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REVIEW ARTICLE

The bacterial adhesion on and the cytotoxicity of various dental cements used for implant-supported fixed restorations

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Abstract

Objective. Bacterial adhesion on and cytotoxicity of eight luting agents used for implant-supported restorations were investigated. ***Materials and method.*** Surface roughness (Ra), surface free energy (SFE) values and three-dimensional images by atomic-force microscopy of circular specimens were determined. Bacterial suspensions of Streptococcus sanguinis and Streptococcus epidermidis were incubated at 37°C for 2 h. Adhering bacteria were examined with fluorescence dye CytoX-Violet, stained with 4',6-diamidino-2-phenylindole (DAPI) and visualized by fluorescence-microscopy. Cytotoxicity-testing was done with WST-1-tests (water soluble tetrazolium). No significant differences, neither with regard to Ra nor regarding SFE were determined. ***Results.*** Adherence of S. sanguinis was less on titanium, TempBondNE and TempBond. TempBond, TempBondNE, RelyX Unicem and Implantlink Semi Classic presented low amounts of S. epidermidis. WST-testing showed high cytotoxic potential of Harvard, Aqualox, TempBondNE and TempBond. No combination of low adherent bacteria with low cytotoxicity was found. ***Conclusion.*** From a biological *in-vitro* perspective, none of the cements may be recommended for implant-supported restorations.

Key Words: *Streptococcus sanguinis, streptococcus epidermidis, dental implant, surface roughness, surface free energy, dental cement*

Introduction

In modern dentistry, missing teeth are frequently replaced by dental implants so that removable prostheses can be avoided. Such implant restorations can be either screw-retained or cement-retained, but cement-retained prostheses have nowadays become the restoration of choice [1]. In clinical practice, cemented implant restorations often consist of zinc oxide phosphate, glass-monomer, resin-modified glass-ionomer, carboxylate, zinc oxide eugenol, zinc oxide non-eugenol, dual-curing composite, self-adhesive and silicone cements. The determining factors for the retentiveness of some of these luting agents used with metal-based and ceramic-based implant components have already been described in a previous trial [2].

As soon as implant surfaces, abutments and superstructures are exposed to the microbial habitat of the human oral cavity, they are immediately colonized by various micro-organisms [3]. Usually, fixed implant-supported restorations are cemented on the abutment in a 'critical region', in which soft connective tissues enclose the implant and its superstructures. Pathogenic biofilms in these areas and on the cement gap may lead to peri-implant mucositis and peri-implantitis, potentially resulting in peri-implant bone loss and implant failure [4–7]. The different luting systems show considerable differences in their probability of adhering oral biofilms [1,8,9], which mainly depends on their surface properties, such as roughness, morphology, and hydrophobicity. *Vice versa*, adhering bacteria may influence the substratum characteristics of specific cements by surface deterioration [10–12]. An aggravating factor is that luting agents for the cementation of fixed restorations on implants are even harder to remove from both the abutment and

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Table I. Overview of tested implant cements, manufacturers, arithmetic surface roughness values, and surface free energy values.

Brand name	Cement type	Manufacturer	R_a (μm)*	SFE (mJ/m^2)*
Harvard (fast luting)	Zinc oxide phosphate	Harvard, Berlin, Germany	1.07 ± 0.80	46.9 ± 3.1
Ketac cem	Glass ionomer	3M ESPE, Seefeld, Germany	0.32 ± 0.11	43.2 ± 2.5
TempBond	Zinc oxide eugenol (temporary)	Kerr, West Collins, USA	0.16 ± 0.03	43.6 ± 2.1
TempBond NE	Non-Eugenol zinc oxide (temporary)	Kerr, West Collins, USA	0.31 ± 0.25	41.1 ± 1.6
Aqualox	Carboxylate	VOCO, Cuxhaven, Germany	0.55 ± 0.21	44.0 ± 3.2
Panavia F 2.0	Dual-curing composite	Kuraray, Osaka, Japan	0.33 ± 0.11	40.9 ± 6.7
Rely X Unicem	Self-adhesive composite	3M ESPE, Neuss, Germany	0.24 ± 0.08	43.6 ± 3.9
Implantlink semi Classic	Resin-based (temporary)	DETAX, Ettlingen, Germany	0.13 ± 0.03	44.7 ± 2.5
Reference material (machined titanium)		Mechanische Werkstatt Biologie, University of Regensburg, Germany	0.10 ± 0.01	26.1 ± 6.4

*Mean (SD).

the implant structure than from natural tooth tissues. These cement residues, also described as residual excess cement [8], may provide perfect growth conditions for peri-implant biofilms that cause local traumata to the connective tissue [13,14]. In general, the role of implant cement and cement residues in the ethiology of peri-implantitis may have been under-estimated in the past. Therefore, it is of outmost importance for the longevity of implant-supported fixed restorations to use cements with low probability of adhering oral micro-organisms [4,5,15–18].

