Oral Diseases (2007) 13, 279–284. doi:10.1111/j.1601-0825.2006.01279.x

http://www.blackwellmunksgaard.com

ORIGINAL ARTICLE

Candida albicans does not invade carious human dentine

M Maijala[1], R Rautemaa[2,3,4], A Järvensivu[1,2,4], M Richardson[3,4], T Salo[5,6], L Tjäderhane[1,2]

[1]*Institute of Dentistry, University of Helsinki, Helsinki;* [2]*Department of Oral and Maxillofacial Diseases, Helsinki University Central Hospital (HUCH), Helsinki;* [3]*Microbiology Unit of Helsinki University Central Hospital (HUCH) Laboratory Diagnostics, Helsinki;* [4]*Department of Bacteriology and Immunology, Haartman Institute, Helsinki;* [5]*Institute of Dentistry, University of Oulu, Oulu;* [6]*Oulu University Hospital, Oulu, Finland*

AIM: *Candida albicans* has been proposed to be a caries pathogen, but the evidence for its specific role is lacking. To be considered significant in caries progression, a marked amount of yeasts should be present in a lesion. The aim of the study was to investigate the presence of *C. albicans* in dentinal caries lesions.

MATERIALS AND METHODS: To demonstrate the extension of caries and to identify the bacteria in a lesion, sections of 10 carious human teeth were stained with Gram and Giemsa stains. *C. albicans* was detected with periodic acid-Schiff (PAS) staining and by immunohistochemistry using a *C. albicans*-specific antibody 3H8. Thirty sections were used for each staining (in total 120 sections).

RESULTS: Extensive bacterial invasion and intensive staining by PAS occurred in all samples. However, with the *C. albicans*-specific antibody, only 30 (3.3%) sections stained weakly positive, with a few stained cells on the lesion surface. However, the positive identification of *C. albicans,* based on the morphology of the cells, was not possible.

CONCLUSIONS: The results do not support the previous suggestion that *C. albicans* is important in the dentine caries pathology. In addition, because of its unspecific nature, PAS turned out to be an unsuitable method for detecting yeasts in carious tooth samples.

Oral Diseases (2007) **13**, 279–284

Keywords: *Candida albicans*; immunohistochemistry; caries; dentine; humans

Introduction

Yeasts are frequently found in the oral cavity as part of the normal flora. The most dominant species is *Candida albicans*. Yeasts are opportunistic pathogens, i.e. they may cause infections if the environment becomes favourable (Odds, 1988; Samaranayake and MacFarlane, 1990). *C. albicans* can cause mucosal infections such as stomatitis, especially in denture-wearers (Nikawa *et al*, 1998). *C. albicans* can also be a part of the disease process in periodontitis. It has been shown to be able to invade connective tissue after passing through the surface epithelium (Järvensivu *et al*, 2004). *C. albicans* has also been suggested as a possible causative agent of caries (e.g. Sedgley *et al*, 1997; Jacob *et al*, 1998; Moalic *et al*, 2001; Nikawa *et al*, 2003). Although these suggestions have largely been based on studies in which *C. albicans* has been found in plaque or saliva (Russell *et al*, 1991; Coulter *et al*, 1993; Loesche *et al*, 1995; Sedgley *et al*, 1997; Radford *et al*, 2000; Moalic *et al*, 2001; Wall-Manning *et al*, 2002), there are some studies indicating its presence in caries lesions (Jacob *et al*, 1998; Marchant *et al*, 2001; Shen *et al*, 2002). However, it is not known if *C. albicans* is a true cariogenic pathogen or simply an indicator of oral conditions favourable for caries or a structural component of the dental plaque biofilm. *C. albicans* has been isolated from root caries lesions (Marren *et al*, 1996; Shen *et al*, 2002), but whether it has a role in enamel or dentinal caries is unclear.

