Adhesion of Methicillin resistant *Staphylococcus aureus* (MRSA) and *Candida albicans* to Parylene-C coated Poly-Methyl Methacrylate (PMMA)

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Not required

Abstract

Staphylococcus aureus and *Candida albicans*, opportunistic pathogens frequently found within denture biofilms, can cause oral and systemic infections. Parylene-C, frequently used to coat medical implant devices, may induce favourable surface alterations, potentially reducing biofilm formation on dental materials *e.g.* poly(methyl-methacrylate) (PMMA). This study aimed to determine if Parylene-C coated PMMA reduces MRSA and *C. albicans* biofilm formation.

No significant difference in viable MRSA and *C. albicans* recovered from 48 hr single or dual species biofilms grown on PMMA or Parylene-C coated PMMA were noted, indicating that coating PMMA with Parylene-C does not reduce biofilm formation by MRSA or *C. albicans*.

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Introduction:

Intraoral removable prostheses, most commonly made with acrylic resin poly-methylmethacrylate (PMMA), are capable of acting as reservoirs for *Candida albicans* and methicillin resistant *Staphylococcus aureus* (MRSA), opportunistic pathogenic organisms associated with oral and life-threatening systemic infections (1).

Parylene-C, a polymer frequently used for coating medical devices, has been shown to alter PMMA's surface roughness (R_a) (2), a feature which could influence microbial adhesion and colonisation. Parylene-N, which has a similar structure to Parylene-C, was shown to significantly reduce adhesion of *C. albicans* on denture resin (3). This study aimed to determine if the lower R_a, bestowed by a Parylene-C coating onto PMMA, effects biofilm formation by MRSA and *C. albicans* in both single and dual species biofilms.

Methods:

Polished, heat cured PMMA (C&J De-lux, Chaperlin & Jacobs Ltd, Surrey) discs of 10 mm diameter, were coated with 10 µm of Parylene-C (Specialty Coating Systems Ltd, Surrey, England) or left uncoated. The R_a of the discs was measured (Proscan 1000 scanning laser profilometer). Sterilised discs were incubated in 5% CO_2 at 37°C for 48 hours with Methicillin resistant *Staphylococcus aureus,* EMRSA-16 (NCTC 13143) and *Candida albicans* single or dual species cultures made up in artificial saliva (OD_{600nm} 0.5). Discs were dipped into sterile PBS to remove planktonic bacteria, and vortexed for 1 min in 1 mL of neutralising broth to remove the biofilm. To determine the number of viable bacteria or yeast per disc, the bacteria solutions were plated onto Columbia blood agar base supplemented with 5% horse blood (CBA) and amphotericin B and/or Sabouraud agar containing vancomycin and incubated for 24-48 hour at 37°C in a 5% CO_2 atmosphere. Statistical significant was determined using a paired-sample t-test, analysed using SPSS software (Version 24, IBM, U.S.A.) with a 5% level of statistical significance.

Results

There was a significant difference between the mean roughness (R_a) value of the uncoated PMMA discs and Parylene-C coated discs (P = 0.018) (Figure 1). The mean number of viable MRSA recovered from biofilms after 48 hours of growth on Parylene-C coated PMMA discs were not statistically different compared to the number recovered from the uncoated discs (P = 0.168) (Figure 2a). There was also no significant difference in the number of viable microorganisms in the *C. albicans* biofilms formed on the Parylene-C coated PMMA discs compared to uncoated discs (P = 0.404) (Figure 2b). Statistical analysis of the total number of microorganisms recovered from the dual species biofilms containing MRSA and *C. albicans* revealed no significant difference between Parylene-C coated PMMA and uncoated PMMA (P = 0.999) (Figure 3).

