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Induction of caries-like lesions by *Candida albicans* in an artificial mouth

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Authors:

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Introduction

Decades ago *Candida* species, frequently found in the human oral cavity, have been thought to be responsible for caries evolution. The later shift of attention in caries research towards mutans streptococci let the yeasts fall into oblivion. Recently, new microecological concepts of caries research and isolating collagenases from *Candida albicans* have led to a revived interest in *Candida* species and their possible role in the etiopathogenesis of dental caries. This investigation shows some first findings to contribute to the discussion.

Material and Methods

Clinical caries - culture and light microscopy

Extracted teeth with active root caries lesions were fixed and embedded either in epoxy resin or paraffin after decalcification in EDTA. Sections were stained and examined by light microscopy. Immunostaining was performed using a monoclonal antibody. Samples of softened dentin were cultivated on Candiselect agar and *Candida* species were differentiated.

Artificial colonization of dentin - light microscopy

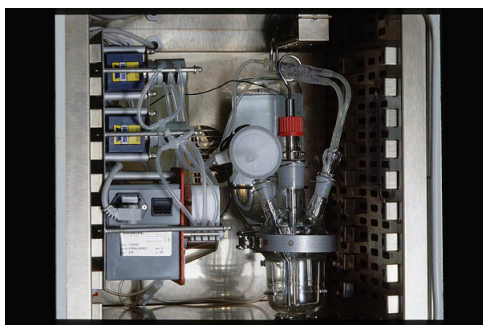
Dentin specimens were contaminated with a *C. albicans* strain SC-5314 and incubated for eight weeks in an artificial mouth. Controlled by a microcomputer, an artificial saliva was added and changed every 60 minutes. Sugar solution was supplemented in the middle of each hour. The purity and pH of the culture were observed. After fixation the specimens were decalcified and embedded in paraffin. Sections were stained and examined under the light microscope.

Artificial colonization of enamel - polarized light microscopy

Enamel slices were partially covered with nail varnish and incubated in the artificial mouth. After demounting and imbibition in water the slices were viewed in polarized light.

Artificial colonization of dentin - electron microscopy

Specimens of EDTA-decalcified human dentin were immersed for 24 hours in a liquid culture of *Candida albicans* and an additional day in HBSS. After fixation the specimens were critical point dried, sputter coated and observed in a SEM.

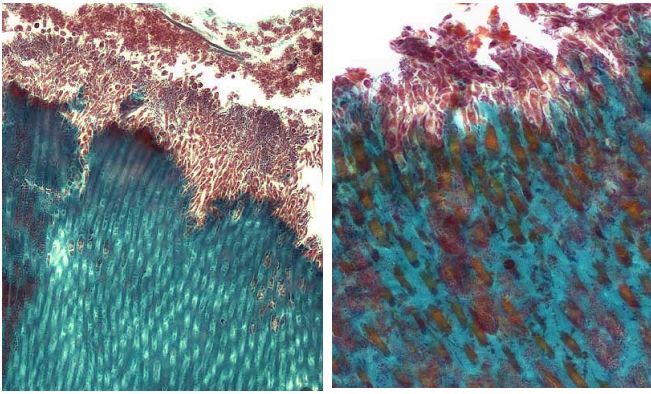


Artificial mouth incubation unit

Results

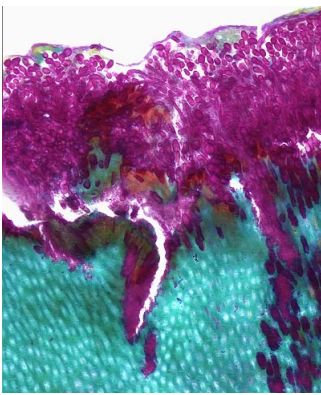
Clinical caries - culture and light microscopy

Dentin was found to be penetrated by *Candida albicans* and other *Candida* species. Both blastospores and hyphae could be discerned. Crater-shaped areas of dentinal decay were observed, which were colonized solely with yeasts. Immunohistochemistry revealed *Candida albicans* penetrating the dentin.

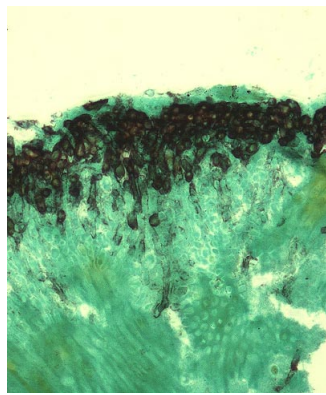


Crater-shaped areas of decay, filled with yeasts; Goldner's tricrome, x400

Yeasts penetrating the dentin; Goldner's tricrome, x400



Funghi amassed in a microbial consortium; PAS/light green, x400



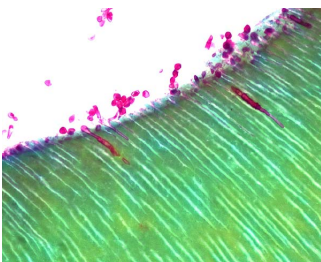
Candida albicans stained with specific antibody; mAb 1B12/light green, x400

Artificial colonization of dentin - light microscopy

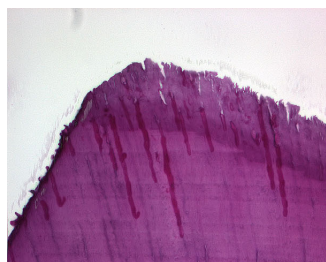
After eight weeks' incubation the specimens were covered by a thick plaque. Supplementation of carbohydrates reduced the plaque pH up to values of 3.45. Light microscopy revealed blastospores and hyphal extensions growing into the dentinal tubules, in some areas infiltrating the whole sample. The cemental structures were heavily infiltrated and loosened by the yeast.