Candida albicans has several properties that make it a potential caries pathogen. It is capable of producing acids under suitable conditions (Samaranayake *et al*, 1986; Peltroche-Llacsahuanga *et al*, 2001). These acids might demineralize enamel and dentine (Nikawa *et al*, 2003). The colonization of oral hard tissues by *C. albicans* may be promoted by the presence of streptococci (Jenkinson *et al*, 1990), which are thought to be the major pathogens in caries. *C. albicans* also readily adheres to both intact and denatured collagen (Makihira *et al*, 2001, 2002), present in dentinal caries lesions. It also produces proteolytic enzymes, which can break the organic collagen matrix of dentine (Hagihara *et al*, 1988; Nishimura *et al*, 2002). It has been shown that *C. albicans* hyphae are able to penetrate enamel fractures and dentinal tubules from outside the tooth (Sen *et al*, 1997a) and from the root canal (Waltimo

Correspondence: Dr Leo Tjäderhane, Institute of Dentistry, University of Helsinki, PO Box 41, 00014 University of Helsinki, Finland. Tel: +358 9 191 27261, Fax: +358 9 191 27517, E-mail: leo.tjaderhane@helsinki.fi
Received 14 February 2006; revised 29 March 2006; accepted 5 April 2006

et al, 2000) *in vitro*. *C. albicans* hyphae and yeast cells have also been seen in dentinal tubules under large caries lesions *in vivo* (Jacob *et al*, 1998). Together these factors indicate that *C. albicans* might be an important pathogen in the progression of dentinal caries.

The aim of this study was to examine the presence and quantity of *C. albicans* in dentinal caries. The hypothesis was based on the premise that if *C. albicans* is an important caries pathogen, significant amounts of yeast would be present in demineralized dentine in large, exposed dentinal caries lesions. Furthermore, due to its capability to invade dentinal tubules (Jacob *et al*, 1998; Sen *et al*, 1997a,b; Waltimo *et al*, 2000), *C. albicans* could also be found in undemineralized dentine beyond the borders of a carious lesion. Stainings for bacteria were also used to demonstrate the extension of the lesion and to confirm the microbial presence in carious dentine.

Materials and methods

Samples

Ten molar teeth with extensive occlusal caries lesions were all extracted as part of the comprehensive dental treatment at the University Student Health Care Center and the Department of Oral and Maxillofacial Surgery, University of Oulu. The molars were used with the patient's informed consent, following the guidelines of the Faculty of Medicine at the University of Oulu for the use of human samples in research. Only one tooth per patient was included in the study. The teeth were stored immediately after extraction in neutral 4% formaldehyde for 48 h. Within the same day, the teeth were also sectioned in the middle of the caries lesion to ensure fixation and to allow precise sectioning of the lesion area later. After fixation, the teeth halves were demineralized in ethylenediaminetetraacetic acid (EDTA), embedded in paraffin and cut into 6-μm sections with a microtome in a standard fashion. Both halves of each tooth were cut, allowing the staining of the sections representing the whole caries lesion. Three sections from each lesion were chosen to be stained with *C. albicans*-specific antibody, one in the middle of the lesion and two in the periphery of the lesion, one on each side. Adjacent sections were stained with Gram, Giemsa and periodic acid-Schiff (PAS) stains. The total number of the sections stained was 12 for each lesion, thus resulting in 120 stained sections, 30 with each stain and 30 with the *C. albicans*-specific antibody.

Agar blocks containing *C. albicans* ATCC28366 were used as controls in the immunohistochemical staining procedure. They have been found suitable for use with this kind of staining (Williams *et al*, 1998; Järvensivu *et al*, 2006). One millilitre of yeast suspension in normal human serum or RPMI1640 was mixed with 1 ml of 2% warm purified agarose (Pharmacia, Copenhagen, Denmark) and poured into individual compartments of plastic Petri dishes (Bibby Sterilin Ltd, Stone, Staffordshire, UK). After solidifying, the embedded agarose material was fixed in 10% formal saline for 24 h before processing through graded concentrations of alcohol and xylene, and embedded in paraffin wax. The blocks were then treated like the tooth samples.

Gram staining

Gram stain is routinely used to detect bacteria in many kinds of specimens (Disbrey *et al*, 1970). The sections were deparaffinized in xylene and rehydrated in graded alcohol series and in water. First, the sections were stained in Lillie's crystal violet for 2 min and rinsed with tap water. Then they were transferred to Lugol's iodine and stained for 1 min, rinsed with acetone–ethanol solution and with tap water. Then the sections were exposed to neutral red for 1 min and rinsed with tap water. Finally, they were dehydrated and mounted in Dako Glysergel mounting medium (Dako Corp., Carpinteria, CA, USA).

