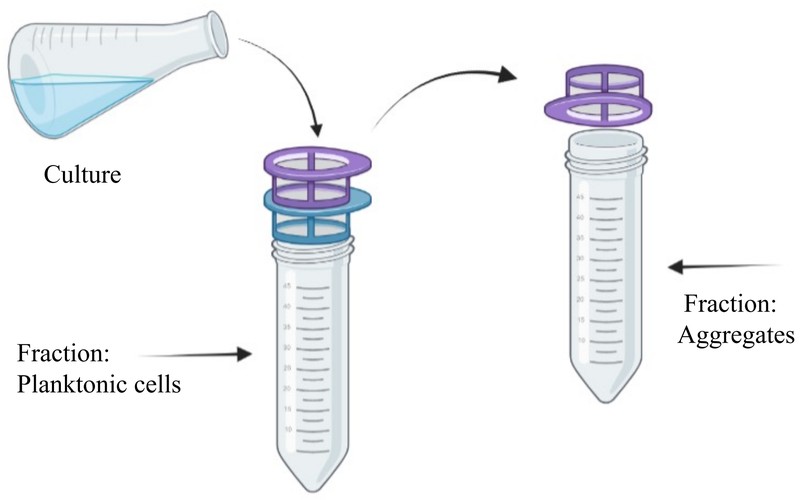

Figure 1: Schematic drawing of the experimental set-up. Filters from Pluriselect with mesh sizes 30 and 10µm are connected to a 50 ml falcon tube. A culture is poured over the filters, planktonic cells and aggregates smaller than >10µm will be in the filtrated liquid collected in the falcon tube and aggregates larger than > 10µm will be collected in the filters. Aggregates larger than >30µm courted in the 30µm filter will be isolated by backwashing the filter into a new falcon tube.

Results:

We show that by this simple mechanical filtration approach, we can enrich aggregates >30µm such that they constitute 92.5% of the viable biomass. Likewise, the filtrated planktonic fraction contained 95.5 % of the viable biomass.

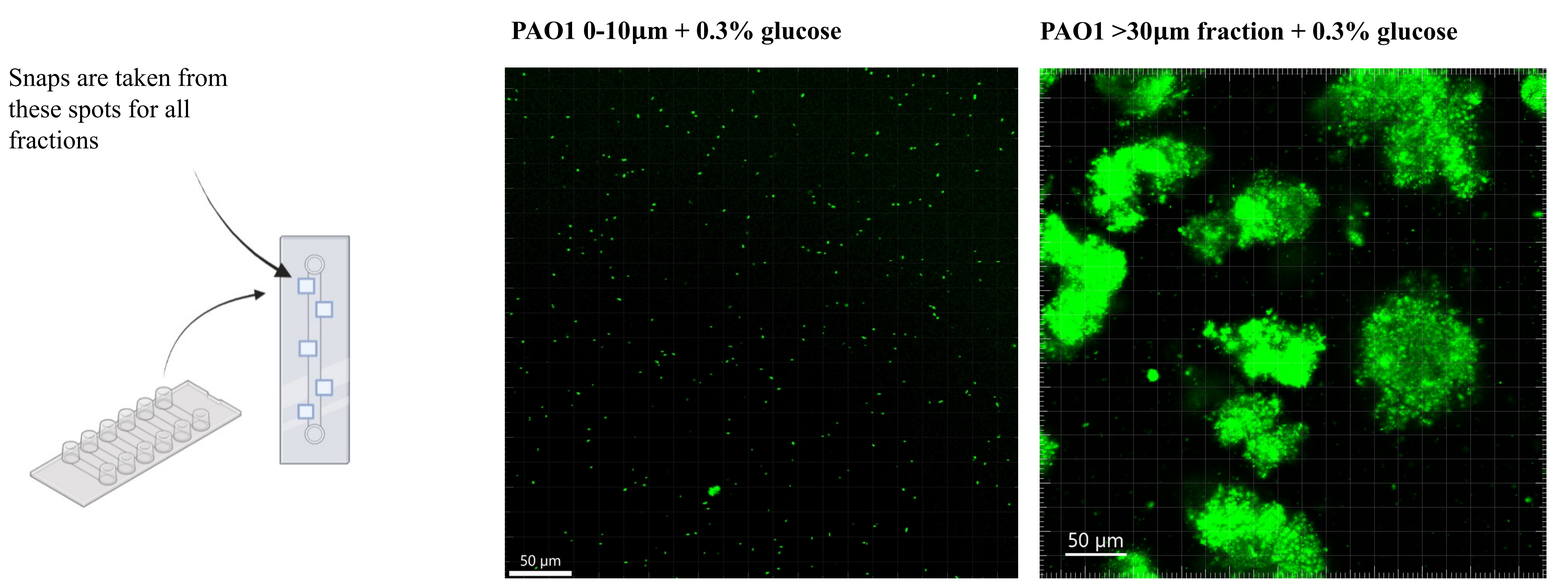

Figure 2: Confocal images of the fractions. 60µl of the fractions stained with Syto9 are added to Ibidis µ – slides and analysed using Zeiss LS880 confocal microscope. Left image: Schematic illustration of an Ibidi µ – slide and where in the wells snaps are taken. Right images: Representative pictures of the fractions 0-10µm and >30µm.