Discussion

The results of this study showed no statistical difference in the number of viable organisms recovered from PMMA and Parylene-C coated or uncoated PMMA, for both MRSA and *C. albicans* single species, and dual species biofilms. These results are in contrast to a recent study in which Parylene-N reduced adhesion of *C. albicans* to coated silicone elastomer and denture PMMA-based resins (3). This difference could be explained by Parylene-N's superior penetration abilities to those of Parylene-C, due to its unique molecular movement during deposition (4). However, Parylene C fulfils ISO10993 and USP Clave VI tests and is more appropriate for use with intraoral prostheses than Parylene N.

A surface roughness of 0.2 µm, thought to be the limit to which gingival plaque microbes can adhere, would not however be representative of the fitting surfaces of dentures, where R_a usually exceeds 3.4 µm (5). The PMMA discs in this study were sanded to a more realistic target R_a value of 3.0 µm comparable to the R_a values obtained by Bourlidi *et al* (2). To achieve the lowest possible R_a values with the Parylene-C coating, the discs were coated with

10 µm of Parylene-C (2). However, despite the mean roughness of the Parylene-C coated discs (1.45 µm) being significantly lower than the uncoated (2.95 µm) PMMA discs (P = 0.018), this was insufficient to result in a significant reduction in biofilm formation by MRSA or *C. albicans* on the coated discs.

Conclusion

Coating PMMA with Parylene-C despite significant reduction R_a does not lead to any statistically significant difference in biofilm formation by the opportunistic pathogens, *C. albicans* and MRSA.

(700 word limit | 692 words achieved)

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Figures

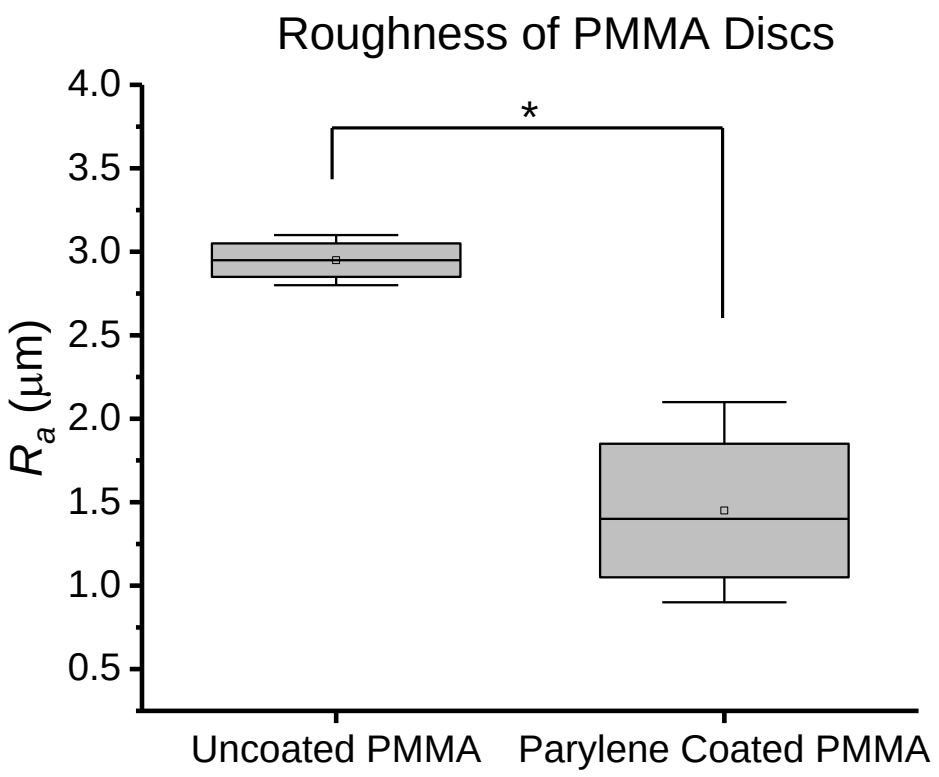

Figure 1

Graph showing the roughness (R_a) values (µm) of Parylene-C coated PMMA discs and uncoated PMMA discs. Error bars represent 1.5 Interquartile Range; - = median; ◦ = Mean (*****, P<0.05).