In addition to patient-specific factors, such as the character of salivary proteins and the sufficiency of oral hygiene, the quantity and composition of microbial biofilms on a specific substratum in the human cavity is influenced by the physico-chemical characteristics of the surface [6,19–22]. Both surface-free energy and surface roughness play essential roles in this process [6,23–25], in which surface roughness is assumed to be the predominant parameter [23,25,26]. High surface roughness values further bacterial adhesion by reducing the influence of shear forces on initially attaching bacteria [27–29]. The literature contains contrary reports regarding the influence of surface hydrophobicity on biofilm formation. However, most authors have suggested that implant substrata with high surface-free energy (SFE) enhance bacterial adhesion [29–33]. Other trials have shown that micro-organisms with high SFE are expected to preferentially adhere to surfaces with high SFE, whereas low SFE surfaces are mainly adhered to by low SFE micro-organisms [34,35].

Besides mechanical properties and the probability of adhering pathogenic biofilms, the biological compatibility and cytotoxicity of luting systems must be taken into consideration [36]. De Souza Costa et al. [37] investigated the cytotoxic effects of glass-ionomer cements on odontoblast cells and showed that conventional glass-ionomer cements had significantly lower cytopathic effects than resin-modified glass-ionomer cements. In cement-containing resin components, unbound free monomers released after polymerization are responsible for cytotoxic effects [38]. The release of unbound monomers or additives is caused by defective photopolymerization or by thermal, mechanical and chemical factors [38]. Approximately 15–50% of methacrylic groups remain unreacted [39]. In addition, the surface of composite resins exposed to oxygen during curing produces a non-polymerized surface layer containing cell toxic formaldehyde [40]. Therefore, the aim of the present *in vitro* trial was to investigate the probability of eight widely-used dental cements for implant-supported fixed restorations accumulating oral bacteria and to examine their cytotoxicity.

Materials and methods

Specimen preparation and surface characterization

In this trial, we assessed eight widely used luting systems for implant-supported fixed restorations and machined pure titanium as reference material (see Table I). Circular specimens measuring 6 mm in diameter and 2 mm in thickness were prepared according to the manufacturers' instructions using a custom metal mould with calibrated circular holes with a chemical balance (Sartorius, Göttingen, Germany). We mixed the components of the luting agents according to the manufacturers' mixing ratios, accepting a tolerance limit of ± 0.1 mg. The materials were inserted into a mould and immediately covered with a glass slide (Alfred Becht GmbH, Offenburg, Germany) to obtain smooth specimen surfaces. Additionally, all specimens were polished to a high gloss using a polishing machine (Motopol 8; Buehler Ltd.,

Coventry, UK) and wet abrasive paper discs (Buehler, Lake Bluff, IL) with a grit of 1000, 2000 and 4000.

The surface roughness of three specimens ($n = 3$) of each of the eight materials (8 × 3), which was determined at three different sites with a stylus instrument (Perthometer S6P; Perthen, Göttingen, Germany), is shown as the arithmetic average peak-to-valley value (R_a). The total surface-free energy (SFE) as well as its dispersive and polar components were calculated from automated contact angle measurements (OCA 15 plus; Dataphysics Instruments, Filderstadt, Germany) [41]. Therefore, three liquids with differing surface tension were used: deionized water, diiodomethane (Sigma-Aldrich, St. Louis, MO) and ethylene glycol (Merck KgaA, Darmstadt, Germany). Four drops of each liquid (2 µl) were examined on five randomly selected specimens ($n = 5$) of each material (× 8). The left and right contact angles of each drop were averaged. We calculated SFE according to the Owens, Wendt, Rabel and Kaelble [42] method. Three-dimensional images of each test and reference surface were obtained by means of atomic force microscopy (AFM) using the tapping mode scan of an AFM VEECO machine (Plainview, New York, USA); this method was also used to determine the surface topography. We scanned several randomly selected areas measuring either 3 × 3 µm or 30 µ 30 µm for each of the investigated substrata.

Automated fluorescence-based quantification of initial biofilm formation

A *Staphylococcus epidermidis* strain culture (AC-Acession: AF270147) was isolated from the skin of one of the authors; the sample was identified and confirmed by 16S rDNA-nucleotide comparison (IDNS® version v3.1.63r14 © SmartGene 2005 Molecular Mycobacteriology, Lausanne, CH). After isolation, *S. epidermidis* was proliferated in a BHI–culture medium (Bacto™ Brain Heart Infusion, BD Becton, Dickinson and Company Sparks, MD). We added glycerin and stored the bacterial cultures at 80°C ($n = 15$ per cement and bacteria, total number of samples = 240). Prior to testing, cultures were defrosted and incubated at 37°C overnight. *Streptococcus sanguinis* (strain 20068; DSMZ) was cultivated in sterile trypticase soy broth (Tryptic-Soy Broth; BD Diagnostics, Sparks, MD) supplemented with yeast extract (Sigma-Aldrich, St. Louis, MO).

