Preliminary study into the effects of tobacco smoke on *Candida albicans*.

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INTRODUCTION

Denture-stomatitis (DS) affects approximately 65% of denture wearers and is one of the most common forms of oral candidosis. The main species involved in oral candidosis is *Candida albicans,* which is an opportunistic fungal pathogen that is problematic for people who are immunocompromised or have implanted medical devices. Environmental factors are important in the modulation of *C. albicans* biofilms, and investigating how such factors influence *C. albicans* pathogenicity will give a better insight into the risk factors for oral candidosis. DS has increased prevalence in cigarette smokers[1,2] and tobacco condensate has been shown to increase *C. albicans* adhesion, growth, biofilm formation, expression of the virulence genes[3] and hyphal production[4].

Hypothesis and aims:

We hypothesise that the chemically characterised tobacco condensate on denture acrylic surfaces will modify *C. albicans* biofilm development and alter expression of certain virulence characteristics. Assessment of modulation will involve investigating effects on adherence, biofilm quantity, virulence gene expression and hyphal transformation.

METHODS

***Candida* strains:**

Six strains of *C. albicans* were investigated including *C. albicans* SC5314, originally a clinical specimen from human infection and five clinical isolates (Table 1).

Table 1. *C. albicans* clinical isolates studied including the source of isolation, and their efficacy at surface colonisation and invasion.

Strain	Isolate source	Surface colonisation*	Invasion*
PB1/93	Normal oral mucosa	++	Low
408/99	SCC tongue	++	Low
480/00	SCC oral mucosa	++	Low
PTR/94	CHC buccal mucosa	+++	High
705/93	CHC buccal mucosa	+++	High

*based on reconstituted human oral epithelium infection[5]; SCC, squamous cell carcinoma; CHC, Chronic hyperplastic candidosis; Adapted from [5]

Poly(methyl methacrylate) (PMMA) Coupon Discs:

PMMA is a thermoplastic used to construct denture prostheses and orthodontic retainers.

8 mm ⌀ PMMA discs were prepared using Oracryl Cold Cure polymer SW9053 powder (Bracon Dental Laboratory Products, Etchingham, UK) and the Oracryl Cold Cure Liquid monomer at a 2:1 ratio by weight. The mixture was poured into a prepared mould and allowed to cure for 30 mins under a weighted glass plate. Following sterilisation, they provided a solid substratum on which to grow mono- and polymicrobial biofilms.

Preconditioning PMMA coupons with tobacco condensate:

PMMA coupons (⌀ 8 mm) and 50 mL of artificial saliva were placed into the smoking apparatus (Fig. 1). Cigarettes with the filters removed were inserted into the holder indicated by an orange circle (Fig. 1), lit and the smoke was drawn through the apparatus by the vacuum pump. A dreschel head released the smoke into the artificial saliva before it was drawn through the rest of the apparatus. Twenty cigarettes were 'smoked' to generate the condensate.

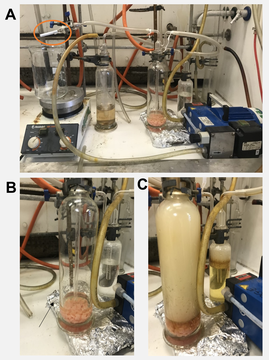


Fig 1. Tobacco preconditioning apparatus

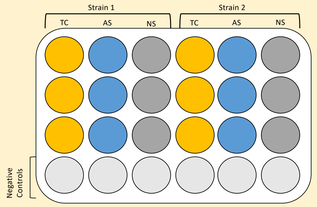


Fig 2. Plate layout for adherence and biofilm static growth.

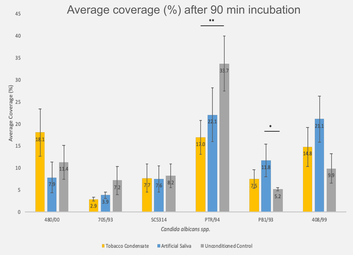
The type of coupon present in the well was: TC Tobacco preconditioned, AS artificial saliva preconditioned and NS no preconditioning. Negative controls were conditioned but had no *Candida* added.