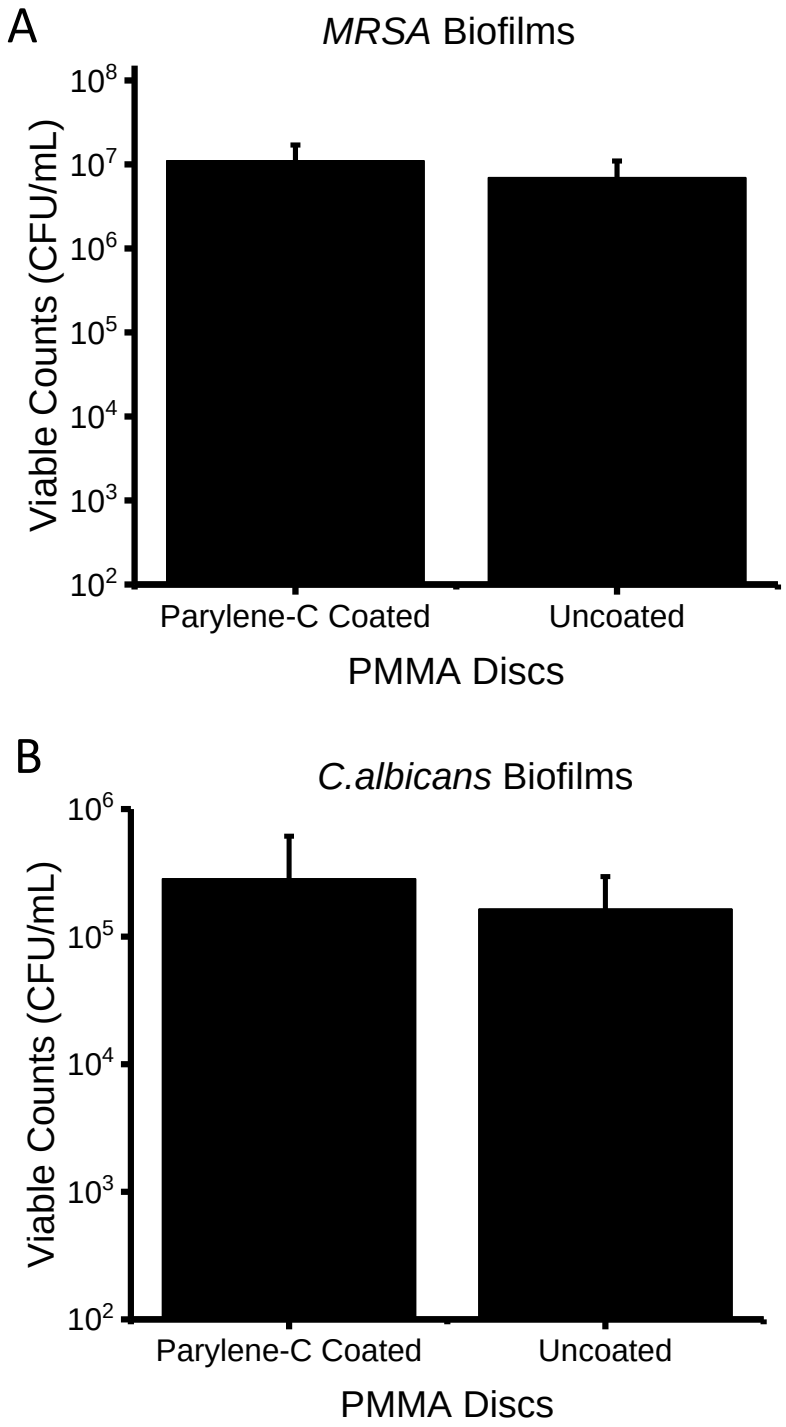

Figure 2

Single species biofilm formation (CFU/mL) (A) Viable counts of an MRSA single species biofilms formed on Parylene-C coated and uncoated PMMA discs (B) Viable counts of a *Candida albicans* single species biofilms formed on Parylene-C coated and uncoated PMMA discs. All experiments were repeated in triplicate. Error bars represent standard deviations.

