The Influence of Connection on the Microleakage Development of Implant-Supported Fixed Bridges

Keywords

E.coli
Microleakage
Implant-level
Abutment-level
Bacterial Penetration

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ABSTRACT

Purpose: If bacteria penetrate through the implant-abutment connection, they may initiate inflammatory reactions in the peri-implant tissue. It seems that the type of connection plays a key role in the development of peri-implantitis. The aim of the present in vitro study was to compare the microleakage of implant-supported fixed complete arch bridges at the levels of abutment and implant. Methods: Ten identical polyurethane model bases containing six implants each were produced using an edentulous model of the upper jaw. These models were prepared with two types of implant-supported complete arch prostheses. Five specimens were fixed at implant level and five at abutment level. The inner parts implants were inoculated with Escherichia coli (E.coli). Each implant was surrounded with closed bacteria-proof vessels to observe bacterial migration from the inner parts of implants to the nutrient solution. Samples of nutrient solution were taken at different time points up to 2 to 10 days and colony forming units were determined. Results: The bacterial accumulation in the implant-supported bridges at the implant level was significantly lower than at the abutment-level (p=0.00953). Conclusion: For implant-supported fixed complete arch prostheses, bacterial accumulation was lower at the implant level than at the abutment-level.

INTRODUCTION

Microbial leakage at the implant-abutment connection of two-part implant systems is one of the major factors that influence peri-implant inflammatory reactions of soft and hard tissue.[1] Due to modern manufacturing techniques, the connection between implant and abutment can cause microgaps. A few studies evaluated different implant-abutment systems and analyzed microgaps between both components of 2-50 µm.[2-5] In general, oral microorganisms are between 1.1 µm and 1.5 µm in length and 0.1 µm to 0.5 µm in diameter – so they are able to pass through microgaps at the implant-abutment connection.

If bacterial leakage at the implant-abutment interface could be minimized, this might help to prevent such inflammatory reactions and optimize bone stability. The formation of microgaps between implant and abutment cannot be prevented, but the spectrum of gaps can be influenced by various technical parameters. One of the important factors is the fitting accuracy between the two parts, as stable fitting can reduce micromovements during loading. The interface between abutment and implant can be externally or internally connected, but restorations with internal connection are more common.[6,7] Previous studies have reported that the

connection is more stable and tighter with an internal conical implant to abutment connection than with other designs – such as flat-to-flat or tube-in-tube connections.[8,9] Moreover, axial and extra-axial loading during mastication can cause changes in the stability of the implant-abutment connection, which can lead to a so-called "pumping effect".[8] This pumping effect can enhance exchanges between the implant-abutment interface and the oral environment. Hence, microorganisms, their nutrients and metabolites can all be transported; this can promote the development of peri-implantitis or reinforce an existing inflammatory reaction.[1,5,10,11]

Mechanical loading in a chewing simulator can be used to simulate conditions in the oral cavity *in vitro*, in this complex system. Many studies have shown that microleakage can also be detected even when masticatory loading is not simulated.[12-17] For example, Nascimento *et al*. compared different implant systems under static and dynamic loading conditions and concluded that loading significantly increased the incidence of microleakage, but microleakage was also found in all systems investigated - even without loading.[5]

Carinci *et al*. already demonstrated that bacterial leakage can be influenced by different implant designs.[18] Due to modern production methods, multi-span bridges can now be manufactured directly at the implant level, thus saving material and time. However, manufacturing tolerances can lead to microgaps at the interfaces. No study of microleakage in isolated implants with implant-supported fixed full arch bridges has been conducted yet. The aim of the present *in vitro* model study was therefore to compare microleakage in implant-supported fixed complete arch prostheses at the implant and abutment levels, using *Escherichia coli* (*E.coli*) as test species in two groups. This gram-negative enteric rod was also used in prevoius microleakage studies and it is associated with peri-implantitis and may be partly responsible for early implant loss.[19-21]

MATERIALS AND METHODS

All test specimens in the current study were based on ten model bases with six implants each. Five model bases were implemented with bridges at the implant level (group_1_il). Another five models were given implant-supported fixed bridges at the abutment level (group_2_al). Based on the literature, the microbial penetration of bacteria into the sterile environment of microbial inoculated implants was proven.[12,15-17,22-24] In each model, four implants were inoculated with *E.coli* and two implants were used as controls. For the investigation of bacterial penetration, all 60 implants were isolated for 10 days. Sample size was calculated as in comparable investigations.[1,12] In each model, four implants were inoculated with *E.coli* and two implants were used as controls. For the investigation of bacterial penetration, all 60 implants were isolated for 10 days. Sample size was calculated as in comparable investigations.[1,12] The number of 60 implants examined, including 40 inoculated implants, can be considered as a meaningful scope of testing. Comparable studies on single implants usually use less than 40,[12-14,17,23] also 40[15,19,22,24] or rarely more than 40[5] inoculated implants. Similarly, the use of 20 control implants can be assumed to have a low probability of false-positive results.

