Method for detection and quantification of bacterial adherence to wound dressings using qPCR and gene expression analysis

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Introduction

The mode of action for commercial wound dressings may aim to physically remove bacterial load from a wound through attachment to a dressing surface. Analysing transcription of key genes involved with the attachment process alongside bacterial quantification can aid in the understanding of dressing mode of action.

Aim

To quantify *Pseudomonas aeruginosa pelA* and *rsaL* adhesion gene transcription following application of a commercial wound dressing when compared to a standard gauze.

Methodology

A *P. aeruginosa* inoculum (1x10^6 CFUmL^{-1}) was prepared and 100 µL applied to the surface of a commercial wound dressing, standard gauze and a negative control (plastic film). After 24 hours static incubation at 37°C, planktonic bacteria were removed by washing. Adhered bacteria were recovered in 5mL of sterile PBS by sonication, and mRNA was extracted for gene transcription profiles using RT-qPCR.

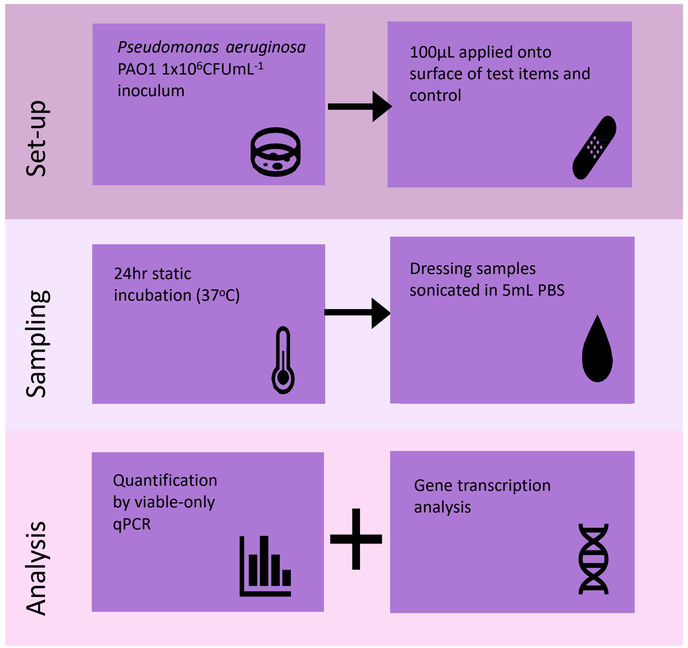

Figure 1. Methodology for the set-up, sampling and analysis of gene regulation and quantification following up to 24 hour *Pseudomonas aeruginosa* incubation on the surface of a wound dressing.

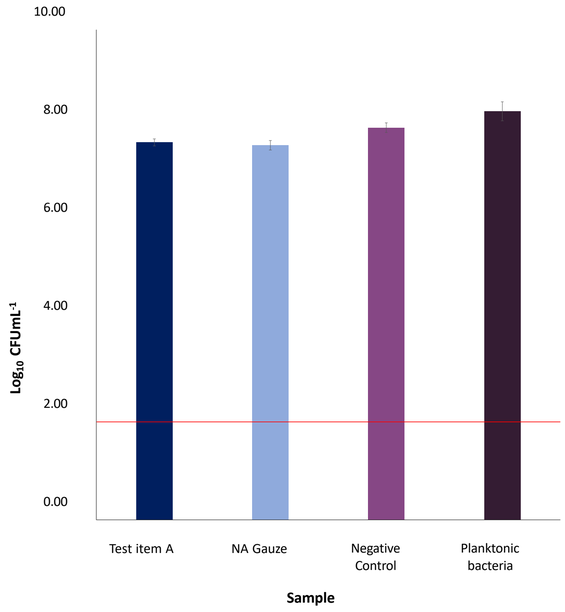

Figure 2. Quantification of *Pseudomonas aeruginosa* using viable-only qPCR, following 24 hours incubation at 37°C. Data is presented as the mean ± standard deviation (N=3). Red line indicates the qPCR limit of detection. No significant difference noted between samples (p> 0.05).

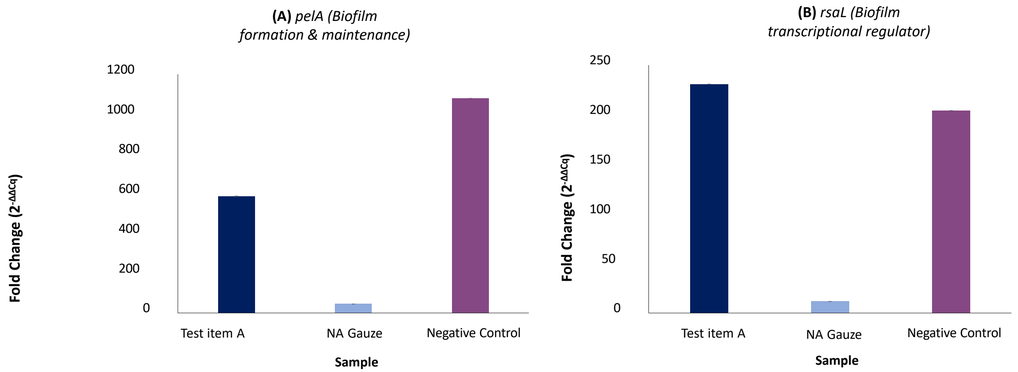

Figure 3. Individual gene transcription profiles for *Pseudomonas aeruginosa* **(A)** *pelA* and **(B)** *rsaL* following 24 hours incubation at 37°C. Data is presented as the fold change difference in biofilm sample when compared to planktonic inoculum.

Test Item	Details
Test Item A	Dialkylcarbamoyl chloride wound dressing
NA Gauze	Standard Medical Gauze
Negative control	Plastic film
Planktonic bacteria	*P. aeruginosa* culture in TSB

Table 1. Test item details used in this study. TSB = Tryptone soy broth

Results

Viable-only qPCR quantification

- No significant difference was detected in bacterial quantification between recovered samples following 24 hours incubation (p>0.05) (Figure 2)

***pelA* (Biofilm formation & maintenance)**

- Transcription of *pelA* was significantly up-regulated (586.10 fold increase) for the commercial wound dressing when compared to the standard gauze (45.43 fold increase) (Figure 3A).

***rsaL* (Biofilm transcriptional regulator)**

- Transcription of *rsaL* was significantly up-regulated (230.72 fold increase) for the commercial wound dressing when compared to the standard gauze (11.75 fold increase) (Figure 3B).

Conclusions

- Established methodology for detection and quantification of bacterial adherence to a wound dressing using two molecular techniques
- Despite similar bacterial load, transcription data differentiated between test items
- Significantly higher expression of both adhesion related genes from the commercial wound dressing recovery compared to standard gauze.

This project was carried out by Perfectus Biomed Group Now Part of NAMSA
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