Assessing the formation of biofilms us a UKAS accredited CDC reactor model and qPCR method to detect the upregulation of biofilm forming genes

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Introduction

Bacteria existing as part of a biofilm have increased resistance to antimicrobial agents and are more likely to cause persistent infections than planktonic bacteria. This study aimed to track the expression of *Staphylococcus aureus* genes known to be involved in biofilm formation over a period of up to 72 hours and on three different surfaces.

Rho, the transcription termination factor, was selected as a reference gene to normalise sample input. Six genes known to be essential for biofilm formation or involved in biofilm formation, *icaR*, *fnbA*, *spa*, *pyrR*, *clfB* and *ureA*, were selected for testing to cover a range of functions, including metabolism, adhesion and gene regulation. Biofilms were grown in a CDC reactor on polycarbonate, stainless steel and copper coupons to investigate whether gene expression differed for attachment to the different surfaces. Identification of a group of biofilm forming genes with expression which can be clearly detected in a defined situation could allow the assessment of the ability of anti-biofilm products to prevent biofilm formation.

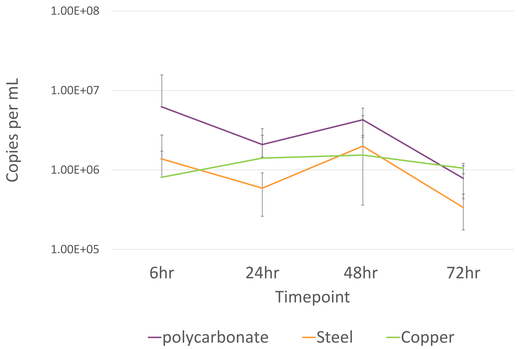
Methodology

- CDC Bioreactor containing polycarbonate, steel and copper coupons was inoculated with 1.0 x 10^7CFUmL^{-1} *Staphylococcus aureus* strain NCTC 8325. was incubated for 72 hours with samples taken at 6, 24 and 72 hours.
- DNA and RNA were extracted using Qiagen's Allprep PowerViral DNA/RNA isolation Kit. RNA samples were treated with Dnase I using Dnase Max kit.
- Reaction number for each samples/timepoint was N=4. All reactions contained 2µL RNA, 10µL RT-SYBR green Master Mix, 0.2µL Reverase transcriptase 5.8µL RNase Free water 1µL and a final reaction concentration of 0.5µM of each Forward and Reverse primer
- The reaction was started with reverse transcription at 50°C for 10 mins, initial denaturation at 95°C for 2 mins, and 540 amplification cycles of 95°C for 5 seconds and 60°C for 10 seconds. qPCR products were analyzed by melting curve for unspecific products or primer dimer formation all genes produced single peaks and single products.
- Expression of 6 selected biofilm related genes at 24, 48 and 72 hours was calculated relative to the calibration at 6 hours and the reference gene (*rho*) to normalise sample input.

Results

The quantity of viable organisms attached to each coupon surface was assessed at each time point and the DNA copy number per mL was measured. This was shown to have very little variation between the time points for each surface. The recovery from polycarbonate coupons was slightly higher than stainless steel and copper, suggesting that this surface is more favourable for bacterial attachment.

The adhesion gene *clfB* shows dramatically differing patterns by surface type. Polycarbonate surfaces showed dramatic increase up to nearly 1000 fold expression compared to 6 hours. The steel surface remained relatively consistent and close to expression at 6 hours with minor fluctuations over 72 hours. The expression of *ureA* showed a similar pattern of expression over 72 hours on all three surfaces; however, expression was considerably higher in cells attached to the polycarbonate coupons.

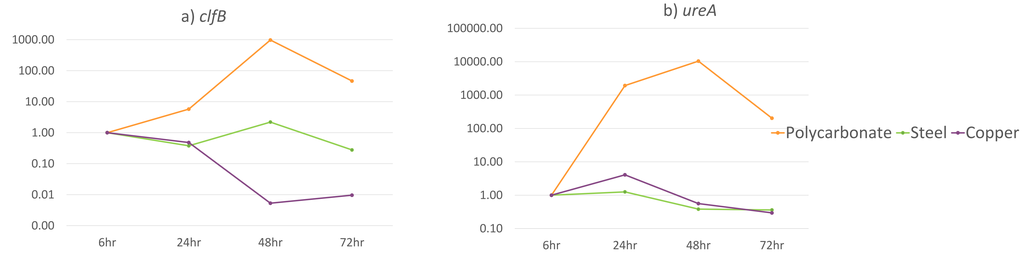


Graph 1: DNA copy number per mL isolated from each coupon surface over time.

Relative quantity of biofilm genes compared to reference gene *rho*

Gene	Type	Polycarbonate				Steel				Copper			
		6hr	24hr	48hr	72hr	6hr	24hr	48hr	72hr	6hr	24hr	48hr	72hr
rho	**Ref**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
fnbA	TAR	1.00	0.07	0.07	0.06	1.00	0.43	1.22	0.44	1.00	0.48	1.49	0.20
pyr R	TAR	1.00	1.81	21.57	1.53	1.00	0.29	0.20	0.01	1.00	797.60	108.00	36.33
clfB	TAR	1.00	5.75	975.30	46.54	1.00	0.38	2.20	0.28	1.00	0.48	0.01	0.01
ureA	TAR	1.00	1924.00	10410.00	203.30	1.00	1.26	0.38	0.36	1.00	4.07	0.56	0.29

Table 1: Relative quantity change in transcriptional levels of biofilm target genes in culture of *S.aureus* isolated from polycarbonate steel and copper surfaces at 24, 48 and 72 hours. Ref = Reference Gene, Tar = Target gene of interest. Relative quantities were calculated by relative standard curve method. The 6-hour timepoint was chosen as the calibrator samples for analysis. Data are representative of two independent experiments the representative values were means of quadruplicates. All analysis was performed using QREX (Qiagen) Gene expression software.



Graph 2: Relative quantity of biofilm forming genes per gene. a) *clfB* b) *ureA*. Fluctuation of relative quantities of 6 biofilm gene transcripts over 72 hours. Data are representative of two independent experiments the representative values were means of quadruplicates.

Discussion and Conclusions

These results suggest that the polycarbonate surface would be the optimal surface for assessment of the expression of biofilm forming genes. Polycarbonate coupons have an uneven surface which would be more favourable for bacterial attachment.

Assessment of the expression of a biofilm forming gene, for example *ureA*, with and without a treatment would give an indication of whether a product was effective at preventing biofilm formation.

This project was carried out by Perfectus Biomed Limited
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