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In vivo study of the initial bacterial adhesion on different implant materials

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ABSTRACT

Objective: Biofilm formation on implant materials plays a major role in the aetiology of periimplantitis. The aim of this study was to examine *in vivo* the initial bacterial adhesion on six different implant materials.

Methods: The implant materials Ti-m, TiUnite®, ZiUnite®, ATZ-m, ATZ-s, TZP-A-m were tested using bovine enamel slabs as controls. All materials, fixed on splint systems, were examined after 30 min and 120 min of oral exposure. DAPI staining was used for quantitative analysis of the initially adherent microorganisms. Initial adherent microorganisms were visualised by fluorescence *In situ*-hybridisation (FISH) and quantified by confocal laser scanning microscopy (CLSM). The targets of the oligonucleotide probes were *Eubacteria*, *Veillonella* spp., *Fusobacterium nucleatum*, *Actinomyces naeslundii* and *Streptococcus* spp.

Results: DAPI analysis showed that increasing the time of oral exposure resulted in an increasing amount of initial adherent bacteria. The highest level of colonisation was on ZiUnite®, with the lowest occurring on the bovine enamel, followed by Ti-m. This early colonisation correlated significantly with the surface roughnesses of the materials. FISH and CLSM showed no significant differences relating to total bacterial composition. However, *Streptococcus* spp. was shown to be the main colonisers on each of the investigated materials.

Conclusion: it could be shown that within an oral exposure time of 30 min and 120 min, despite the salivary acquired pellicle initial biofilm formation is mainly influenced directly or indirect by the material surface topography. Highly polished surfaces should minimise the risk of biofilm formation, plaque accumulation and possibly periimplantitis.

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1. Introduction

The vast majority of the inserted oral implants today are made out of titanium.[1] However, the development of implant materials also shows that high-performance ceramics are increasingly able to perform the role of titanium, allowing a single material to be used for an entire restoration.[2] The advantages of ceramics are their chemical stability, biocompatibility, high mechanical strength and resistance to corrosion. Furthermore, they are non-allergenic.[3] As early as 1993 a metal-free abutment-system consisting of highly pure aluminium oxide was developed, but it gradually became clear that fractures occurred due to its unfavourable elasticity coefficient.[4] Bioactive glass-, resorbable tricalciumphosphate (TCP)- and hydroxyapatite (HA)-ceramics show a good bonding to bone as seen with aluminiumoxide, but the manufacturing procedure and susceptibility to fracturing are problematic.[5] Through the further development of ceramic as a material to be used in implantology zirconium oxide, especially the so-called tetragonal zirconia polycrystal (TZP), has come into favour. It is characterised by a high fracture strength and fracture toughness that are twice as high as that found in aluminium oxide.[6]

Since biofilm is formed on all surfaces of the oral cavity, there is an increased need for more information about possible differences in the initial rates of colonisation of bacteria on different dental materials.[7] The surface roughness, chemical composition, hydrophobicity, surface charge and surface energy of the material not only determine the rate of colonisation but also the structure and strength of biofilms.[8] In cariology, the formation of biofilms on tooth surfaces and restorative materials is considered to be an etiologic factor in the initial formation of caries, whereas in the field of implantology biofilms play a major role in the aetiology of periimplantitis.[9,10] The examination of oral biofilms is methodologically complex and difficult because biofilm structure is susceptible to mechanical, physical and chemical effects.[11] Sandig et al.[12] showed that through direct mechanical removal from the tooth surface biofilm breaks and thus changes its structure. In recent years, it has been shown that it is very important to use an intact plaque layer as a basis for research because *in vivo* formed biofilm differs significantly from the *in vitro* formed samples.[13,14] The oral cavity with its complex mix of germs, its shearing forces and the antimicrobial properties of saliva cannot be precisely imitated, which at least partially explains the difference seen in biofilms formed *in vivo* and *in vitro*.[15] The influence of the salivary acquired pellicle on bacterial adhesion *in vivo* is still unclear and under discussion.[13]

The aim of this study was to examine initial bacterial adhesion on six different implant materials *in vivo* using bovine enamel slabs as a control. The bovine enamel slabs are well-suited for their use as a control because of the structural similarity to human enamel.[16] DAPI staining was used for quantitative analysis, which enabled a simultaneous staining of all microoganisms present in the biofilm. Additionally, the initial adherent microorganisms were visualised by fluorescence *in situ*-hybridisation (FISH) and quantified by confocal laser scanning microscopy (CLSM). The combination of FISH and CLSM enables a non-destructive, three-dimensional examination and evaluation of biofilm structure.[15,17,18]

2. Materials and methods

2.1. Implant materials and enamel slabs

For the examination of initial bacterial adhesion *in vivo* the following six different oral implant materials (5 mm in diameter, 1 mm in thickness) were used: machined titanium (Ti-m) (Nobel Biocare, Gothenborg, Sweden), modified titanium (TiUnite®) (Nobel Biocare), ZiUnite® (Nobel Biocare), machined alumina-toughened zirconia (ATZ-m) (Metoxit, Thayngen, Switzerland), sandblasted alumina-toughened zirconia (ATZ-s) (Metoxit), and machined zirconia (TZP-A-m) (Metoxit).

As a control, bovine enamel slabs, made from freshly extracted, caries- and BSE-free front teeth from the lower jaw of cows (slaughterhouse, Freiburg, Germany) were used. The enamel slabs were manufactured as previously described.[19]

The BSE-free bovine enamel samples were disinfected by ultrasonication for 2 min in 2% sodium hypochlorite followed by ultrasonication in 70% ethanol for another 2 min. The samples were then washed twice and stored in sterile distilled water. The discs of implant material, 5 mm in diameter and 1 mm in thickness, were washed in distilled water and cleaned in 70% alcohol. Subsequently, the discs were then washed twice in distilled water.

The implant surface morphology was determined using scanning electron microscopy (SEM) and atomic force microscopy (AFM). Furthermore, hydrophilicity or hydrophobicity of the materials was determined by measurement of the contact angle using water. All of these values were already determined in a prior study concerning mature biofilm formation *in situ* on the same assortment of materials.[19]

2.2. Patient collective

Six healthy volunteers between the ages of 23–54 with DMFT (decayed, missing, filled teeth) values of 4.5 ± 3.5, saliva flow rates of 1.2 ± 0.2 ml/min and lactate formation rates of 3 ± 0.6 (scale from 1 to 9) were selected for the clinical experimental trial. Diseases of the salivary gland or general disorders were not present in any of the volunteers. In addition, no carious defects or inflammations of the marginal periodontium existed. A complete dentition with sufficient place for the slabs was required for a stable maintenance of the splint. Exclusion criteria for the volunteers were alcohol-, nicotin-, or drug-consumption, the use of antibacterial mouth rinses or antibiotics within the 3 month period prior to the study, as well as the implementation of oral hygiene activities in the 2 h period before and during the investigations. In addition, eating was not allowed during the wearing of the splint.

The volunteers consented to be included in the study, which was approved by the ethics commission (EK-63-07, University Freiburg, Germany) before the examinations started.

2.3. Splint system

For each of the volunteers an individual, intraoral splint system for the upper jaw was manufactured which contained