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SPIDERS: Surface Printing to Investigate Drug Effects on Real Surfaces

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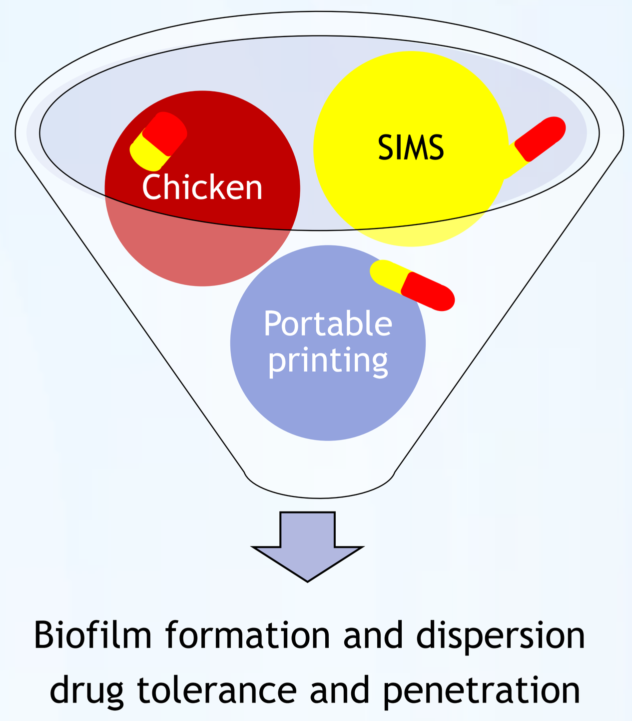
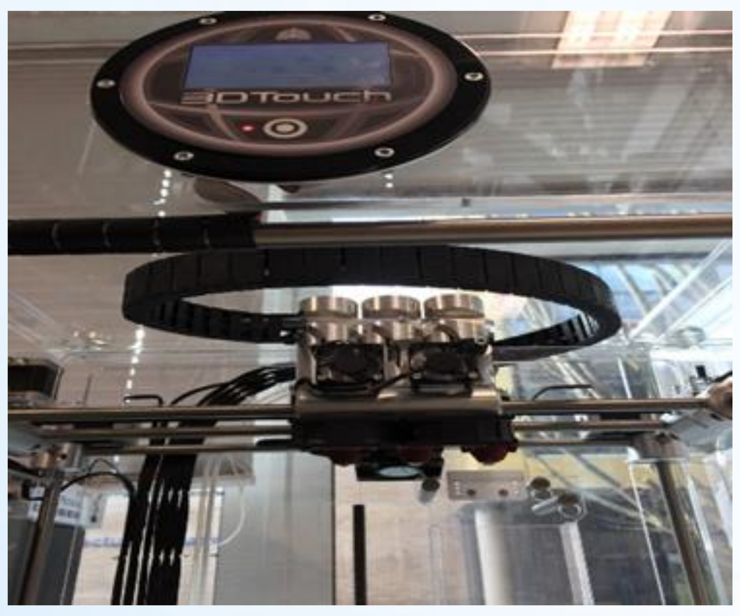
Introduction

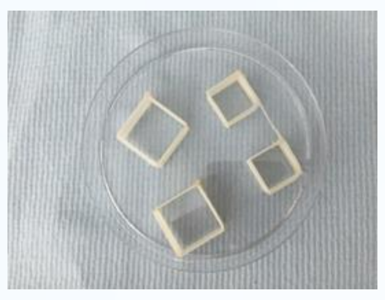
Bacterial resistance to antimicrobials is exacerbated by their propensity to form biofilms on inorganic and organic surfaces. Bacterial resistance and tolerance of antimicrobials has been studied in 'flow cells': compartmentalised devices in which bacteria are suspended and grown and to which antimicrobials can be introduced in a controlled manner. Introducing the real world into flow cells and recreating natural bacterial biofilms in them is difficult. We have used 3D additive manufacture to print flow cells directly onto the biofilm of interest or on a natural surface, which we can then grow biofilms on. Using our bespoke multi-chambers we have so far demonstrated biofilm formation, liquid-tight seals onto biofilms, dynamic biofilm dispersal across chamber bridges, and we are currently investigating antibiotic tolerance and resistance.