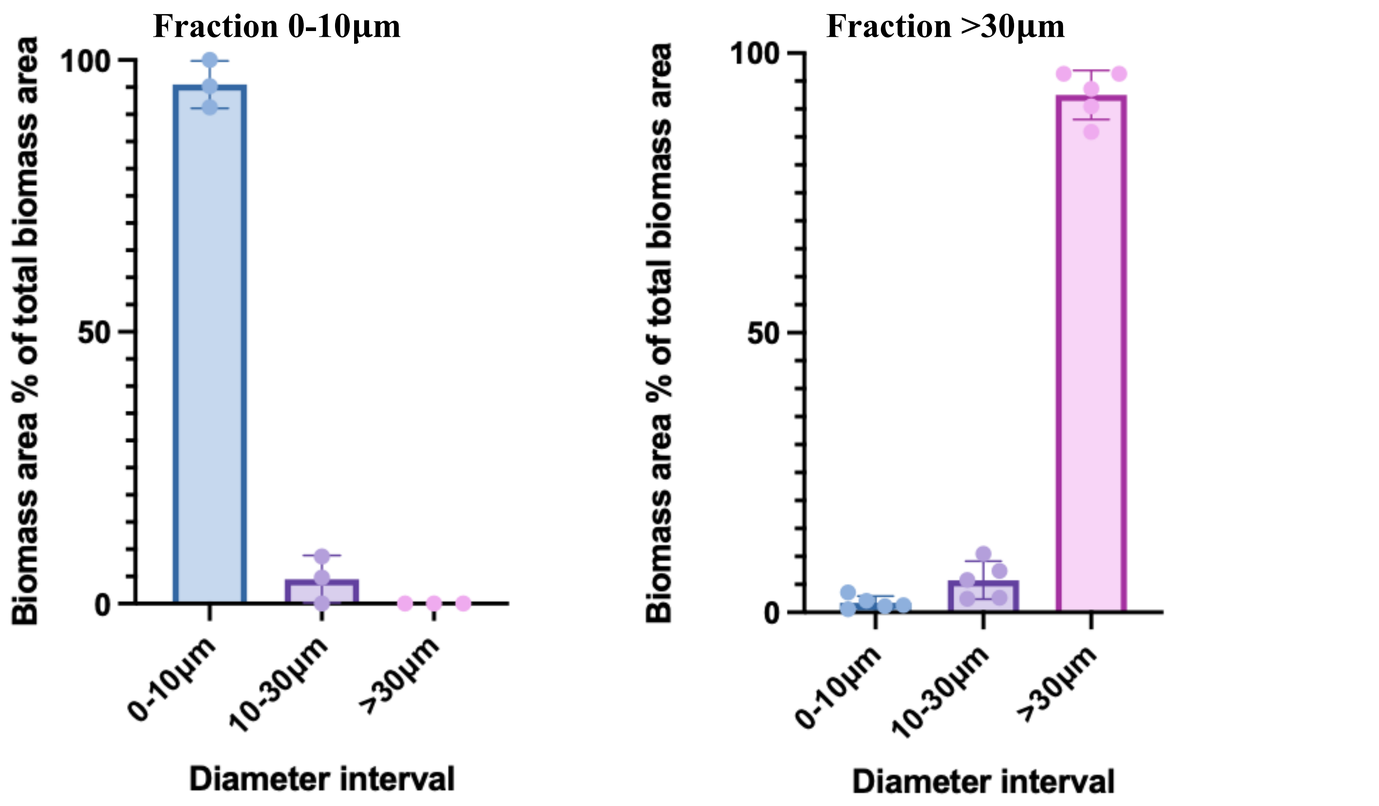

Figure 3: Biomass distribution within the fractions. The total biomass and the amount of biomass that belongs to different diameter intervals of aggregates are determined using R Studio. Fraction 0-10**µm:** 95,5% of the total biomass within the fraction corresponds to cells and aggregates with a diameter between 0 and 10µm**,** 4,5% of the total biomass of the fraction corresponds to aggregates with a diameter between 10 and 30µm, and none of the biomass within the fraction corresponded to aggregates larger than 30µm, results originate from 3 biological replicates. **Fraction >30 µm** 92,5% of the biomass corresponds to aggregates larger than >30µm, and 5,7% corresponds to aggregates with a diameter between 10 and 30 µm. Lastly, 1,8% of the biomass corresponds to cells and aggregates with a diameter between 0-10µm, results originate from 5 biological replicates.

Conclusion:

This simple filtration technique makes it feasible to isolate biofilm aggregates from planktonic cells. The culture is fractionated into aggregates and planktonic cells, enabling one to investigate the effect of planktonic- and/or biofilm lifestyle in multiple settings.