For both types of bacteria, cells were harvested by centrifugation, washed twice in PBS (Phosphate buffered saline, Sigma-Aldrich) and re-suspended in normal saline. We adjusted the cells by densitometry (Genesys 10S; Thermo Spectronic, Rochester, NY) at 600 nm to a MacFarland 0.4 standard optical density that equalled the bacterial concentration of ~ 5 × 10^9 cfu (colony forming units)/ml. The quantity of bacterial adhesion was determined with a fluorescence dye. We recorded the CytoX-Violet Cell Proliferation Kit (Epigentek Group Inc., New York, NY) and fluorescence intensities with an automated multi-detection reader (Fluostar optima; BMG labtech, Offenburg, Germany) at wavelengths of 560 nm excitation and 590 nm emission. High relative fluorescence intensity indicates a high number of viable adhering bacteria; 1 ml of bacterial suspension was added to each well and incubated at 37°C for 120 min on an orbital shaker. After biofilm formation, the bacterial solution was extracted by suction and the specimens were washed once with PBS to remove non-adherent bacteria. All samples were transferred to new 48 well plates. For each well, 200 µl PBS and 20 µl CytoX-Violet (indicator solution) were added and the well-plates were incubated at 37°C for 120 min in darkness; 190 µl of the indicator solution from each well was transferred to sterile black 96-well plates and fluorescence intensities were recorded. We investigated 10 specimens for each tested cement and bacteria. As references, we used the fluorescence values of pure phosphate buffered saline (0-control), buffer and CytoX-Violet (dye-control) and pure bacterial solution (bacteria-control).

Visualization of adherent bacteria

Adhering *S. epidermidis* and *S. sanguinis* were stained with DAPI (4',6-diamidino-2-phenylindole) and directly visualized on the test substrata via fluorescence microscopy. We conducted the same procedure as described above and extracted the bacterial suspensions after incubation.

Each specimen was carefully positioned on a glass slide and stored in the dark at 4°C until further processing. Fluorescence emission was determined with a fluorescence microscope (BX61; Olympus GmbH, Hamburg, Germany) in combination with the image processing software cell P (Olympus GmbH). For each specimen, we captured the fluorescent microscopic images of five randomly selected sites with a digital camera (U-CMAD3; Olympus GmbH) connected to the microscope. The areas covered by cells (fluorescent) were calculated as the percentage of specific standard microscopic fields (420 × 320 µm = 0.13 mm^2) with the image analysis software Optimas 6.2 (Meyer Instruments, Houston, TX).

Evaluation of cytotoxicity—WST-1-test

Two specimens of each test material were produced ~ 24 h before incubation and stored under dry conditions for further processing. Specimens were transferred to 24-well cell clusters (24 Well Cell Culture Cluster, Corning Inc., Corning, NY); we added 1 ml of Dulbecco's Modified Eagle Medium (PAA, Pasching, Austria) to each well, which was incubated at 37°C for 24 h in a thermo shaking device

(OrbitalShaker; ThermoForma, Marietta, OH). After exactly 24 h, specimens were transferred to new 24-well cell clusters with a fresh medium (1 ml per well) and incubated at 37°C for additional 48 h. The samples were removed and we used the conditioned 24 h-media and 48 h-media as growth media in the cytotoxicity testings.

Human dental follicle cells were cultured in Dulbecco's Modified Eagle Medium (PAA, Pasching, Austria) supplemented with 10% foetal bovine serum and Penicillin/Streptomycin in a humidified atmosphere at 37°C and 5% CO_2. For cytotoxicity evaluation, cells in passage 6 were seeded in 96-well plates at a density of 5000 cells per cm^2. After 2 days, we changed the medium to the conditioned media for 24 h and added the cell proliferation reagent WST-1 (Roche Diagnostics GmbH, Mannheim, Germany). After incubating the cells for 2 h, we measured the absorbance at 450 nm with a microplate reader. Experiments were done in pentaplicate. Viable cells reduce the WST-1 reagent to a water-soluble formazan dye. Thus, absorbance correlates to the number of viable cells, i.e. high extinctions indicate high relative cell viability.

Statistical analysis

All calculations and graphic displays were done with SPSS 16.0 for Windows (SPSS Corporation, Chicago, IL). Means and standard deviations were calculated.

We used three-way analysis of variance (ANOVA) to analyse the influence of cement type, R_a, and SFE on the adherence of *S. sanguinis* and *S. epidermidis*. The Tukey–Kramer multiple comparison test was applied for post-hoc analysis. The level of significance was set at $\alpha = 0.05$.