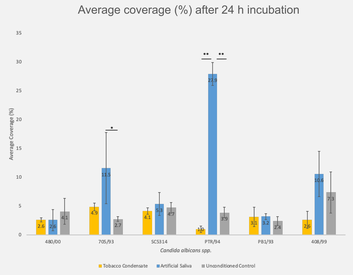
PMMA discs preconditioned with tobacco, artificial saliva or no preconditioning (Fig. 2) were incubated statically at 37°C in Yeast Nitrogen Base medium inoculated with *C. albicans* (n=6) for either 90 min or 24 h to facilitate adherence and biofilm formation, respectively.

Candida attached to the discs were subsequently stained with calcofluor white and imaged by confocal laser scanning microscopy (CLSM).

RESULTS

Fig 3. Average percentage coverage /1550µm² (n=15):





* indicates a P value < 0.05 and ** indicates a P value < 0.00. P values were obtained by a One-Way ANOVA with Tukey post-hoc test.

Fig 4. Representative CLSM images

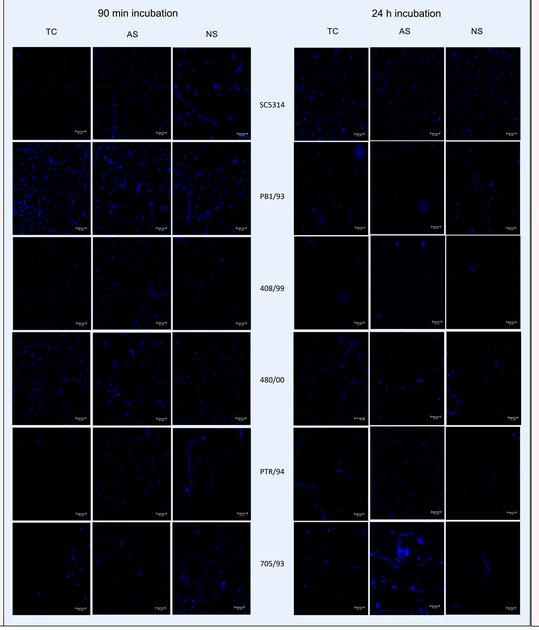
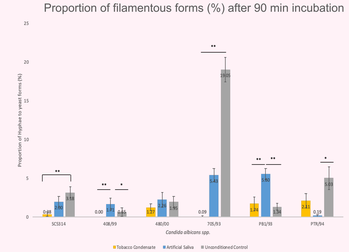
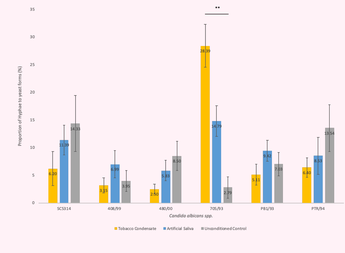


Fig 5. Proportion of filamentous forms /100µm² (n=15):





* indicates a P value < 0.05 and ** indicates a P value < 0.00. P values were obtained by a One-Way ANOVA with Tukey post-hoc test.

RESULTS/ CONCLUSIONS

- Level of *C. albicans* adherence and biofilm development to acrylic was strain dependent (Fig. 3).
- *C. albicans* adherence to tobacco condensate coated surfaces was generally lower than controls, with exception of strain 480/00 where tobacco condensate increased adherence.
- Biofilm coverage was highest on artificial saliva coated surfaces for *C. albicans* strains 705/93, SC5314 PTR/94 and 408/99. Strain 480/00 had highest biofilm coverage on uncoated surfaces.
- Hyphal adherence (90 min) to tobacco coated PMMA was again generally lower compared to control surfaces. After 24 h, the proportion of filamentous forms per unit area for *C. albicans* 705/93 was higher on the tobacco coated surface compared to controls, suggesting promotion of hyphal growth on this surface (Fig 5).

FUTURE WORK

- Work is ongoing to ascertain the significance of these effects upon *C. albicans* pathogenicity.
- Changes in gene expression in response to the type of conditioning of virulence and hyphal associated genes is currently underway.
- Chemical analysis of the components adsorbed by the PMMA preconditioned with tobacco is ongoing.

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