Giemsa staining

Giemsa is a stain for all bacteria. It does not distinguish Gram-positive from Gram-negative bacteria (Disbrey *et al*, 1970). The sections were deparaffinized and rehydrated, exposed to 4% Giemsa solution, kept in a humid chamber for 45 min at 20°C, and mounted with Mountex® mounting medium (Histolab Products Ab, Gothenburg, Sweden).

PAS staining

Periodic acid-Schiff stain is widely used to detect fungi in different kinds of tissue samples. The sections were deparaffinized and rehydrated as described previously. They were first exposed to periodic acid and then stained with Schiff's leuco-fuchsin reagents, rinsed and mounted as above.

Immunohistochemical staining

The primary antibody used to detect *C. albicans* was a mouse monoclonal antibody 3H8 (IgG1; 2.5 mg ml^{-1}; kindly provided by Prof. R. Robert at Société de Recherche et de Réalisations Biotechnologiques, Paris, France). It was raised against a zymolyase-solubilized preparation from blastoconidia cell walls of *C. albicans* ATCC26555. The antibody recognizes mannoproteins of high molecular mass present in the *C. albicans* cell wall (Marcilla *et al*, 1999; Ellepola and Morrison, 2005). The sections were deparaffinized in xylene, rehydrated in graded alcohol series and in water, incubated with pepsin for 45 min in a humid chamber at 37°C and washed three times for 4 min with phosphate-buffered saline (PBS). To inhibit endogenous peroxidase activity the sections were incubated with H_2O_2 in methanol for 30 min at 20°C. They were then washed three times for 4 min with PBS. To inhibit non-specific staining the sections were first incubated with normal horse serum from the Vectastain® ABC kit [1:50 in 2% bovine serum albumin (BSA)/PBS] for 20 min in a humid chamber at 20°C, and then incubated with the primary antibody against *C. albicans* (3H8 1:500 in 1% BSA/PBS), first for 30 min at 37°C, then for 60 min at 20°C. Then the sections were kept overnight in a humid chamber at 4°C. For negative controls, 1% BSA/PBS was used instead of

a primary antibody. The next day, the sections were first washed with PBS three times for 4 min, and incubated with anti-mouse biotinylated antibody (1:200 in 0.1% BSA/PBS) from the Vectastain® ABC kit for 30 min at 37°C in a humid chamber. The sections were then washed with PBS three times for 4 min and incubated with the kit reagent for 30 min at 37°C in a humid chamber. After that, they were washed again with PBS three times for 5 min. Peroxidase-binding sites were revealed by keeping the sections in 3-amino-9-ethylcarbazole (AEC) with H_2O_2 for 15 min at 20°C, washed with tap water for 10 min and counterstained with Mayer's haematoxylin for 3 min 45 s. Finally, the sections were rinsed with tap water for 10 min and mounted with Dako Glysergel mounting medium.

Results

Bacterial stainings

With Gram staining ($n = 30$), all the lesions were stained intensively, indicating a strong invasion of dentinal tubules by Gram-positive bacteria (Figure 1a). Giemsa staining ($n = 30$) was similarly strongly positive in all the specimens demonstrating bacteria under the surface of the lesion, which extended deep into dentine (not shown).

PAS staining

With PAS staining ($n = 30$) all the lesions of all the specimens were positive. There were intensive stainings observed in the dentinal tubules in the lesions, resembling the result of Gram and Giemsa stainings (Figure 1b).

Anti-C. albicans immunohistochemical staining

Positive staining for *C. albicans* was detected in only one of the sections (of 30) of one lesion. In this section, weak positive staining was restricted to two small isolated areas on the surface of the lesion (Figure 1c–e). All the other sections were negative. Positive controls stained intensively and *C. albicans* cells could be clearly seen (Figure 1f). The negative controls showed no staining (not shown).

Discussion

Candida albicans is regarded as the most potential pathogenic fungus normally found in the oral cavity. It is known to possess adhesive, acidogenic and proteolytic properties essential for dentinal caries progression and it has been reported to be capable of invading dentinal tubules (Sen *et al*, 1997a,b; Jacob *et al*, 1998; Waltimo *et al*, 2000). In this study, extracted human molar teeth with large, exposed dentinal caries lesions were used to study the role of *C. albicans* in dentinal caries *in vivo*. The virtual absence of staining with the *C. albicans*-specific 3H8 antibody in the lesions strongly indicates that yeasts in general, and *C. albicans* especially, are not present in dentinal lesions. It may be argued that *C. albicans* cells would not have survived the decalcification process. However, the loss of *C. albicans* structures in the EDTA processing seems unlikely, as the bacteria were widely present inside the dentinal tubules, and *C. albicans* has previously been shown to resist dentine decalcification (Waltimo *et al*, 2000).