MODELS

A plaster model of an edentulous upper jaw was used as foundation for the production of test specimens. The middle of the jaw and of the alveolar ridge was determined and a wax-up with denture teeth was prepared. The positions of teeth 12, 14 and 16 were marked on the model using the wax-up as a jig. The positions of teeth 22, 24 and 26 were marked by mirroring (*Figure 1*). Using a splint, a 3.5 mm diameter hole was drilled at all six marked positions *(Figure 2*) and implant model analogs were fixed with screwed copings. Ten plaster models were then prepared. Marking drillings surrounding the implants were prepared for the next step in these models *(Figures 3 and 4*). This model with impression copings was duplicated with flowable silicone material and the implants on the impression copings were fixed with the prescribed torque of 20 Ncm. The implants used were slightly conical, self-tapping screw implants with optional platform switching. This gave ten models consisting of polyurethane 5mm deep holes were drilled around each implant, guided by the previously prepared marker drillings.

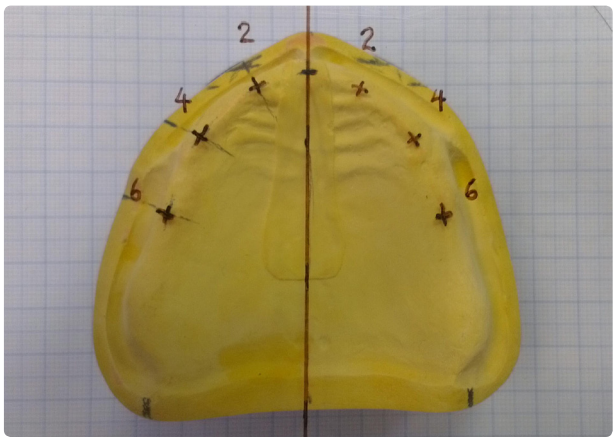

Figure 1: Marked positions of implants.

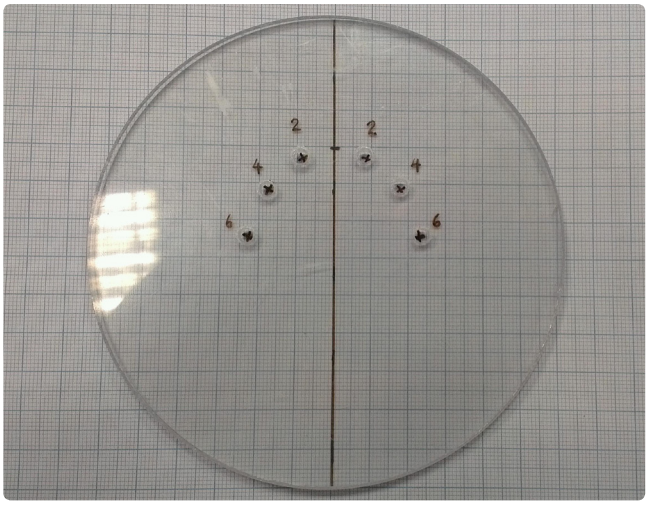

Figure 2: Positions of all six implants on the splint.

Figure 3: Marking drillings surrounding the implants.

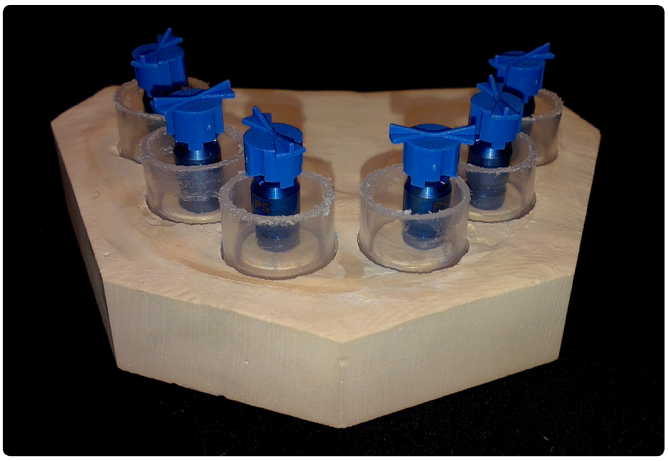

Figure 4: Fixed impression copings on the model.

