Development of an in-vitro Denture Plaque Biofilm to Model Denture Malodour

Abstract

This study aimed to develop an in-vitro denture plaque biofilm to model denture malodour. No previous studies have attempted to characterise the malodour associated with dentures and the effect of *Candida* spp. (main aetiological agent of denture related stomatitis) on malodour. Pooled denture plaque microcosms and 'model' denture plaque biofilms (pooled saliva supplemented with additional microbial species) with and without addition of candida were grown aerobically at 37 °C for up to 13 d in a constant depth film fermenter (CDFF) on denture acrylic discs. Sample discs were removed, rinsed in sterile water and placed in phosphate buffered saline (PBS). The discs were vortex mixed to remove the biofilms, diluted in PBS and plated in duplicate onto general and selective media. The composition and stability of the biofilms over time was assessed. CDFF grown microcosms and 'model' denture plaque biofilms were relatively stable in composition, with streptococci remaining the dominant microbial group. Model denture plaque biofilms were comparable in composition to denture plaque microcosms. This model system has the potential for evaluation of agents that might affect these parameters such as denture cleansers and other oral hygiene treatments.

Introduction

Oral malodour refers to a disagreeable odour originating from the mouth itself and is a common and often distressing condition, yet is poorly explored in denture wearers. Although the proportion of people reliant on dentures is decreasing, over 25 % of the UK wears complete or partial dentures, with 13 % of the population currently edentulous (Kelly *et al*., 2000). In comparison, less than 15 % of Australian adults wear dentures, with only 6.4 % of the population currently edentulous (Slade *et al*., 2007). In a recent report (Petersen & Yamamoto, 2005), the UK was ranked as the sixth highest WHO region/country with regards to prevalence of edentate elderly. There are currently 15 million denture wearers in the UK (Verran, 2005), representing a significant consumer base and unique healthcare consideration. Due to the artificial nature of the denture, many edentulous patients express concern that they may produce a distinct malodour (Fiske *et al*., 1995) and can thus be identified as 'denture wearers'.

The oral cavity provides a diverse number of surfaces including soft shedding non-keratinised buccal mucosal epithelia, the keratinised mucosa of the gums, the highly papillated mucosa of the tongue and the hard non-shedding surfaces of the teeth. In comparison with the dentate individual, the mouth of the denture wearer presents additional hard non-shedding areas and new environments (tissue-fitting surface) to support growth of microorganisms and development of plaque. Plaque biofilm formation on dentures is important in the development of oral candidosis and denture related stomatitis, which are common infections suffered by denture wearers.

Dirty dentures contribute to malodour (Neill, 1968), which is generally acknowledged in the dentate to be caused in part by volatile sulphur compounds (VSCs) including hydrogen sulphide, methyl mercaptan and dimethyl sulphide (Tonzetich, 1977). These VSCs cause a fetid or putrid odour and are produced by Gram negative bacteria, particularly anaerobic species such as

Porphyromonas spp., *Prevotella* spp., and *Fusobacterium* spp. (Mink *et al*., 1983; Rolla *et al*., 1999) in tongue coating. The ecology and pathology of denture biofilms, including the tongue, is relatively unknown. In the oral cavity obligate anaerobes are found as part of the normal flora, yet their prevalence and identification to species level within denture plaque has been poorly defined in the literature. In addition, denture plaque hosts a range of acidogenic lactobacilli, streptococci and *Candida* spp.

The microbiology of denture plaque has received little attention in comparison with dental plaque, yet it differs in location and composition (Verran, 1999). Denture plaque (fig. 3) is known to harbour increased proportions of *Candida* spp. (main aetiological agent in denture stomatitis) in comparison to dental plaque. Denture related stomatitis is a condition present in 10-75 % of denture wearers (Arendorf & Walker, 1987; Kulak-Ozkan *et al*., 2002) and is often linked with acute pseudomembraneous oral candidosis or 'thrush' (Webb *et al*., 1998). An odour dissimilar to oral malodour has been detected from the prostheses rather than the oral cavity itself, and is more frequently associated with dirty dentures (fig.1; 37 %) than visibly clean dentures (19 %) (Coulthwaite & Verran, 2005). Only a limited number of people maintain effective oral hygiene and the majority of denture wearers are failing to keep their dentures clean (Kulak-Ozkan *et al*., 2002; Budtz-Jorgensen & Bertram, 1970; Dikbas *et al*., 2006).