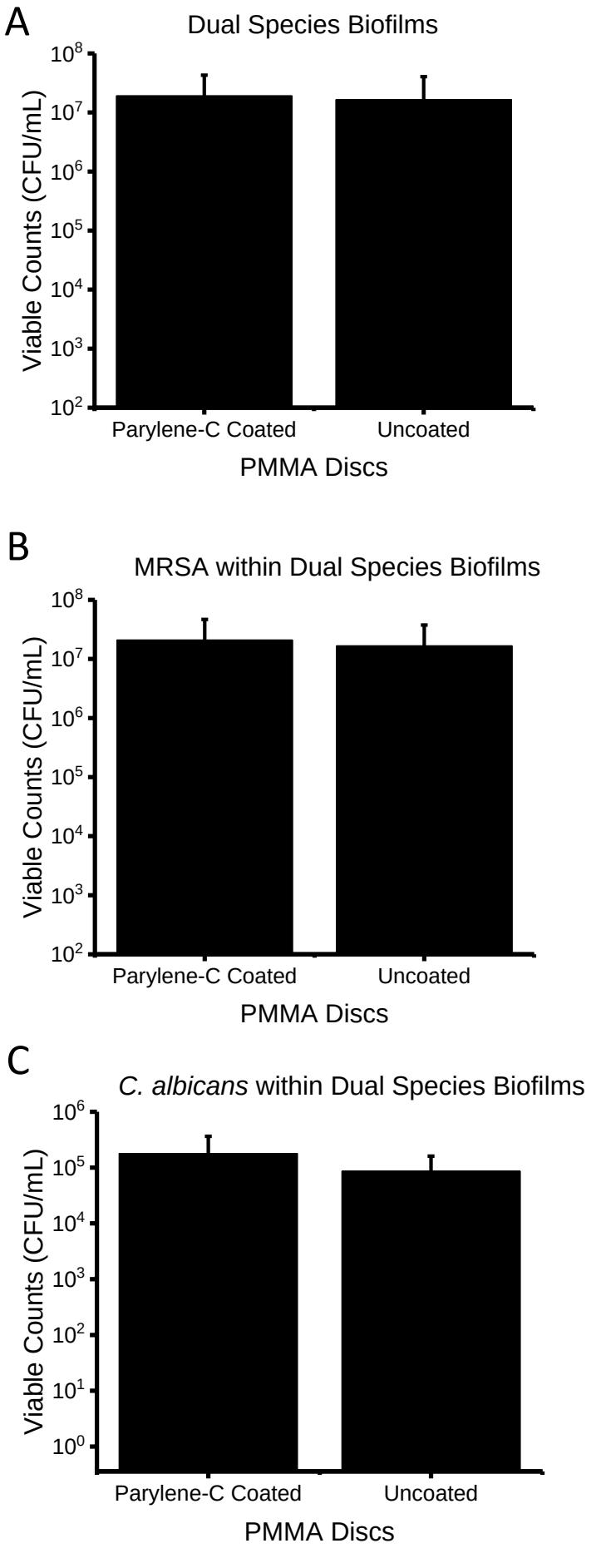

Figure 3

Dual species biofilm of MRSA and *C. albicans* (CFU/mL) (A) Total viable counts of MRSA and *Candida albicans* within dual species biofilms formed on Parylene-C coated and uncoated PMMA discs (B) Viable counts of MRSA within dual species biofilms formed on Parylene-C coated and uncoated PMMA discs. (B) Viable counts of a *Candida albicans* within dual species biofilms formed on Parylene-C coated and uncoated PMMA discs.

All experiments were repeated in triplicate. Error bars represent standard deviations.