Results

Surface characterization

Surface roughness (R_a). Table I indicates the mean surface roughness values and standard deviations for all surfaces tested. The highest mean value of R_a was observed for Harvard cement (1.07 ± 0.8 µm, $p < 0.01$ for all comparisons). Lower surface roughness values between 0.10 ± 0.01 µm and 0.55 ± 0.21 µm were found for Ketac Cem, TempBond, TempBond NE, Aqualox, Panavia F2.0, Rely X Unicem, Implantlink semi, and titanium. No significant differences could be found between these eight surfaces ($p > 0.05$ for all 28 comparisons).

Surface-free energy (SFE). SFE could be calculated by means of the goniometer measurements (Table I). No significant differences were observed between the tested cements ($p > 0.05$ for all 28 comparisons). The mean values varied between 40.9 ± 6.7 mJ/m^2 and 46.9 ± 3.1 mJ/m^2. The SFE value of the reference material titanium was significantly lower than those of the other cements (26.1 ± 6.4 mJ/m^2, $p < 0.05$ for all eight comparisons).

Atomic force microscopy (AFM). Examples of the tapping mode atomic force micrographs are given in Figures 1 (30 µm × 30 µm = 900 µm^2 scan area) and 2 (length = 1 µm). Small and narrow grooves (500 nm; Figure 1) could be observed on Ketac Cem. The other materials showed higher amplitudes (1.2–4 µm; Figure 1), whereas titanium seemed to be almost plane (Figure 1). Furthermore, the microstructure of Ketac Cem and Panavia F 2.0 seemed to be more irregular than that of the other materials (Figure 2).

Automated fluorescence-based quantification of initial biofilm formation

Streptococcus sanguinis. Figure 3 depicts the mean relative fluorescence intensities and standard deviations, i.e. the amount of adhering *S. sanguinis*, on all tested cements. The highest quantities of streptococci were found on Harvard cement (22.8 ± 5.9 relative fluorescence units/rfu) but without any significant difference to Ketac Cem (21.8 ± 3.9 rfu; $p > 0.99$) and Panavia F2.0 (20.0 ± 3.0 rfu). Medium quantities of bacterial cells were observed on Aqualox (17.0 ± 2.1 rfu), RelyX Unicem (14.8 ± 4.0 rfu) and Implantlink semi Classic (10.6 ± 6.0 rfu). Of all tested cements, the significantly lowest amounts of *S. sanguinis* were present on TempBond (4.5 ± 1.6 rfu) and TempBond NE (4.9 ± 2.0 rfu; $p > 0.99$ for comparison between the two temporary cements and $p < 0.05$ for comparison with all other tested cements). The quantity of streptococci was significantly lower on the reference material titanium (628 ± 770) than on all cements tested ($p < 0.05$ for all comparisons).

Staphylococcus epidermidis. The quantities of *S. epidermidis* on all tested substrata after 120 min of incubation are given in Figure 4. We found the lowest fluorescence intensity on the reference material titanium (5.0 ± 2.8 rfu). Low amounts of adhering staphylococci without any significant difference to titanium were observed on TempBond (6.7 ± 977.8 rfu; $p = 0.871$), TempBond NE (5.1 ± 1.8 rfu; $p > 0.99$), RelyX Unicem (7.9 ± 2.1 rfu; $p = 0.219$) and Implantlink semi Classic (5.5 ± 2.4 rfu; $p > 0.99$). Significantly higher quantities of *S. epidermidis* were found on Harvard cement (9.2 ± 1.6 rfu; $p = 0.01$), Ketac Cem (11.2 ± 1.5 rfu; $p < 0.01$), Aqualox (9.0 ± 1.2 rfu; $p = 0.02$) and