Artificial colonization of enamel - polarized light microscopy

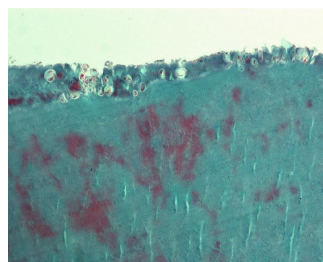
Below the fungal plaque the uncovered enamel surface became demineralized, and this was followed by a total enamel breakdown. It can be assumed that remineralizing conditions were insufficient because of the thickness of the plaque; therefore a clear subsurface demineralization did not occur.



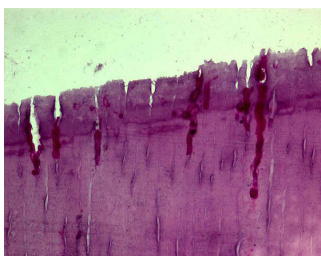
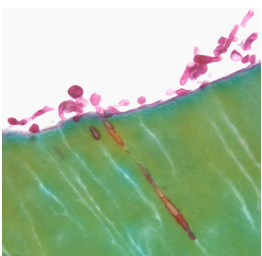
Hyphal extensions advancing into dentin; PAS/light green, x400



Infiltration of dentin by C. albicans; PAS x400



Fungi loosening cement structures; Goldner's tricrome, x400



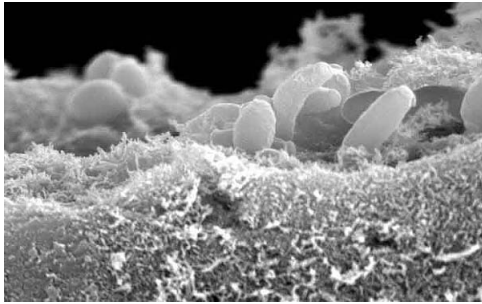
Candida growing into a dentinal tubule by budding; PAS/light green, x600

Tubules enlarged by fungal activity; PAS x400

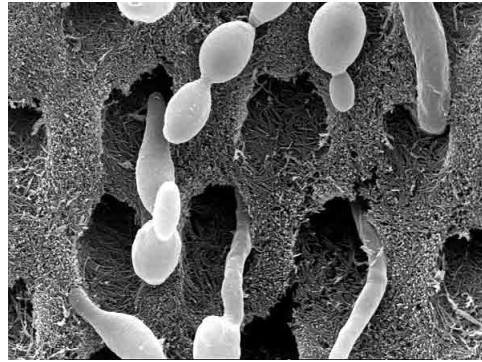
Deminerlization and breakdown of enamel; Polarized light/water, x25

Artificial colonization of dentin - electron microscopy

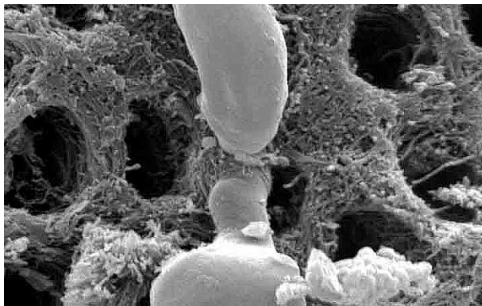
Blastospores of *C. albicans* adhered in high amounts to the demineralized dentin surface. Hyphae regularly moved into the dentinal tubules. The close contact of a hypha with the dentin blurred the structure of the collagen fibrils. This may be due to an excreted substance or to a collagenolytic activity of the yeast.



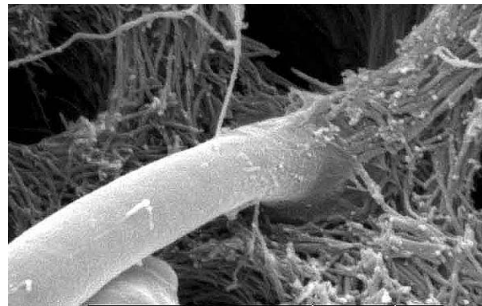
Blastospores adhering to the surface



Hyphal extensions growing into dentinal tubules



Yeast in close contact with collagen fibrils



Hypha affecting the alignment of collagen fibrils

Discussion

Although their presence is not essential to the development of dental caries, yeasts can be found in carious dentin not only in the zone of necrosis but also penetrating the dentin together with other microorganisms. Crater-shaped areas of dentinal decay, observed to be exclusively filled with yeasts, support the hypothesis of their active role in dentin caries pathogenesis. The ability to substantially lower the environmental pH makes them suitable for participation in the demineralization of dental hard tissues. These assumptions are confirmed by the artificial mouth experiment, in which the incubation of tooth segments with a *Candida*-monoculture caused demineralization, penetration and partially matrix destruction. Collagenolytic activity of *Candida albicans* has been shown by several authors. Further research is required with respect to the relevance of fungal enzyme secretion in the caries process. With regard to morphological phenomena a possible role of *Candida* species in the microecology of dental caries can be assumed.

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Abbreviations

HBSS - Hanks Balanced Salt Solution

Acknowledgement

The authors want to thank Prof. Dr. R. Sentandreu, Fac. de Pharmacia, Universitat de València, for the provision of the antibody.

This Poster was submitted by Dr. Thomas Klink.

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Poster Faksimile:

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Department of Conservative Dentistry of University Clinic Carl Gustav Carus, Dresden, Germany



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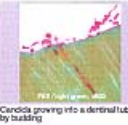

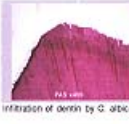
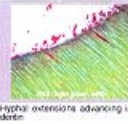



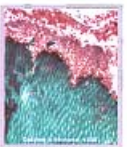
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Conclusion

With regard to morphological phenomena a possible role of *Candida* species in the microecology of dental caries can be assumed.