Little work has been published on denture-associated malodour, but many species capable of producing malodorous compounds are found in denture plaque (Verran, 2005). Any effect of increased numbers of *Candida* spp. on malodour is unknown, as is any effect of the denture material itself. Yonezawa et al. (2003) reported a reduction in candida levels on the tongue without a significant reduction in VSCs, indicating candida does not play a major role in VSC production in the oral cavity. However, the production of other volatile compounds by *Candida*

species and their overall effect on oral malodour in denture wearers has not been investigated to the authors' knowledge. Dentures are particularly associated with malodour if they are worn overnight (Krepsi *et al*., 2006), thus an effective oral hygiene regimen is important to control denture plaque and contribute to control of associated malodour. There are agents that specifically target oral malodour generation, although few developed with the denture wearer in mind. Many commercial denture products claim breath freshening properties; but the scientific evidence to support this is limited (Myatt et al., 2002). Relevant in vitro models could provide information on such agents, such as mechanism of action, and required treatment duration (Williams, 1999) before progression to clinical trials.

Oral biofilm models allow greater numbers of samples to be evaluated more rapidly, safely, and at lower cost than in vivo models. There are two complementary microbial approaches to generating and studying model biofilm systems: construction of a synthetic plaque-like consortium with major plaque species (Marsh, 1995; Kinniment *et al*., 1996); and growth of plaque microcosms from the natural oral microflora (Lamfon *et al*., 2005). Several in vitro methods have been developed to successfully model oral biofilms on various materials including batch culture systems, filter based fermentation systems (McBain *et al*., 2005) and chemostats (Marsh *et al*., 1983). Batch culture systems are not representative of in vitro plaque biofilms. Steady state in vitro biofilm models used for the production of oral biofilms include the constant depth film fermenter (CDFF) (Kinnement *et al*., 1996; Pratten *et al*., 1998a; McBain *et al*., 2003; Pratten *et al*., 2003) for modelling non-shedding surface plaque biofilms, and the Sorbarod perfusion system (Spencer *et al*., 2007). Pratten *et al*., (2003) used a CDFF to model oral malodour production from tongue biofilms and showed a correlation between VSC concentration

and viable counts of H_2S producing bacteria, and the positive effect of anti-malodour compounds on VSC production.

There have been very few studies on mixed denture plaque biofilms generated in vitro with and without the presence of candida. When producing biofilm models the inoculum is often pooled saliva from healthy dentate volunteers (Pratten *et al*., 2000; McBain *et al*., 2003). This would not be representative of the denture wearing population microbiota, nor indeed of microbial biofilms that would exist in vivo. However, such inocula are easier to obtain and dispense. The CDFF is a suitable system for modelling plaque development on denture acrylic, and to the author's knowledge, has not been used to study the effect of the presence of *Candida albicans* on denture plaque formation and composition, development of steady state biofilms, and odour generation.

Gas chromatography and mass spectroscopy (GC-MS) has been used to detect and identify volatile organic compounds (VOCs) produced in headspace gas of microbial cultures, dental and tongue plaque samples, and trapped breath gas (Greenman, 1999). However, the VOC profiles of *Candida* spp., denture plaque with and without candida, and denture breath gas have not yet been characterised. The GC-MS technique may enable identification of certain profiles and species within mixed biofilms from the mass spectra of volatile compounds produced.

To the authors' knowledge, no previous studies have attempted to characterise the malodour associated with dentures and the effect of *Candida* spp. on malodour. The aim of this study was to develop a stable model denture plaque consortium to enable detection of malodour, and investigation of factors affecting denture-associated malodour in vitro.

Materials & Methods

Denture plaque and saliva

Prior to the start of this study ethical approval was obtained from the Department of Biological Sciences at Manchester Metropolitan University. Healthy edentate denture wearing volunteers were invited to take part in the study. Volunteers were given information sheets and signed informed consent was obtained. The study was conducted in accordance with the Declaration of Helsinki (1964) and subsequent amendments. Volunteers were informed of their right to withdraw from the study at any time. Denture plaque samples were taken using sterile plastic toothpicks and placed in 5 ml of reduced transport fluid (RTF; Coulthwaite & Verran, 2005), the plaque samples were screened for the presence of *Candida* spp. and pooled into two groups, one group containing cultivable candida, and the other without cultivable candida. The pooled plaque samples were vortex mixed and divided into 1 ml aliquots and frozen at -20 °C. Un-stimulated whole human saliva was collected from 10 healthy volunteers (5 female, 5 male, mean age 32; no current oral problems or medication being taken) by expectoration into sterile universals on ice. Equal volumes of saliva (5 ml) from all 10 volunteers was pooled, mixed, and aliquoted into 1ml volumes and frozen at -20 °C.