BRIDGES

Coping cylinders were screwed into the implant model and 12-membered bridges were designed by using modelling resin *(Figures 5 and 6)*. The implant bridges were manufactured in cooperation with Heraeus-Kulzer, Hanau, Germany. For this step, bridges of pattern resin were scanned and ten bridges of CoCr alloy for each model were milled by the CAD/CAM technique. Five bridges were produced on the implant level and the other five bridges on the abutment level, using Vario SR abutments *(Figure 7)*.

PRODUCTION OF NUTRIENT SOLUTION VESSELS

The top 15 mm of polypropylene tubes were isolated and positioned around the implants as nutrient vessels. Silicone was applied to create seals between the model base and the nutrient vessels *(Figures 8 and 9)*.

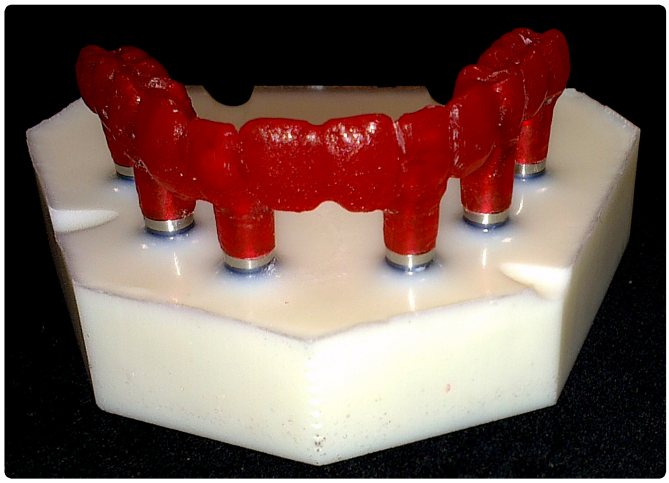

Figure 5: Example of one bridge designed by using pattern resin.

Figure 6: Example of one bridge designed by using pattern resin with marking drillings.

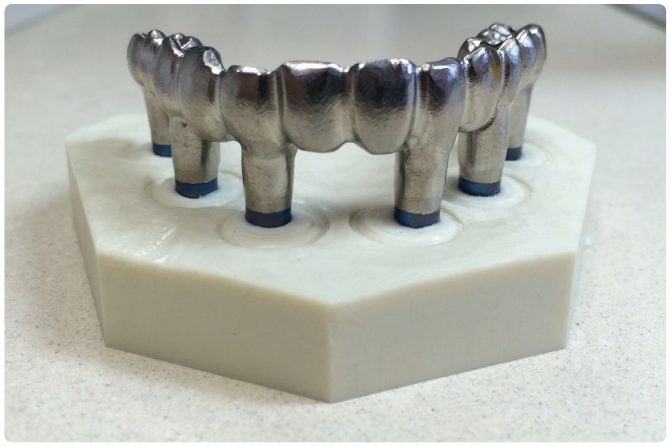

Figure 7: Example of CoCr bridge on implant level.

MICROBIOLOGICAL LEAK TESTING

The method used in the current study to evaluate bacterial penetration was based on Callan *et al.* who applied a similar test setup to examine the implant-abutment connection of 43 two-part implant systems for 8 germs.[25] Models, abutments, bridges, connecting screws and tools were welded into

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Figure 8: Complete set-up.

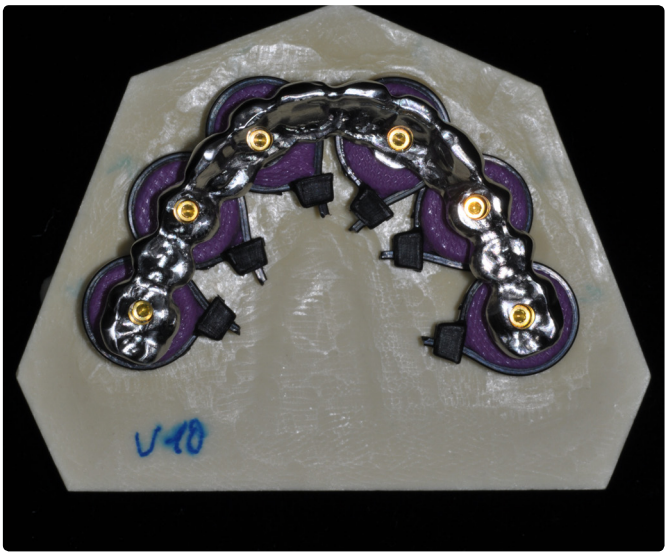

Figure 9: Complete set-up.