It has been suggested that *C. albicans* could have a role in the infrastructure of dental microbial plaque and in its adherence to the oral surfaces. Unlike most microbes, *C. albicans* is rather tolerant to the immune defence mechanisms of the oral cavity as reflected by its ability to colonise oral surfaces. In our previous studies *C. albicans* was typically found in the outer layers of the plaque and it seemed to act as a barrier between the host immunity and the inner layers of the mixed biofilm (Järvensivu *et al*, 2004). Therefore, *C. albicans* could have a role in the immune evasion of the plaque in oral infections, although it may not directly impact on the development of dental caries lesions.

In mucosal samples, PAS staining is a useful tool for identification of fungi, although *C. albicans*-specific antibodies are significantly more sensitive (Järvensivu *et al*, 2004). In addition to fungi, a wide variety of structures containing carbohydrates, e.g. in saliva will stain positively with this stain (Disbrey *et al*, 1970). PAS staining was found to be unsuitable for detection of yeasts in dentinal caries because of its unspecific nature. Positive staining could be detected throughout the lesions but the PAS stain did not highlight any microbial structures. All the sections stained with PAS showed intensive staining in the dentinal tubules, while adjacent sections of the same lesion stained with anti-*C. albicans* immunohistochemical stain were negative. Anti-*C. albicans* immunohistochemical staining was found to be functional and specific, as the positive controls were stained intensively and the negative controls remained unstained, confirming the previous findings (Järvensivu *et al*, 2004). It is highly unlikely that other yeasts, unrecognized by *C. albicans*-specific antibody, would be present in caries lesions in quantities large enough to be responsible for the extensive PAS staining observed. The positive staining for PAS may reflect the presence of salivary proteins rich in carbohydrates. Salivary mucopolysaccharides may gain entry to the tubules, because the lesions were extensive and the acids from bacterial metabolism had etched the outer ends of the tubules open. Alternatively, positive staining may indicate alterations in the structure of the dentine matrix during the progression of caries, possibly demonstrating the presence of depolymerized components of the material coating the collagen fibres (Lormée *et al*, 1986). It is also noteworthy that all the lesions included in the study were coronal lesions, and the possibility that *C. albicans* would be present in a larger amount in root caries lesions still remains. However, all the teeth included in the study had extensive open caries lesions, the dentine was widely exposed, and the access of salivary or plaque *C. albicans* to enter the dentine surface and dentinal tubules was evident. Thus we believe that the same applies to root caries, although further stainings would be needed to confirm this assumption.

Candida albicans-specific antibody 3H8 has successfully been used to identify *C. albicans* in several studies (e.g. Iranzo *et al*, 2003; Marot-Leblond *et al*, 2004;