For investigation of cultivable microbial composition; 1 ml of the pooled plaque samples were individually serially diluted in phosphate buffered saline (PBS; Oxoid, Basingstoke, UK) and plated out in duplicate onto: Columbia blood agar (COLBA; Oxoid) for total aerobes; fastidious anaerobe agar (FAA; Lab M, Bury, UK) for total anaerobes; streptococcus blood agar (SBA) using COLBA base with colistin and oxolinic acid (Oxoid) for presumptive streptococci, Wilkins Chalgren agar (WC) with vancomycin and naladixic acid (Lab M) for presumptive Gram negative anaerobes (GNA; underrepresented in most studies of denture plaque); de Man Rogosa Sharpe agar (MRS; Oxoid) for presumptive lactobacilli (increased proportions in denture plaque);

and Sabouraud dextrose agar with chloramphenicol (Lab M) for presumptive *Candida* spp. (increased proportions in denture plaque). Saliva samples (1 ml) were serially diluted in PBS and plated in duplicate onto FAA, SABC and MRS. COLBA, FAA, SBA and WC media were supplemented with 5 % sterile horse blood (TCS Biosciences). Plates were incubated aerobically (COLBA, SABC), in elevated CO_2 (5 %; SBA, MRS) and anaerobically (FAA, WC) for up to 6 d at 37 °C. White/cream shiny domed colonies on MRS that were catalase negative Gram positive rods were deemed *Lactobacillus* spp., colonies on SBA that were catalase negative and Gram positive cocci were deemed *Streptococcus* spp. and colonies on WC that were Gram negative were deemed to be Gram negative obligate anaerobes (GNA) and any pigmented colonies were counted and further identified. The effect of freezing and thawing samples on the microbial composition was investigated. Frozen saliva samples were thawed and spiked with 100 µl (1.0 x 10^6 cfu ml^{-1}) *C. albicans* GDH 2346 to increase the proportions of Candida to that representative of denture plaque with candida, and then termed 'model denture plaque'.

The constant depth film fermenter

A constant depth film fermenter (CDFF; University of Wales, Cardiff, UK) as described by Wilson (1999) and Wimpenny (1999), was used to grow in vitro biofilms aerobically at 37 °C. Briefly, the CDFF consists of a main turntable rotating at 3 rpm containing six polytetrafluoroethylene (PTFE) pans (each 20 mm diameter), beneath two spring-loaded PTFE scraper bars, which continually smear medium over the pans. Each pan has five cylindrical holes (5.0 mm diameter), which contained autopolymerised denture acrylic (polymethyl methacrylate; PMMA; Metrodent Ltd. Huddersfield, UK) discs placed on top of PTFE plugs. Acrylic discs were recessed below the height of the pan allowing for biofilm growth up to 300 µm, regulated

by the scraper bars removing excess material. The nutrient medium is pumped at a controlled rate into the system and allowed to drop directly onto the main turntable.

Continuous culture in the CDFF

The mixed microbial sample (either pooled denture plaque with and without candida, or 'model denture plaque'; 1 ml) was inoculated into 500 ml of sterile artificial saliva (Pratten *et al*., 1998b), placed on a magnetic stirrer and pumped into the CDFF over 8 h at 37 °C, at a rate of 1 ml min^{-1} using a peristaltic pump (Watson-Marlow) as the CDFF turntable rotates at 3 rpm. After 8 h a sterile artificial saliva reservoir was connected and pumped into the CDFF at a rate of 0.5 ml min^{-1} (0.72 l d^{-1}), similar to the resting flow rate of whole saliva in healthy individuals (Dawes, 2004).

Denture plaque microcosms developed for up to 192 h (8 d), and a single pan was removed at 72, 96, 120, 144, 168 and 192 h. Individual acrylic discs were removed, rinsed in sterile distilled water to remove non-adherent cells, added to 5 ml PBS, vortex mixed and sonicated to remove the adherent biofilms. Cell suspensions were serially diluted in PBS and plated in duplicate onto COLBA, FAA, WC, SBA, MRS and SABC for reproducibility within pans, and investigation of composition changes and/or stability over time as the biofilm matures. The discs were processed for viable counts on all previously described media. For the 'model denture plaque' biofilms, discs were removed for viable counts after 13 d, suspended and diluted in PBS and plated in duplicate onto FAA, MRS and SABC, for total anaerobes, lactobacilli and candida respectively. These media were deemed to support growth of the majority of the organisms, since the majority of organisms in the oral cavity are facultative anaerobes. Lactobacilli on MRS and candida on SABC were target organisms of interest due to their increased prevalence in denture plaque in

comparison to dental plaque. Organisms were distinguished by colony morphology on selective media and Gram-stained to give a preliminary indicator of their identification.

Statistical analyses

The Anderson-Darling Normality Test was used to ensure data fit a normal distribution and therefore parametric analyses can be applied. To compare the means between two independent samples an unpaired 2-tailed t-test was used. Analysis of variance (ANOVA; F-test) was used to determine whether all group means are the same between replicate discs within the same pan and between pans over time. Where there was a significant difference between means according to ANOVA, a Tukey test was used to establish which specific means are significantly different from one another by comparing the means using 95% simultaneous confidence intervals.