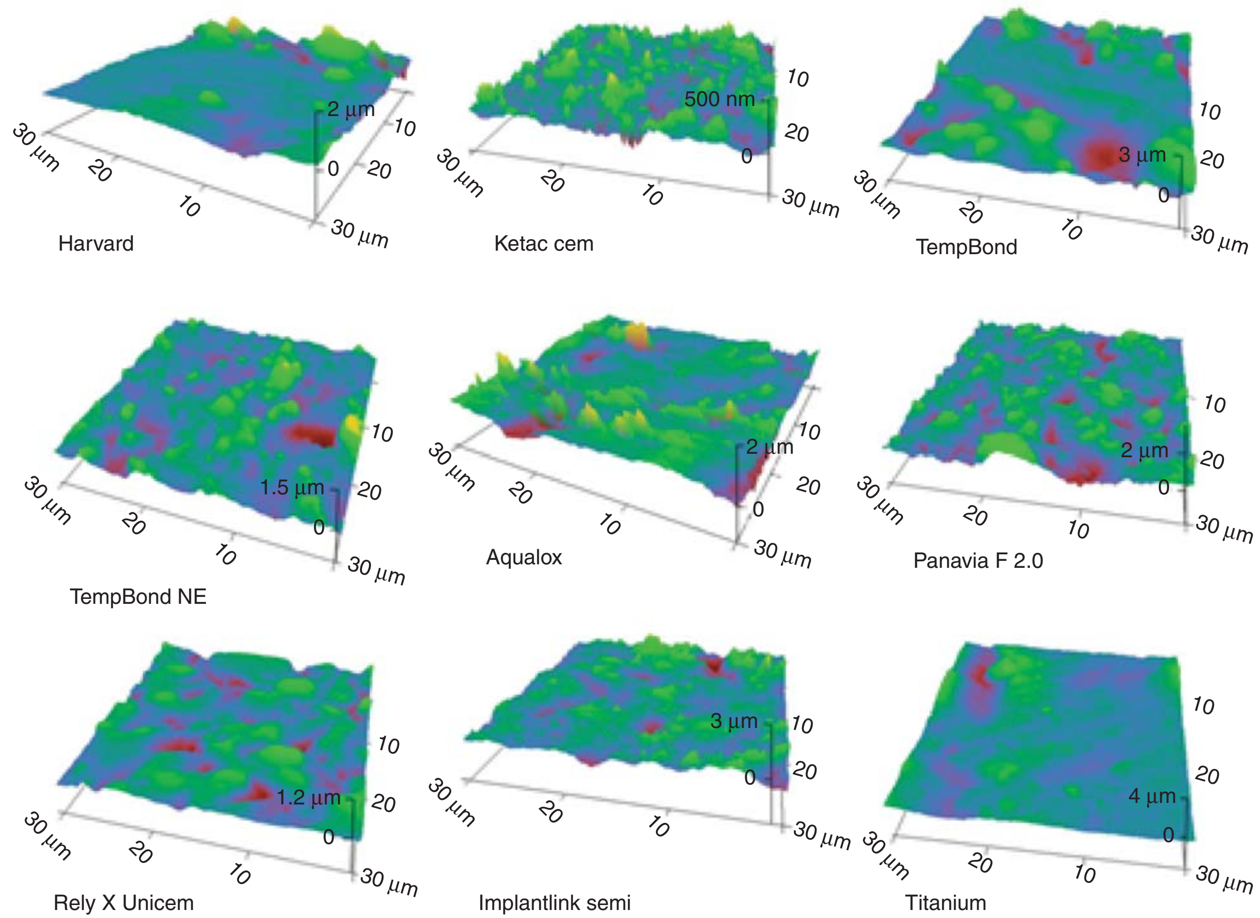

Figure 1. AFM images of 30 × 30 µm scan areas of eight tested implant cements and the reference material titanium.

Panavia F2.0 (9.7 ± 2.8 rfu; $p < 0.01$) than on the reference material titanium.

Visualization of adherent bacteria

Adhering staphylococci and streptococci were visualized via direct staining with DAPI and fluorescence microscopy to ensure a uniform spatial distribution of the tested bacteria in one single layer on all surfaces. We found an homogeneous statistical spreading of single bacterial cells and typical strains and pairs of *S. epidermidis* and *S. sanguinis* on all surfaces tested (Figure 5).

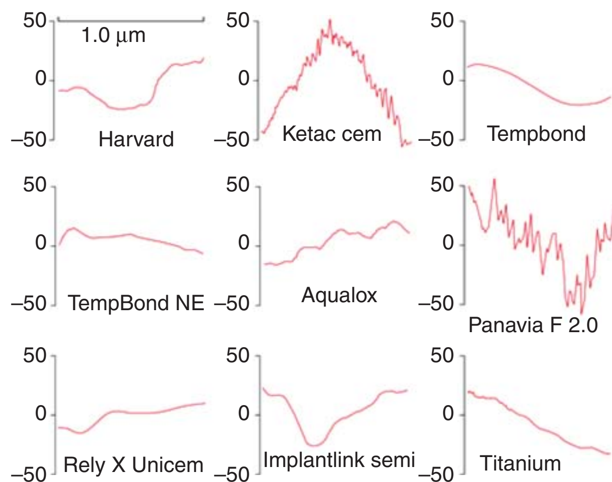

Figure 2. AFM surface profiles (length: 1 µm of eight tested implant cements and the reference material titanium).

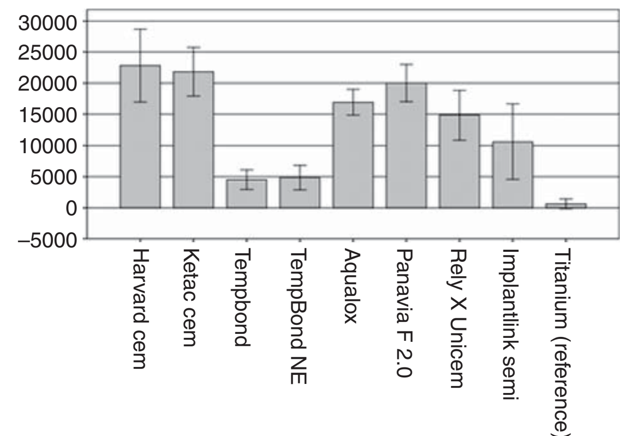

Figure 3. Relative fluorescence intensities of *S. sanguinis* on titanium and eight tested implant cements (means and standard deviations).