sterilizable bags and steam sterilized (121°C, 2 bar, 15 min). Due to their plastic deformability, the nutrient solution vessels were disinfected for 30 min in isopropanol and dried under a sterile workbench. *E.coli* strain *DH5 alpha* was cultivated over night for 16 h at 37°C under agitation (150 rpm) in lysogeny broth nutrient solution. On the next day bacteria were pelletized by centrifugation and washed twice with LB broth to remove all metabolic products. In order to obtain reproducible bacteria concentrations, the cultures were adjusted to a final optical density at 600 nm (OD_{600}) of 1 in LB broth. To determine the number of colony forming units per milliliter (CFU/ml), the adjusted overnight cultures were serially diluted 10-fold over a range of 10^{-1} to 10^{-7}. 1 ml of the 10^{-6} and 10^{-7} dilutions, were spread on TSA plates and incubated for 24 hours at 37°C before the colony forming units were counted.

All six implants of each bridge were numbered according to their position on the model base *(Figures 8 and 9*). With the exception of implants at position 1 and 4, the implants were inoculated with 5 µl bacteria solution (OD_{600}= 1), which corresponds to 3.23 x 10^8 (± 6.77 x 10^7) CFU/ml *E.coli*, at the deepest point of the internal geometry. The implants on positions 1 and 4 were used as negative controls and were inoculated with sterile nutrient solution. The sleeves were then fitted, and the bridge structures assembled and tightened to the specified torque (abutments screw 20 Ncm, prosthodontic screw 15 Ncm). The nutrient solution vessels were filled with 3 ml sterile nutrient solution and the complete setup was incubated at 37°C. After incubation for 0, 2, 4, 6, 8 and 10 days, samples were taken of the nutrient solutions surrounding the implants. 1 ml of the samples were plated on a TSA plates and the nutrient vessels were refilled and incubated again. After a cultivation time of 24 hours at 37°C, the number of CFU/ml was determined. After the last sampling on day 1, the superstructures were removed and the internal section of the implants rinsed with 20 µl nutrient solution. This was used as a positive control and evaluated after 24 hours incubation.

STATISTICAL ANALYSIS

Statistical analysis was performed using the SPSS for Windows, version 12.0. The Fisher Yates test was used to test for statistically significant differences in microleakage between the different groups. The level of significance was set at 0.05 for the analysis.

RESULTS

GROUP_1_IL: BRIDGES AT THE IMPLANT LEVEL

At the beginning of the analysis, one of the implants of group_1_il (No.2.6, bridge No.2) was excluded because of bacterial penetration on day 0. Thus, the number of test specimens at the implant level was reduced to n=19. Three of five bridges showed no bacterial migration. The other two specimens exhibited microleakage in two implants on both 2 and day 4. From day 6, no bacterial penetration was evident *(Figure 10*). It could be concluded that 4 of 19 tested implants exhibited bacterial accumulation at the implant-abutment interface. The negative controls remained free of bacteria. During the separation of the components, the inner parts of implants exhibited detectable colonization with vital bacteria. Overall, 21% of the implants in group_1_il were penetrated by bacteria.

GROUP_2_AL: BRIDGES AT THE ABUTMENT LEVEL

In all five bridges, bacterial penetration was found at the abutment level. 13 of 20 specimens showed penetration on days 2 to 10 *(Figure 10*). The negative controls and nutrient solutions remained free of bacteria. As with specimens in group 1, the inner parts of implants exhibited detectable colonization with vital bacteria. In group_2_al, 65% of implants exhibited microleakage.

After verification using the Fisher Yates test, the difference between the two groups was statistically significant (p=0.00953). The test specimens of group_2_al showed microleakage more frequently than the implants of group_1_il.