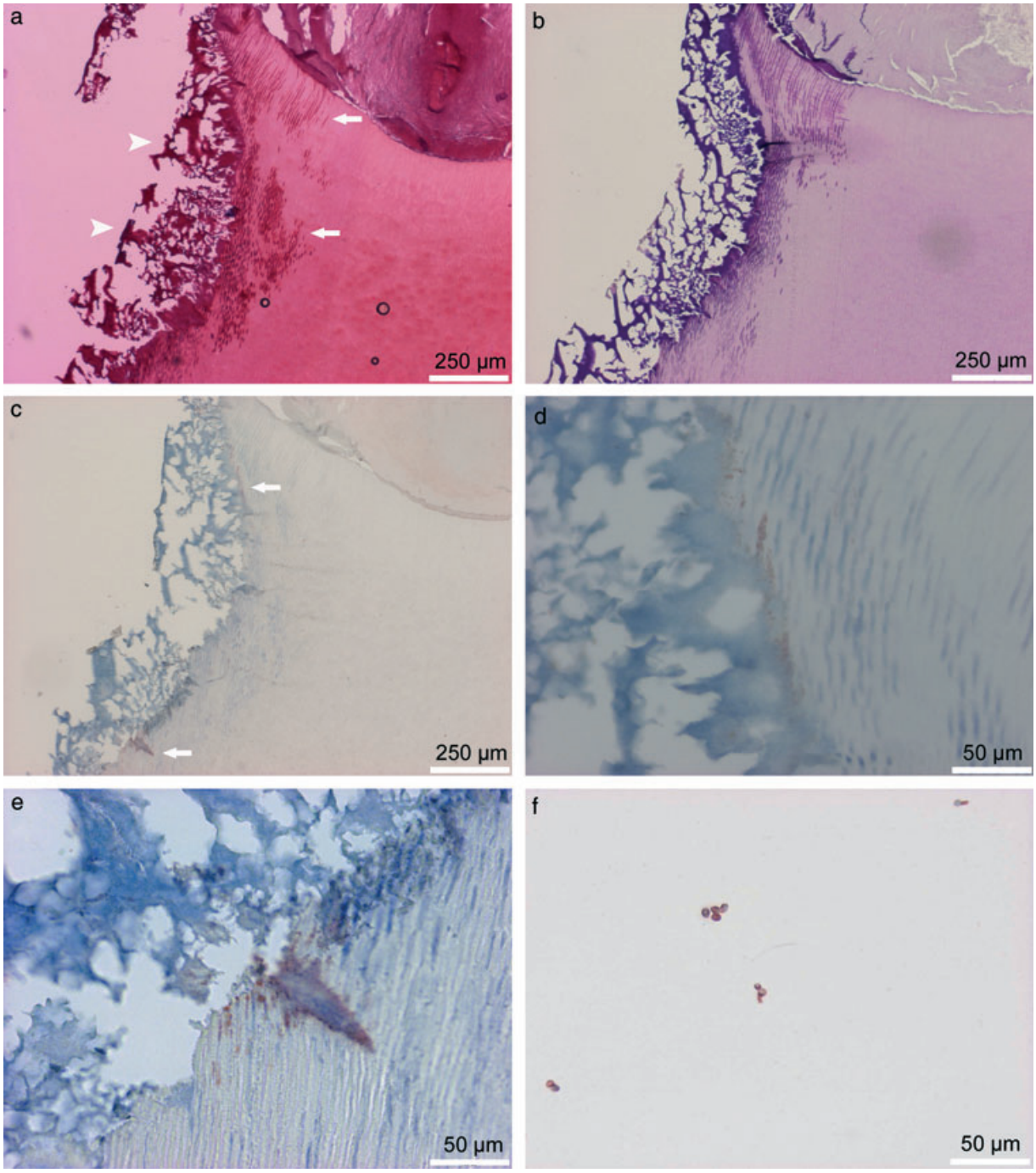

Figure 1 The results of the stainings in the only sample in which *C. albicans* was detected with the *C. albicans*-specific antibody. (**a**) Gram staining demonstrated extensive destruction of dentine on the surface of the dentinal caries lesion (arrowheads), with bacterial penetration surrounding the lesion surface (arrows). Giemsa staining provided similar outcome (not shown). Magnification: 4×. (**b**) Adjacent section to the one in (**a**), stained with PAS. Overall, the staining pattern essentially followed the one seen with Gram staining in (**a**). Magnification: 4×. (**c**) Adjacent section stained with *C. albicans*-specific 3H8 antibody. Two isolated areas with faint staining were observed (arrows). Magnification: 4×. (**d**–**e**) Higher magnification of the areas showing positive staining in (**c**). The selective staining with *C. albicans*-specific 3H8 antibody of the structures mostly located in dentinal tubules, indicated the presence of *C. albicans*, although the normal structures of *C. albicans* could not be readily identified. Magnification: 20×. (**f**) In positive controls, intensive staining demonstrating normal form of *C. albicans* with *C. albicans*-specific antibody was readily identified. Magnification: 20×