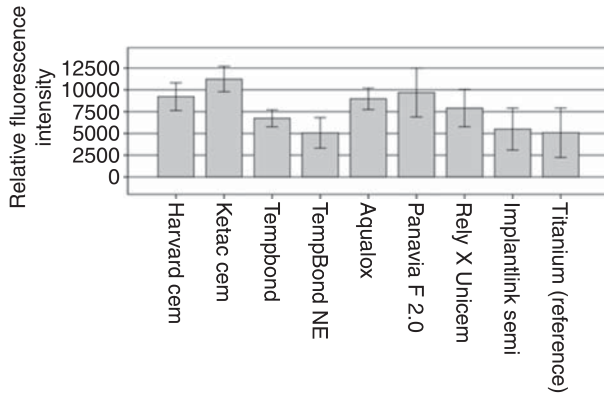

Figure 4. Relative fluorescence intensities of *S. epidermidis* on titanium and eight tested implant cements (means and standard deviations).

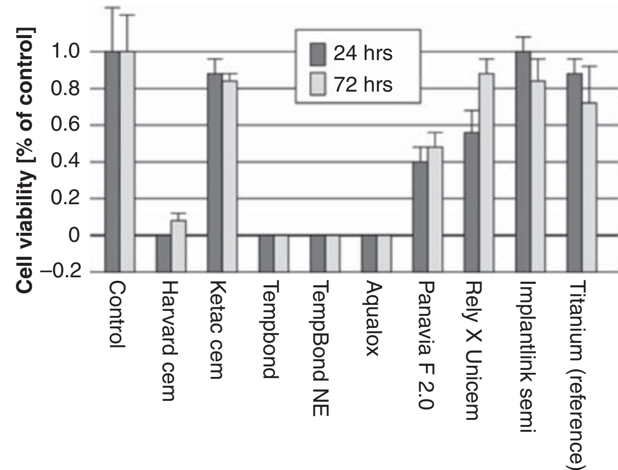

Figure 6. Colourimetric WST-1-assay. Extract extinction of titanium and eight tested implant cements in relation to negative control after 24 and 72 h of conditioning (means and standard deviations).

Evaluation of cytotoxicity—WST-1-test

Figure 6 summarizes the results of the cell vitality of human dental follicle cells on the reference material titanium and the eight implant cements after 24 h and 72 h of conditioning. Apart from RelyX Unicem ($p = 0.023$), none of the tested cements showed any significant difference in cell viability after 24 h and 72 h of conditioning. Ketac cem, Implantlink semi Classic and titanium showed mean (SD) extinctions of 0.88% (± 0.05), 1.01% (± 0.09) and 0.89% (± 0.19) after 24 h of conditioning and 0.84% (± 0.08), 0.86% (± 0.07) and 0.72% (± 0.09) after 72 h of conditioning; these values were not significantly different from negative control. We found lower extract extinctions for RelyX Unicem (0.57% ± 0.08 after 24 h, 0.86% ± 0.1 after 72 h) and Panavia F2.0 (0.39% ± 0.06, 0.49% ± 0.06). The significantly lowest extinctions (without any statistical differences among each other) were observed for Harvard cement (0.01% ± 0.01, 0.06% ± 0.01), Aqualox (0.01% ± 0.002, 0.01% ± 0.003), TempBond (0.01% ± 0.01, 0.01% ± 0.003) and TempBond NE (0.002% ± 0.004, 0.01% ± 0.003).

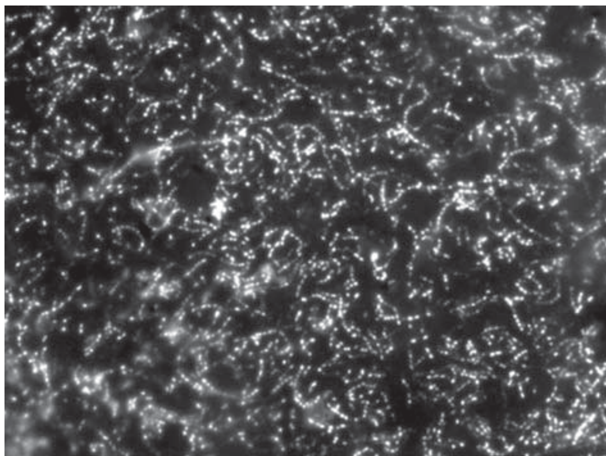

Figure 5. Fluorescence micrograph (DAPI) of a Panavia F 2.0 surface after 2 h of incubation in *S. sanguinis*. Homogeneous distribution of single cells, pairs and chains of streptococci (110 × 80 μm).