Results

Denture plaque and saliva composition

Growth on all general and selective media demonstrated a mixed microbial composition typical of the edentulous oral flora. Streptococci were the predominant microorganisms in pooled denture plaque with candida and without candida (fig. 1). Of the total viable facultative anaerobes in denture plaque with candida, streptococci represented 80.16 %, lactobacilli 18.02 %, GNA 15.95 % and yeast represented 0.42 %. Of the total viable facultative anaerobes in denture plaque without candida, streptococci represented 60.05 % and GNA represented 2.39 %, with no yeast or lactobacilli recovered. In fresh saliva, *Candida* spp. represented <0.01 % and lactobacilli 0.048 % of the total facultative anaerobes. In comparison to the fresh pooled saliva, denture plaque with

candida had increased *Candida* spp. ($t=8.79$, $p<0.05$), increased *Lactobacillus* spp. ($t=17.04$, $p<0.005$) and decreased total anaerobes ($t=17.66$, $p<0.05$) (fig. 2). After freezing and thawing the saliva, the lactobacilli composition increased, representing 13.69 % and the yeast composition decreased, still representing <0.01 (3.85×10^{-5}) % of the total facultative anaerobes. A significant 4 log increase in *Candida* spp. was obtained after spiking frozen saliva with *C. albicans* GDH 2346 ($t=149.83$, $p<0.001$), resulting in *Candida* spp. comprising 1.38 % of the total anaerobes. There was a significant decrease in lactobacilli counts after spiking the saliva with *C. albicans* ($t=8.22$, $p<0.05$), dropping the composition to 7.77 % of the total facultative anaerobes.

Denture plaque microcosms

Denture plaque microcosms were reproducible at any time point and over time (fig. 3a,b). There was an increase in viable counts at 192 h but the percentage composition remained stable. The presence of *Candida* spp. affected microbial growth. Lower counts were obtained for all the investigated microbial groups, and no lactobacilli were recovered in the absence of candida. Streptococci were the predominant organisms in biofilms both with and without candida, although numbers were lower in the absence of candida.

Model denture plaque biofilms

Model denture plaque biofilms developed from pooled saliva were comparable in composition to denture plaque microcosms with mean proportions of lactobacilli representing 2.9 % at 13 d and 192 h respectively. The levels of candida in the denture plaque microcosms at 96 h comprised 1.5 %, yet only 0.005% in the model plaque biofilms after 13 d. However, this was representative of the composition of yeast found in denture plaque in vivo, comprising <0.1%

even in stomatitis. For all target groups of microorganisms, there was no significant difference in counts within the same pan at a single time point, indicating reproducibility between discs. Viable counts of streptococci, total aerobes, total anaerobes, GNA and lactobacilli differed over time as the microcosms developed, however they represented similar proportions to those of the inoculum. Quasi-steady state population proportions were reached by 120 h in the microcosm plaque biofilms with *C. albicans*. Microbial composition of the denture plaque microcosms without candida was more variable but streptococci remained the dominant group.

Model denture plaque biofilms were not reproducible between runs with high standard deviations obtained (table1). However the percentage composition represented by these key groups of microorganisms remained relatively stable, anaerobes comprising 96-99 %, lactobacilli 0.2-1.6 % and Candida spp. <0.02 %. The proportion of microorganisms were similar to that seen in the original inoculum, with total counts of anaerobes in the region of 10^8, lactobacilli 10^6-10^7, and Candida 10^3 cfu ml^{-1}.

Discussion

In this study a model denture plaque has been developed, which more simplistically and reproducibly reflects the in vivo conditions and compositions of denture plaque- advantages of the use of the CDFF to study oral biofilms (Wilson, 1999; Pratten *et al*., 2003). The CDFF was operated aerobically in order to replicate the oral environment, although anaerobic microenvironments will develop within the plaque biofilm as in natural plaque, supporting the growth of more fastidious microorganisms. The proportions of the genera present were comparable to those observed in in-vivo studies (Theilade & Budtz-Jorgensen, 1980; Budtz-

Jorgensen *et al*., 1981) where streptococci were the predominant organisms, with low proportion of Gram negative obligate anaerobes and yeast. However, more recent molecular methods enable detection of these difficult to cultivate microorganisms and may indicate higher proportion than estimated by culture. The diverse inocula from pooled saliva used to produce microcosm plaques result in biofilms that appear to contain a far less diverse flora than saliva itself (Pratten *et al*., 2001).

The relationship between lactobacilli and *Candida* spp. is relatively unexplored. Studies of denture plaque do not usually investigate the presence of lactobacilli alongside candida (Theilade & Budtz-Jorgensen, 1980; Budtz-Jorgensen *et al*., 1981), yet both are acidogenic, and might be expected in the denture plaque, where the presence of *Candida* spp. is associated with a higher acidogenic ratio (Verran *et al*., 1999).