Weissman and Kornitzer, 2005). All morphological forms of *C. albicans* stain positive for the cell wall mannoprotein recognized by the antibody 3H8 (Järvensivu *et al*, 2006), and it is a more sensitive detector of *C. albicans* in dental plaque samples than culturing (Järvensivu *et al*, 2004). Based on this technique, a positive correlation between the yeasts and caries could not be identified. The results indicate that yeasts do not have an important role in the

progression of caries in the sense that they would populate caries lesions, penetrate into and demineralize dentine or degrade demineralized dentine matrix. The apparent discrepancy between our finding and the previous results indicating the presence of *C. albicans* in caries lesions (Marchant *et al*, 2001; Shen *et al*, 2002) may be explained by the different techniques applied. In previous studies, carious dentine was collected for microbial sampling, and the plaque was either not removed (Marchant *et al*, 2001) or plaque was only superficially swabbed away (Shen *et al*, 2002). It is thus highly possible, that the samples contained microbes originally situated in the plaque, and not in the body of lesion. This assumption is supported by the fact that the proportion of *C. albicans* in the samples was very low, although the isolation frequency was very high in both studies (Marchant *et al*, 2001; Shen *et al*, 2002). Jacob *et al* (1998) claimed that *C. albicans* was present in dentinal tubules under caries lesions, based on PAS staining and scanning electron microscopy. However, our results clearly demonstrate that PAS is not a reliable method to detect yeasts in carious dentine. On the other hand, SEM is a descriptive method for the morphology of the yeast, and absolutely reliable only in *in vitro* studies with monoinfection (Waltimo *et al*, 2000). Therefore, the importance of *C. albicans* as a caries pathogen may be overestimated in the previous studies. It may still have a supportive role, especially in the early phases of caries lesion formation, as *C. albicans* is known to be able to participate in the formation of microbial biofilms (Sen *et al*, 1997a), and it is known to be able to produce acids (Samaranayake *et al*, 1986; Peltroche-Llacsahuanga *et al*, 2001) possibly participating with the demineralization.

Acknowledgements

We thank E-M Kiljander and M Kivekäs for their excellent assistance in laboratory work. This study was funded by the Academy of Finland (grant no. 104 337), the Finnish Dental Society Apollonia, and The Finnish Woman Dentists' Association.

References

Coulter WA, Murray SD, Kinirons MJ (1993). The use of a concentrated oral rinse culture technique to sample oral candida and lactobacilli in children, and the relationship between candida and lactobacilli levels and dental caries experience: a pilot study. *Int J Paediatr Dent* **3:** 17–21.

Disbrey BD, Rack JH (1970). Histological Laboratory Methods. E & S Livingstone, London.

Ellepola AN, Morrison CJ (2005). Laboratory diagnosis of invasive candidiasis. *J Microbiol* **43** (Spec No)**:** 65–84.

Hagihara Y, Kaminishi H, Cho T, Tanaka M, Kaita H (1988). Degradation of human dentine collagen by an enzyme produced by the yeast *Candida albicans*. *Arch Oral Biol* **33:** 617–619.

Iranzo M, Canizares JV, Sainz-Pardo I, Aguado C, Ponton J, Mormeneo S (2003). Isolation and characterization of an avirulent *Candida albicans* yeast monomorphic mutant. *Med Mycol* **41:** 43–52.

Jacob LS, Flaitz CM, Nichols CM, Hicks MJ (1998). Role of dentinal carious lesions in the pathogenesis of oral candidiasis in HIV infection. *J Am Dent Assoc* **129:** 187–194.

Järvensivu A, Hietanen J, Rautemaa R, Sorsa T, Richardson M (2004). Candida yeasts in chronic periodontitis tissues and subgingival microbial biofilms in vivo. *Oral Dis* **10:** 106–112.

Järvensivu A, Rautemaa R, Sorsa T, Richardson M (2006). Specificity of the monoclonal antibody 3H8 in the immunohistochemical identification of *Candida* species. *Oral Dis* **12:** 428–433.

Jenkinson HF, Lala HC, Shepherd MG (1990). Coaggregation of *Streptococcus sanguis* and other streptococci with *Candida albicans*. *Infect Immun* **58:** 1429–1436.

Loesche WJ, Schork A, Terpenning MS, Chen Y-M, Stoll J (1995). Factors which influence levels of selected organisms in saliva of older individuals. *J Clin Microbiol* **33:** 2550–2557.

Lormée P, Weill R, Septier D (1986). Morphological and histochemical aspects of carious dentine in Osborne-Mendel rats. *Caries Res* **20:** 251–262.

Makihira S, Nikawa H, Tamagami M, Hamada T (2001). Bacterial and candidal adhesion to intact and denatured collagen in vitro. *Mycoses* **45:** 389–392.

Makihira S, Nikawa H, Tamagami M, Hamada T, Samaranayake LP (2002). Differences in *Candida albicans* adhesion to intact and denatured type I collagen in vitro. *Oral Microbiol Immunol* **17:** 129–131.