Discussion

Because cement-retained prostheses offer distinct advantages, such as the elimination of screw loosening [43] and improved aesthetics due to missing screw access holes [44], cementation on implants is often preferred over screw-fixed restorations. On the other hand, disadvantages of cementation are impeded replacement and REC with possibly enhanced microbial colonization. Micro-organisms are able to colonize on implant surfaces before or after surgical interventions or by haematogenous spread from infections elsewhere in the body [45]. Due to eating, speaking, drinking and swallowing bacteria would be washed off without adhesion. The initial adhesion is the crucial link between the surface and rest of the biofilm [46]. Those adhesive, pathogenic biofilms may lead to peri-implant mucositis and peri-implantitis followed by possible implant loss [4–7]. Generally, the choice of cement is crucial for the success rate of indirect dental restorations [1]. One biological factor determining the long-term survival rate of implant-based restorations is the quantity and quality of biofilm accumulation on the cement gap and possible REC. Secondly, the interaction between the cement (including potentially cytotoxic components) and the connective tissue is crucial for the integrity of soft tissues and the surrounding bone. Surprisingly, hardly any literature reports exist on the biofilm formation on and the cytotoxicity of luting agents used for cemented implant-restorations. Therefore, this trial aimed at finding suitable cements for fixed implant restorations based on the biological performance of these materials.

Because of its high biocompatibility and excellent mechanical properties, titanium is most commonly used for the fabrication of implants and very frequently for the fabrication of implant abutments. Some authors have reported on the antibacterial effects of titanium, which may further explain the rather low amounts of adhering bacteria on titanium [47,48]. For this reason, we used titanium as reference material in the present trial. In terms of other studies the clinical process of bacteria to colonize surfaces ('race for the surface') depends largely on the surface structure [49,50]. With regard to surface roughness (R_a) and surface free energy values, we did not find any significant differences among all eight luting systems tested. Therefore, the influence of these two surface properties on bacterial adhesion could be neglected in this trial. Furthermore, differences in bacterial adhesion could be directly related to the chemical composition of the cements themselves. This indicates that the 'race for the surface' could be interfered with by the chosen cement [50,51]. *S. epidermidis* and *S. sanguinis* are frequent components of the human oral microflora. Both types of bacteria reside on the mucous membranes and can bind to hard surfaces in the oral cavity [52]. *S. epidermidis*, which has recently been detected in pathogenic biofilms of failing dental implants [53,54], plays a major role in biomaterial-associated infections and in implant failure in all body areas [54,55]. *S. sanguinis* is often present in the human oral cavity and acts as a pioneer colonizer in oral biofilm formation, especially on implant surfaces [56–59]. Therefore, we chose these two types of bacteria as test micro-organisms in our trial. Although both types of bacteria play very different roles in the pathogenesis of peri-implant biofilms and have different surface properties, their probability to adhere to the test and reference materials were almost similar, showing the lowest bacterial adhesion on titanium, TempBond and TempBond NE. Dental materials can be evaluated in simulated clinical conditions with *in vivo* biofilms. However, *in vitro* and *in vivo* trials conducted to evaluate the cytotoxic effects and biocompatibility of dental materials may yield contrasting results [56–58]. Contrary to traditional microbial quantification methods, systems based on biofluorescence have become more important because of their simplicity, precision, reproducibility and highly sensitive procedure for quantifying adhering micro-organisms [59–64]. Fluorometric techniques allow the quantitative investigation of a high number of specimens in a short period of time [6,65]. In our trial, we measured the amount of viable bacteria adhesion with the CytoX-Violet Cell Proliferation Assay Kit. The fluorometric changes of the indicator solution show the activity of the cellular dehydrogenases. The changes are also directly proportional to the cell viability of the adherent bacteria. However, this method does not allow the detection of vital adhering bacteria, so that cultivable and non-cultivable vital cells cannot be differentiated. This fact is very important because large amounts of dead bacteria (up to 40%) have been found after short incubation times [65]. The visualization of adherent bacteria via direct staining with DAPI and fluorescence microscopy showed that each tested surface presented homogeneous spreading of single bacterial cells with typical stains and pairs.