The CDFF is a continuous-culture system where the population reaches a quasi-steady state. It allowed production of large numbers of reproducible samples under conditions similar to those found in the oral cavity, replicating in vivo biofilm formation and composition. The CDFF runs with pooled denture plaque were used to establish reproducibility within and between pans, assess biofilm development and time taken to reach steady state using a variety of media. Model denture plaque biofilms developed from pooled saliva were comparable in composition to denture plaque microcosms. This model plaque was engineered to more appropriately represent the concentration of yeast in denture plaque, which is higher than that usually recovered in the saliva of healthy dentate individuals as also shown in this study.

A further use of saliva in relation to malodour is the salivary incubation test (Quirynen *et al*., 2003) where aliquots of saliva are incubated with a range of chemical compounds to show their anti-malodour efficacy. However, this is based on the study of planktonic bacteria derived from

the saliva of healthy dentate volunteers and does not effectively represent the plaque biofilms found on the denture.

A fetid odour was detected from all CDFF grown microbial films. Headspace gas analysis of VSC levels using a Halimeter and of other volatile compounds using high pressure liquid chromatography (HPLC) have been used previously to investigate in vitro volatile gas production by microorganisms (Goldberg et al., 1997; Quirynen et al., 2003). Solid phase microextraction (SPME) is to be investigated for sample pre-concentration in order to detect low levels of volatile compounds produced by key microorganisms associated with malodour, and *C. albicans*. The GC-MS technique is primarily a research tool with limited clinical application due to required technical operators, preservation and transfer of samples to the specialised laboratory, expense, and time taken for each sample run. GC-MS techniques are currently being developed to characterise the odour profiles of pure cultures in artificial saliva. This technique may ultimately enable identification of individual species within mixed biofilms from the mass spectra of volatile compounds produced.

Many denture cleansing products claim breath freshening properties, yet this has not been specifically investigated and warrants further investigation as to their effect on reduction of volatile organic compounds and microbial species associated with malodour. The CDFF grown 'model denture plaque' biofilms may be more representative of in vivo denture plaque than denture plaque microcosms, the levels of *Candida albicans* can be controlled and they are more easily assessable than true denture plaque.

Conclusions

A stable denture plaque microcosm and ‘model’ denture plaque biofilm with and without *C. albicans* has been established, enabling microbial analysis and detection and analysis of gas production. This model system has the potential for evaluation of agents that might affect these parameters such as denture cleansers and other oral hygiene treatments.

References

Arendorf TM, Walker, D.M. (1987). Denture stomatitis: a review. *Journal of Oral Rehabilitation* **14**(3):217-227.

Budtz-Jørgensen E, & Bertram, V. (1970). Denture stomatitis I: The etiology in relation to trauma and infection. *ACTA Odontologica Scandinavica* **28**:71-92.

Budtz-Jørgensen E, Theilade, E., Theilade, J., Zander, HA. (1981). Method for studying the development, structure and microflora of denture plaque. *Scandinavian Journal of Dental Research* **89**(2)*:*149-156.

Coulthwaite L, & Verran, J. (2005). Denture plaque: A neglected biofilm. In: Biofilms: Persistence and Ubiquity. D Allison, Verran, J., Spratt, D., Upton, M., Pratten, J., McBain, A. editor. Manchester: The Biofilm Club, pp. 311-322.

Dawes C (2004). Factors influencing salivary flow rate and composition. In: Saliva and Oral Health. M Edgar, Dawes, C., O'Mullane, D. editor. London: British Dental Association, pp. 32-49.

Dikbas I, Koksal, T., Calikkocaoglu, S. (2006). Investigation of the cleanliness of dentures in a university hospital. *International Journal of Prosthodontics* **19**(3):294-298.

Fiske J, Davis, D.M., Horrocks, P. (1995). A self-help group for complete denture wearers. *British Dental Journal* **178**(1):18-22.

Goldberg S, Cardash, H., Browning, H., Sahly, H., Rosenberg, M. (1997). Isolation of enterobacteriaceae from the mouth and potential association with malodor. *Journal of Dental Research* **76**(11):1770-1775.

Greenman J (1999). Halitosis. In: Dental plaque revisited: oral biofilms in health and disease. HW Newman, Wilson, M. editor. Cardiff: Bioline, pp. 419-442.

Kelly M, Steele, J., Nuttall, N., Bradnock, G., Morris, J., Nunn, J., Pine, C., Pitts, N., Treasure, E., White, D. (2000). Adult dental health survey:oral health in the United Kingdom 1998. *The Government Statistical Service*.

Kinniment SL, Wimpenny, J.W.T., Adams, D., Marsh, P.D. (1996). Development of a steady-state oral microbial biofilm community using the constant-depth film fermenter. *Microbiology* **142**:631-638.