Marchant S, Brailsford SR, Twomey AC, Roberts GJ, Beighton D (2001). The predominant microflora of nursing caries lesions. *Caries Res* **35:** 397–406.

Marcilla A, Monteagudo C, Mormeneo S, Sentandreu R (1999). Monoclonal antibody 3H8: a useful tool in the diagnosis of candidiasis. *Microbiology* **145:** 695–701.

Marot-Leblond A, Grimaud L, David S *et al*. (2004). Evaluation of a rapid immunochromatographic assay for identification of *Candida albicans* and *Candida dubliniensis*. *J Clin Microbiol* **42:** 4956–4960.

Marren PV, Beighton D, Lynch E (1996). Isolation of yeasts from root caries in an elderly population. *J Dent Res* **75** (Spec issue)**:** 343.

Moalic E, Gestalin A, Quinio D, Gest PE, Zerilli A, Le Flohic AM (2001). The extent of oral fungal flora in 353 students and possible relationships with dental caries. *Caries Res* **35:** 149–155.

Nikawa H, Hamada T, Yamamoto T (1998). Denture plaque – past and recent concerns. *J Dent* **26:** 299–304.

Nikawa H, Yamashiro H, Makihira S *et al* (2003). In vitro cariogenic potential of *Candida albicans*. *Mycoses* **46:** 471–478.

Nishimura M, Nikawa H, Yamashiro H, Nishimura H, Hamada T, Embery G (2002). Cell-associated collagenolytic activity by *Candida albicans*. *Mycopathologia* **153:** 125–128.

Odds FC (1988). *Candida* and candidosis. 2nd edn. Bailliére Tindall: London.

Peltroche-Llacsahuanga H, Hauk CJ, Kock R, Lampert F, Lütticken R, Haase G (2001). Assessment of acid production by various human oral micro-organisms when palatinose or leucrose is utilized. *J Dent Res* **80:** 378–384.

Radford JR, Ballantyne HM, Nugent Z *et al*. (2000). Caries-associated micro-organisms in infants from different socio-economic backgrounds in Scotland. *J Dent* **28:** 307–312.

Russell JI, MacFarlane TW, Aitchison TC, Stephen KW, Burchell CK (1991). Prediction of caries increment in Scottish adolescents. *Community Dent Oral Epidemiol* **19:** 74–77.

Samaranayake LP, MacFarlane TW (1990). *Oral candidosis*. Wright: London.

Samaranayake LP, Hughes A, Weetman DA, MacFarlane TW (1986). Growth and acid production of *Candida* species in human saliva supplemented with glucose. *J Oral Pathol* **15:** 251–254.

Sedgley CM, Samaranayake LP, Chan JC, Wei SH (1997). A 4-year longitudinal study of the oral prevalence of enteric gram-negative rods and yeasts in Chinese children. *Oral Mircobiol Immunol* **12:** 183–188.

Sen BH, Safavi KE, Spångberg LSW (1997a). Colonization of *Candida albicans* on cleaned human dental hard tissues. *Arch Oral Biol* **42:** 513–520.

Sen BH, Safavi KE, Spangberg LS (1997b). Growth patterns of *Candida albicans* in relation to radicular dentin. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **84:** 68–73.

Shen S, Samaranayake LP, Yip HK, Dyson JE (2002). Bacterial and yeast flora of root surface caries in elderly, ethnic Chinese. *Oral Dis* **8:** 207–217.

Wall-Manning GM, Sissons CH, Anderson SA, Lee M (2002). Checkerboard DNA-DNA hybridisation technology focused on the analysis of Gram-positive cariogenic bacteria. *J Microbiol Methods* **51:** 301–311.

Waltimo TMT, Ørstavik D, Siren EK, Haapasalo MPP (2000). In vitro yeast infection of human dentin. *J Endod* **26:** 207–209.

Weissman Z, Kornitzer D (2005). A family of *Candida* cell surface haem-binding proteins involved in haemin and haemoglobin-iron utilization. *Mol Microbiol* **53:** 1209–1220.

Williams DW, Jones HS, Allison RT, Potts AJC, Lewis MAO (1998). Immunocytochemical detection of *Candida albicans* in formalin fixed, paraffin embedded material. *J Clin Pathol* **51:** 857–859.