Because the tested cements lacked any statistical differences with regard to R_a and SFE, the differences in bacterial adhesion could be directly related to the surface morphology and chemical composition of the luting systems. It has been proposed that residual monomers in composite cements might be oxidized to formaldehyde, which could probably have an unrequested antibacterial effect on adhering micro-organisms [1,66]. These effects could not be observed in the present trial, because all resin-based materials (Panavia F2.0, RelyX Unicem and Implantlink semi) had a rather high probability of adhering staphylococci and streptococci. The generally high probability of biofilm formation on composites was reported in some previous trials [41,67]. Ketac Cem, a glass-ionomer cement, showed large quantities of adhering bacteria, which might have resulted from the highly micro-structured surface observed in the atomic force micrographs and surface profiles. Enhanced microbial colonization on substrata with complex surface morphology was also shown on Panavia F2.0 and Harvard. In another trial, streptococcal adhesion was further increased on Harvard Cement specimens with a higher content of phosphoric acid [1]. In the AFM, the microstructures of Panavia F2.0 and Ketac Cem were more irregular, containing more and deeper pits and grooves than the other materials tested. Initial microbial colonization was shown to start from very small—and not from large-scaled—pits and gullies [25,68,69]. These results support the theory that rougher surfaces and complex surface morphologies promote the adhesion of *S. sanguinis* and *S. epidermidis*. Even if surface roughness values after polishing were low and did not statistically differ among the various materials, AFM showed that all surfaces are rough and, therefore, all cements provide adequate adhesion conditions for microbacterial accumulation [70]. The significantly lowest amounts of adhering bacteria were found on TempBond and TempBond NE. This effect visualized in the AFM profiles may be based on the extremely smooth surfaces of the two provisional cements. Additionally, zinc-containing luting systems, such as zinc oxide eugenol (TempBond) and zinc oxide non-eugenol cements (TempBond NE), have been shown to release zinc ions, which in turn have antibacterial effects by inhibiting various cell activities, such as glycolysis and transmembrane proton translocation that modify the

permeability of cell membranes [71–73]. Harvard Cement, Ketac Cem, Panavia F 2.0, Aqualox and Rely X Unicem showed higher amounts of adhering bacteria. These cements had smoother surfaces as visualized in the AFM profiles. Although Harvard Cement contains zinc and dispenses zinc including its antibacterial effect, bacterial adhesion is high. The increase of accumulation on specimens may depend on a low value for pH [1]. Harvard Cement and Aqualox contain acid liquid. The curing reaction of Ketac Cem and Rely X is an acid-base reaction. The lower bacterial adhesion on Implantlink Semi Classic, Rely X Unicem and Panavia F 2.0 than on Harvard and Ketac Cem might be explained by the released residual monomer that is oxidized to formaldehyde, which also has an unrequested antibacterial effect [1,66]. The results of our trial further indicate that surface roughness values, such as R_a, might not be suitable indicators for bacterial adhesion. The analysis of the three-dimensional microstructure via AFM seems to be more adequate for this purpose, because it characterizes the influence of surface morphology on initial bacterial adhesion. Barbour et al. [74] also observed different bacterial coverage on surfaces with different surface morphology, but the same values for roughness.

Additionally to antibacterial effects, we also tested the cytotoxic potential of the cements. The WST-1-test explores the metabolic activity of the mitochondria as an indicator for the vital status of cells [75]. By this method, cell proliferation as well as indirect cell death can be quantified on a large scale in microtiter plates [75]. The results from the WST-testing showed significantly different (cytotoxic) effects of the tested cements on human dental follicle cells. Harvard, TempBond, Temp Bond NE and Aqualox showed significant devitalizing effects compared to control. Harvard Cement, Aqualox, TempBond and TempBond NE contain and dispense zinc, which—besides its antibacterial effects—is known to be cytotoxic [37,76,77]. Additionally, the cytotoxic effect of TempBond may be attributed to free eugenol liberated from the cement [78,79]. Generally, released metal ions as well as waste or high acid components might have cytotoxic effects. Harvard Cement contains phosphore acid and Aqualox contains polyacrylic acid. Rely X Unicem and Ketac Cem use an acid-base reaction as a curing reaction. Panavia F2.0 and Rely X Unicem also have a devitalizing effect compared to control, but with lower intensity. Residual monomers, which are known to be cytotoxic, might be one reason [80–83]. Although Implantlinc Semi Classic contains methacrylate oligomere and triclosane, which are known to be cytotoxic, it has little cytotoxic effect, and one reason might be the lack of metal ions or lower residual monomer.

The results of our WST-tests showed a change in the cytotoxic potential of Rely X Unicem in the first 3 days. This result may correspond with a slow curing [80]/acid-base reaction of the cement. The lowest extinctions were found for Harvard Cement, Aqualox, TempBond and TempBond NE. The low probability of bacterial adhesion on Temp Bond and the low extinctions of the WST-1-test might result from the presence of eugenol, which is both an antibacterial but also a cytotoxic substance. The cytotoxic potential of Harvard Cement, Temp Bond NE and Aqualox may possibly be explained by their content of residual acids. Unfortunately, cytotoxic effects and antibacterial properties often correlate. Therefore, it is difficult to find a suitable cement that combines low probability of adhering micro-organisms with high biocompatibility.

Conclusion

Within the limitations of an *in vitro* trial, our results indicate that—from a biological point of view—none of the tested cements may be recommended without reservation for the cementation of implant-supported fixed restorations. Although TempBond and TempBond NE showed low probability of adhering oral bacteria, both cements had significant cytotoxic effects. Future trials should examine cytotoxicity after more than 72 h. The arithmetical mean roughness value R_a seems to be inadequate for examining the probability of bacterial adhesion.

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