Krespi YP, Schrime, M G., Kacker, A. (2006). The relationship between oral malodor and volatile sulfur compound-producing bacteria. *Otolaryngology - Head and Neck Surgery* **135**:671-676.

Kulak-Ozkan Y, Kazazoglu, E., Arikan, A. (2002). Oral hygiene habits, denture cleanliness, presence of yeasts and stomatitis in elderly people. *Journal of Oral Rehabilitation* **29**:300-304.

Lamfon H, Al-Karaawi, Z., McCullough, M., Porter, S.R., Pratten, J. (2005). Composition of in vitro denture plaque biofilms and susceptibility to antifungals. *FEMS Microbiology Letters* **242**:345-351.

Marsh PD (1995). The role of microbiology in models of dental caries. *Advances in Dental Research* **9**(3):244-254.

Marsh PD, Hunter, J R., Bowden, G H., Hamilton, I R., McKee, A S., Hardie, J M., Ellwood, D C. (1983). The influence of growth rate and nutrient limitation on the microbial composition and biochemical properties of a mixed culture of oral bacteria grown in a chemostat. *Journal of General Microbiology* **129**:755-770.

McBain A, Bartolo, RG., Catrenich, CE., Charbonneau, D., Ledder, RG., Gilbert, P. (2003). Growth and molecular characterization of dental plaque microorganisms. *Journal of Applied Microbiology* **94**:655-664.

McBain A, Sissons, C., Ledder, RG., Sreenivasan, PK., De Vizio, W., Gilbert, P. (2005). Development and characterization of a simple perfused oral microcosm. *Journal of Applied Microbiology* **98**:624-634.

Mink R, Biemer, TA., Pianotti, RS., Dills, SS. (1983). Volatile sulphur compound production in anaerobic oral bacteria. *Journal of Dental Research* **62**(Spec. Issue abstract 91):180.

Myatt G, Hunt, S.A., Barlow, A.P., Winston, J.L., Bordas, A., El-Maaytah, M. (2002). A clinical study to assess the breath protection efficacy of denture adhesive. *Journal of the Contemporary Dental Practitioner* **3**(4):1-9.

Neill D (1968). A study of materials and methods employed in cleaning dentures. *British Dental Journal* **124**(3):107-115.

Petersen PE, & Yamamoto, T. (2005). Improving the oral health of older people: the approach of the WHO global oral health programme. *Community Dental Oral Epidemiology* **33**:81-92.

Pratten J, Barnett, P., Wilson, M. (1998). Composition and susceptibility to chlorhexidine of multi-species biofilms of oral bacteria. *Applied & Environmental Microbiology* **64**(9):3315-3519.

Pratten J, Wills, K., Barnett, P., & Wilson, M. (1998b). In vitro studies of the effect of antiseptic-containing mouthwashes on the formation and viability of Streptococcus sanguis biofilms. *Journal of Applied Microbiology* **84**:1149-1155.

Pratten J, Andrews, C.S., Craig, D.Q.M., Wilson, M. (2000). Structural studies of microcosm dental plaques grown under different nutritional conditions. *FEMS Microbiology Letters* **189**:215-218.

Pratten J, Spratt, D., Wilson, M. (2001). Microcosm dental plaques. Cardiff: BioLine, pp. 141-151.

Pratten J, Wilson, M., Spratt, DA. (2003). Characterization of in vitro oral bacterial biofilms by traditional and molecular methods. *Oral Microbiology Immunology* **18:**45-49.

Quirynen M (2003). Management of oral malodour. *Journal of Clinical Periodontology* **30**(Suppl. 5):17-18.

Rolla G, Waaler, SM. & Kjaerheim, V. (1999). Concepts in dental plaque formation. In: Oral biofilms and plaque control. HE Busscher, LV. editor. Amsterdam, The Netherlands: Harwood Academic Publishers, pp. 1-17.

Slade GD, Spencer, A.J., Roberts-Thomson, K.F., (eds.). (2007). Australia's dental generations: The national survey of adult oral health 2004-2006. Adelaide, Australia: Australian Institute of Health & Welfare.

Spencer P, Greenman, J., McKenzie, C., Gafan, G., Spratt, D., Flanagan, A. (2007). In vitro biofilm model for studying tongue flora and malodour. *Journal of Applied Microbiology* **103**:985-992.

Theilade J, & Budtz-Jorgensen, E. (1980). Electron microscopic study of denture plaque. *Journal de Biologie Buccale* **8**:287-297.

Tonzetich J (1977). Production of oral malodor: a review of the mechanisms and methods of analysis. *Journal of Periodontology* **48**(1):13-20.

Verran J (1999). Denture plaque, denture stomatitis and the adhesion of *Candida albicans* to inert materials. In: Oral biofilms and plaque control. HE Busscher, LV. editor. Amsterdam: Harwood Academic publishers, pp. 175-191.