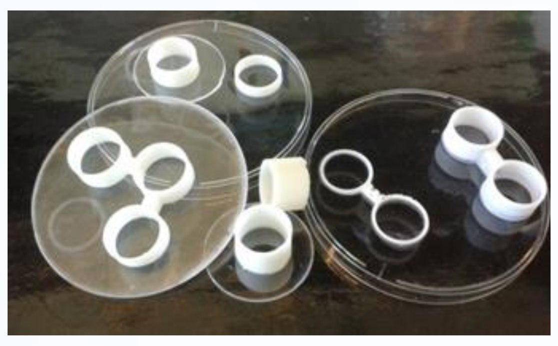


Techniques

3D additive surface printing







3D additive printing, using a 3D touch printer, of square and round devices with single and multi-chambers, connected with open bridges to allow the passage of liquid.

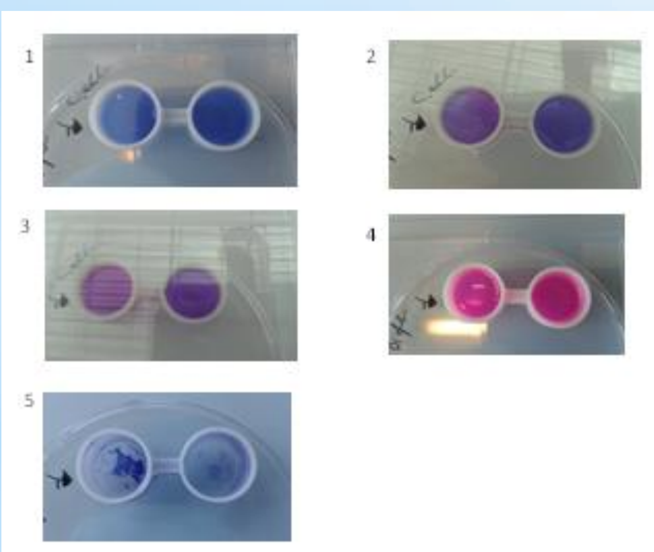
Detection of biofilms on real surfaces

Bespoke multi- chamber 3D additive printed device with bridge connectors on the surface of chicken skin.

Natural biofilm on the surface of chicken skin, stained with crystal violet dye. The rod shapes are the individual bacteria within the biofilm.

Generation of biofilm within the 3D printed device

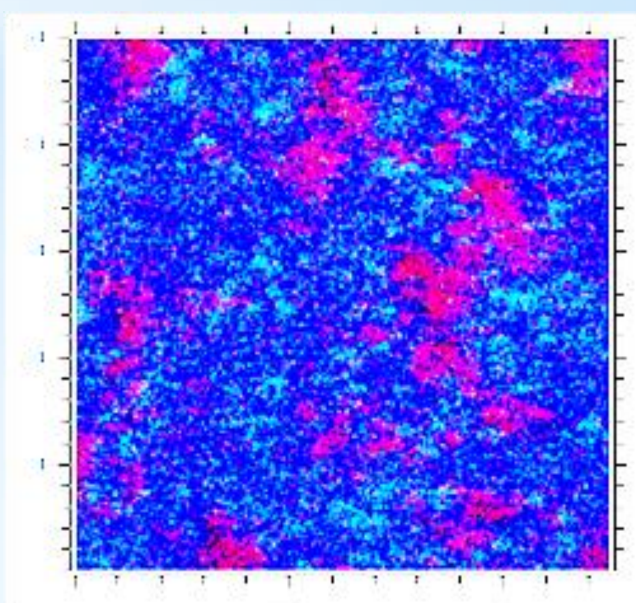
Dispersion and re-establishment of the biofilm detected by measuring the bacterial metabolic activity using resazurin.



Crystal violet staining of all components of a developed biofilm within the printed device

For the 2 well device a biofilm was developed in one side of the device. After 24hrs the planktonic (free floating) bacteria were removed and the device rinsed. Fresh media and resazurin was added to both sides sufficient to cover the bridge. With time the colour changed to pink- measuring the metabolic activity of the bacteria. Gradually the sterile side turned pink indicating the movement of bacteria through the bridge (1-4). After 24hrs both sides were stained with crystal violet showing a new biofilm had been established on the previously empty side (5).

Surface image analysis of a biofilm



The Tof-SIMS (Secondary Ion Mass Spectrometry) analyses image is the overlay image of three ions (blue – phospholipid fragment (common to all bacteria), red – a substrate, green – a lipid fragment of a particular bacterial strain. This was performed on a TOF-SIMS V mass spectrometer (ION-TOF GmbH,Münster, Germany) using a 25keV Bi3+ primary ion beam.

Conclusion

We have demonstrated that we can grow biofilms within our bespoke chambers 3D printed onto surfaces of interest and that we can measure biofilm dispersion through connecting bridges (using rezasurin which measures metabolism colourmetrically).