P0028
Poster Session I
News from the fungal frontier

IMPEDANCE AND TOXIN RELEASE STUDIES OF BACTERIAL BIOFILMS DEVELOPED IN CONTINUOUS FLOW CONDITIONS

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OBJECTIVES

The development of a platform for growing bacterial biofilm in flow conditions is necessary to understand the real behaviour of bacterial adhesion and the toxin release during the biofilm formation. We developed a device with a flow channel and gold electrode sensors in the bottom of this channel to use Electrical impedance spectroscopy (EIS). This approach is very efficient to monitor the biofilm development in real-time and the toxin release with lipid vesicles as a biomarker. This vesicles are synthetized with an internal dye (carboxyfluorescein) and are introduced in the device throw the inlet channel that feed fresh media to the biofilm constantly. When the biofilm becomes toxic the vesicles are lysed and the dye liberation can be detected easily with a fluorescence plate reader. Also absorbance can be monitored at the same time to follow the micro colony liberation from the biofilm. This setup is suitable for a triple detection during the biofilm formation and maturation.

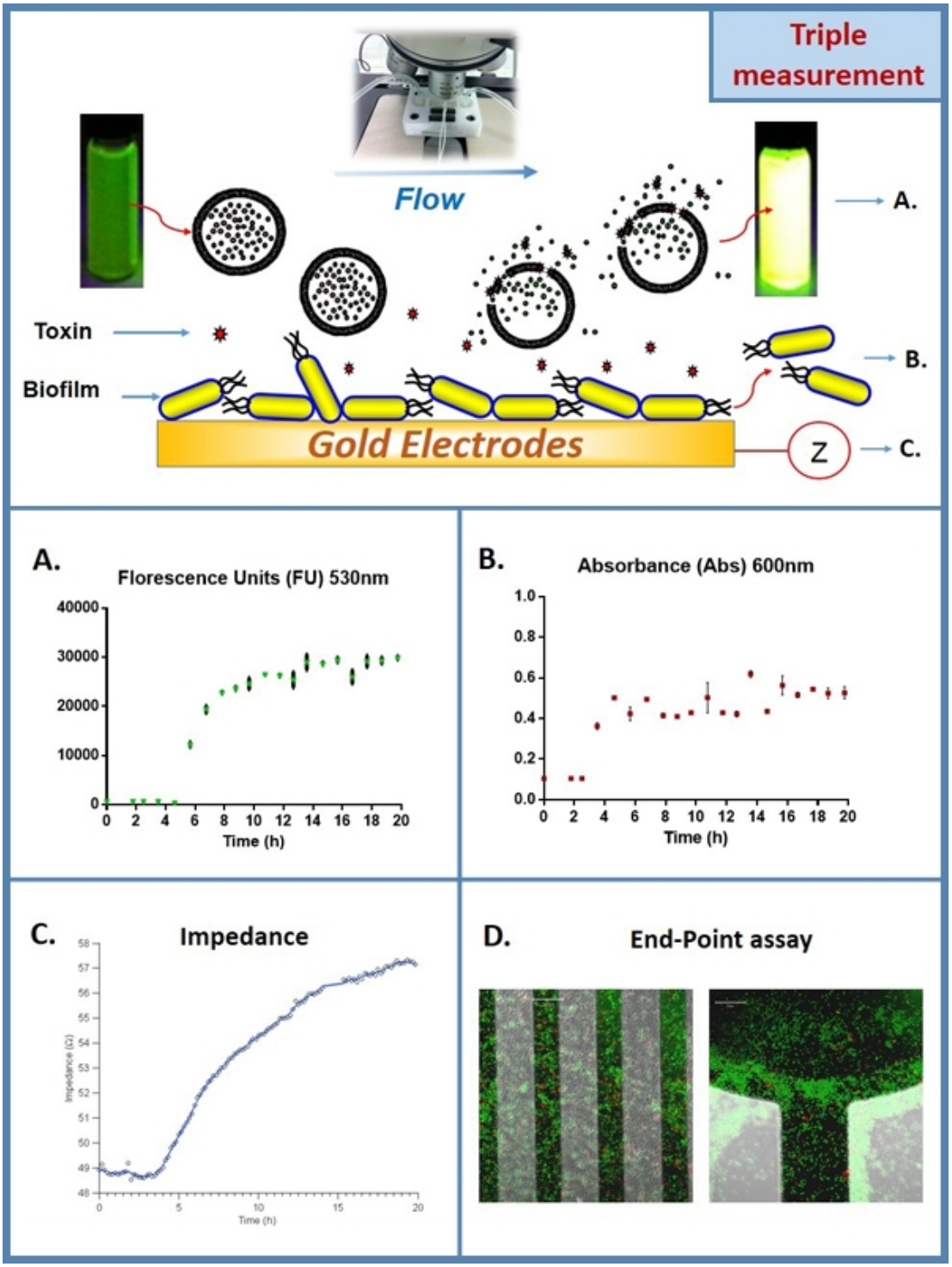
METHODS

The construction of the platform is based on fluidic devices for biofilm growth with flow channel and Impedance data analysis with interdigitated gold electrodes. The design is printed with a 3D printer on Polycarbonate. The assembly of the different parts of the device guaranties a constant flow of Tryptic Soy Broth on a glass surface with gold electrodes. Onto this surface the pathogenic bacterial strain *Staphylococcus aureus* V329 is developed and studied.

The vesicle synthesis was carried out with a mixture of phosphocholine (PC) lipids, phosphoethanolamine (PE) lipids, and cholesterol dissolved in chloroform and following a procedure reported by Marshall, SE., et al, (2013).

RESULTS

The final results from this experiment allow us to measure in real time biofilm development with EIS (Fig 1C) and measure the signal of the toxin release from the attached bacteria (Fig 1A). However the most important result is that we can detect and increase of biofilm toxicity around 6 hours which is the same point of the beginning of the exponential phase in the biofilm profile. Furthermore around 6 hours the Absorbance profile is stable (Fig 1B) which means that micro colonies are being detached to colonize new surfaces. The end point is presented as a adhesion confirmation in Figure 1D.



CONCLUSION

This methodology is suitable to form biofilms rapidly thanks to the continuous feeding of fresh media culture and this property allows the introduction of additional biomarkers to support the EIS data. The results are extremely important because the different profiles of the triple measurement indicates that the stable biofilm detach micro colonies constantly and the exponential growth of the community comes with a constant toxin released to environment to create the perfect niche for a perfect viability of the pathogenic bacterial strain.