Verran J (2005). Malodour in denture wearers: an ill-defined problem. *Oral Diseases* **11**(Suppl. 1):24-28.

Webb B, Thomas, CJ., Harty, DWS., Willcox, MDP. (1998). Effectiveness of two methods of denture sterilisation. *Journal of Oral Rehabilitation* **25:**416-423.

Williams M (1999). Pre-clinical evaluation of plaque control agents: value of in vitro models. In: Oral biofilms and plaque control. HE Busscher, LV. editor. Amsterdam, The Netherlands: Harwood Academic Publishers, pp. 261-275.

Wilson M (1999). Use of constant-depth film fermenter in studies of bioiflms of oral bacteria. In: Methods in Enzymology: Biofilms. RJ Doyle editor. London: Academic Press, pp. 264-279.

Wimpenny J (1999). Laboratory models of biofilm. In: Dental plaque revisited - Oral biofilms in health and disease. HN Newman, & Wilson, M. editor. Cardiff: Bioline, pp. 89-110.

Yonezawa H, Takasaki, K., Teraoka, K., Asaka, T., Sato, C., Tsuchiya, K. (2003). Effects of tongue and oral mucosa cleaning on oral Candida species and production of volatile sulphur compounds in the elderly in a nursing home. *Journal of Medical and Dental Science* **50**(1):1-8.

Tables & Figures

Table 1. Mean viable counts of model denture plaque biofilms from replicate CDFF runs (n=5).

	Run 1	Run 2	Run 3	Run 4	Run 5	Mean	± SD
Anaerobes	$1.80x10^8$	$1.12x10^8$	$1.5x10^8$	$1.62x10^8$	$1.68x10^8$	$1.54x10^8$	$2.61x10^7$
Lactobacilli	$2.92x10^6$	$1.80x10^7$	$5.7x10^5$	$4.37x10^5$	$5.33x10^5$	$4.49x10^6$	$7.62x10^6$
Candida	$5.0x10^3$	$1.67x10^2$	$3.5x10^4$	$1.67x10^2$	0	$8.07x10^3$	$1.52x10^4$

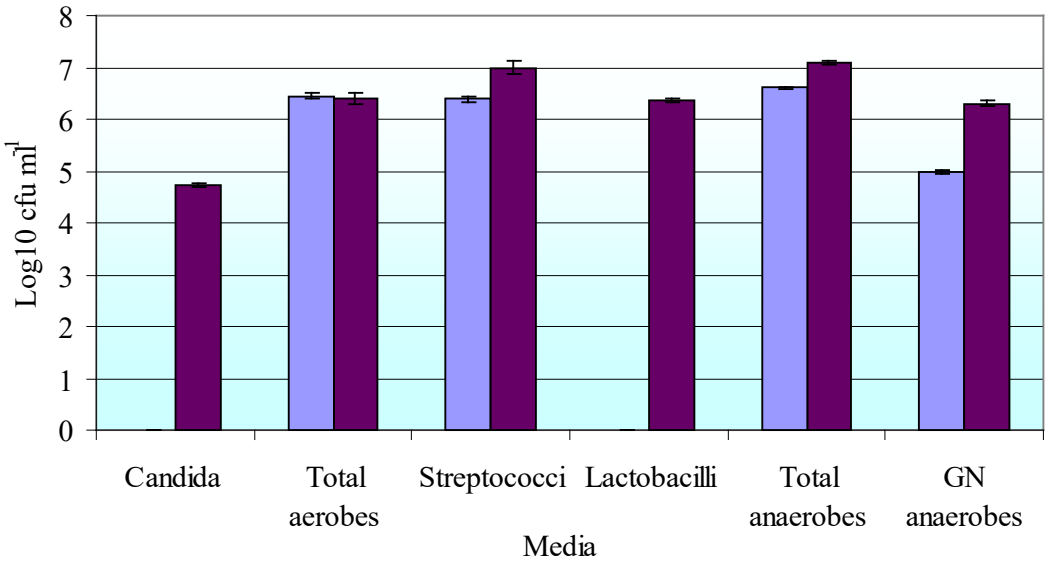


Figure 1. Denture plaque composition without (□) and with (■) *C. albicans*.

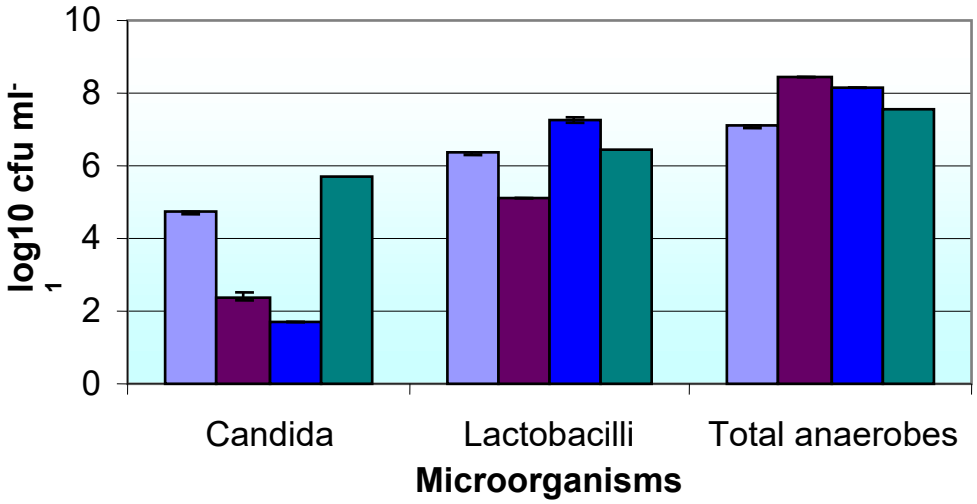
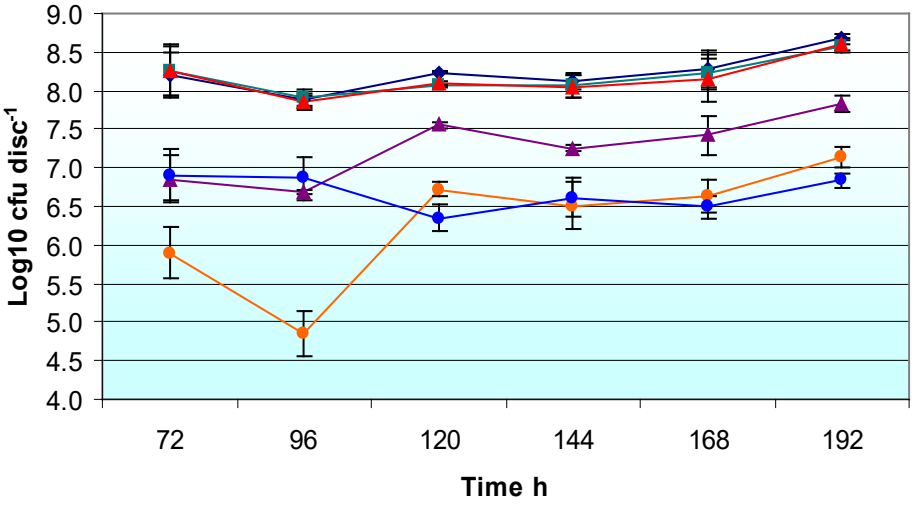
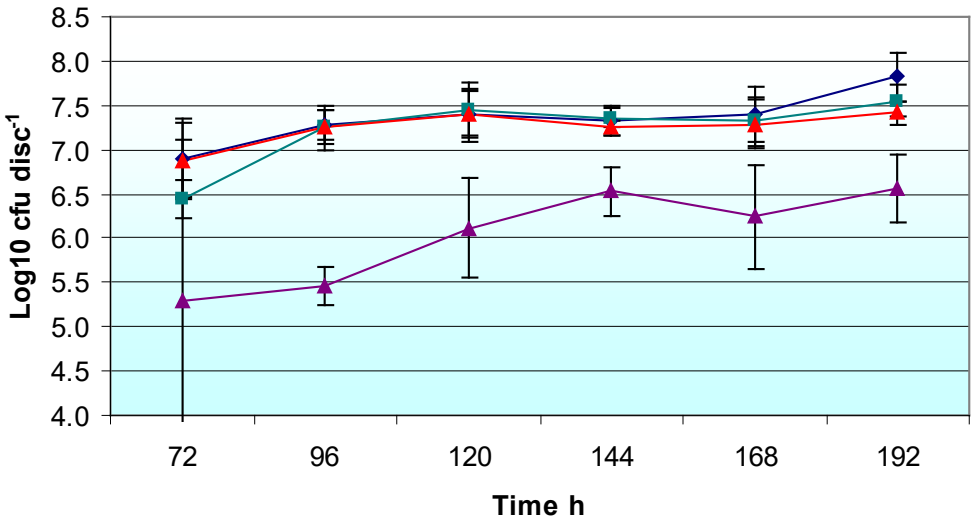


Figure 2. Viable microbial composition (log10 cfu ml^{-1}) ± SE from pooled denture plaque (□), fresh saliva (■), frozen saliva (■), and model denture plaque (■).



a)



b)

Figure 3. Viable microbial composition (log10 cfu disc^{-1}) ± SE from pooled denture plaque biofilms grown on acrylic discs (n=3) in the CDFF over 192 h a) with candida, b) without candida. Total anaerobes (◆), total aerobes (■) (168 n=1, no count at 192h), *Streptococcus* spp. (▲), *Lactobacillus* spp. (●), presumptive Gram negative obligate anaerobes (▲), and *Candida* spp. (●).