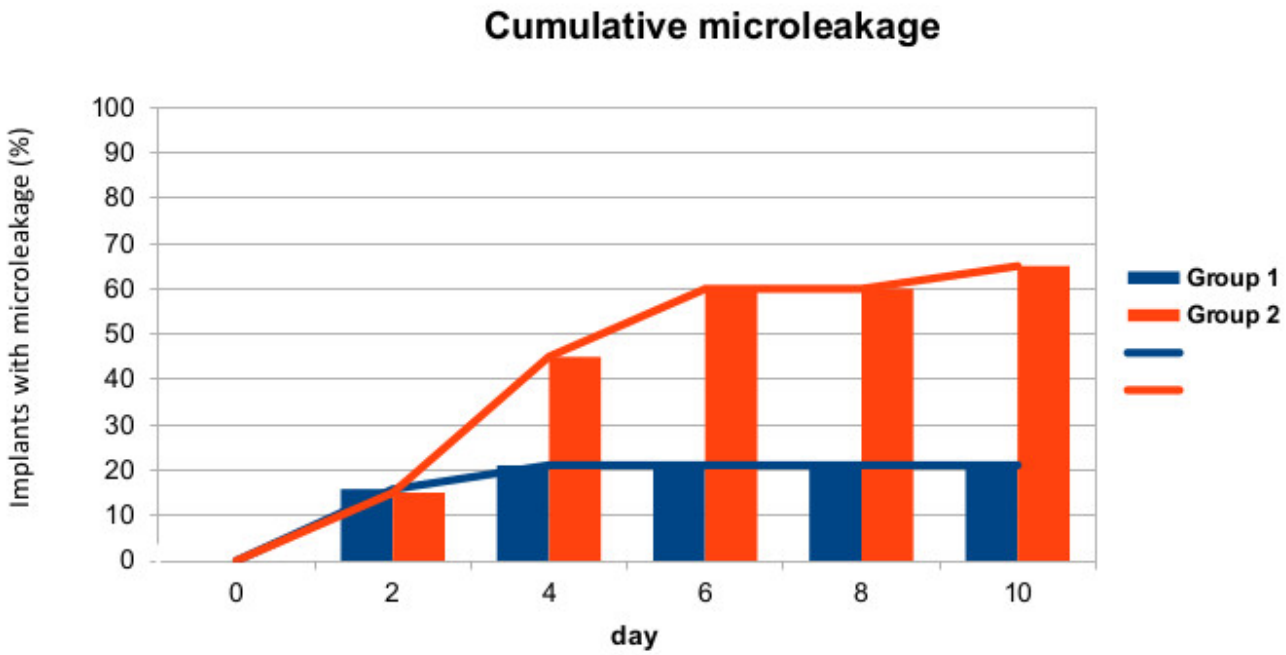

Figure 10: Part of implants in each group with positive bacterial penetration.

DISCUSSION

Two-part implant systems are used frequently. It has been proved that these systems exhibit microgaps at the implant-abutment connection within a few months.[26,27] Release of bacteria and their metabolic products from this reservoir appears to be related to peri-implant diseases.[5,10,11,26] But the extent of the resulting peri-implant inflammation depends not only on the properties of the implant systems used but also on various host factors and site-specific tissue conditions.[28,29] Therefore, it is hardly possible to perform *in vivo* studies to compare the health of the peri-implant tissue with different implant-prosthetic systems and most studies have been conducted *in vitro*. The resistance of an individual implant system to bacterial colonization can be assessed from the incidence and time course of bacterial penetration through the implant-abutment connection.

There have been many published studies on implant-abutment connections with the bacterium *E.coli*.[18-20,30,31] Nevertheless, it is difficult to compare these studies with respect to the microgaps at the implant-abutment interface - primarily due to differences in methodology, as reviewed by Silva-Neto *et al*. in 2012.[32] This is because the cumulative incidence of microgaps is influenced by a variety of factors, including the time of incubation and the microbial system. Dias *et al*. and Rismanchian *et al*. also used *E.coli*, but a different strain (ATC 25922), which is more motile than the DH5 alpha strain used in the current investigation.[12,22] Both authors quantified microleakage as colonyforming units per milliliter of the nutrient solution surrounding the implants. They referred to this as "leaked colonies" and used this parameter in their statistical analyses. This approach is open to criticism, as the generation time of *E.coli* is less than 30 minutes and bacteria would be expected to grow exponentially in the TSB nutrient solution used. Thus, the measured value corresponds not only to the colonies that have passed the microgap, but also to the progeny of the first bacteria which had passed. In addition, neither study included a positive control from the inside of the implant, even though this is important in order to exclude possible transfer by wiping the intra-implant colony during the investigation.

In general, *E.coli* is a typical bacterium used for testing microleakage in the oral cavity. For example, Koutouzis *et al*. investigated microleakage of *E.coli* in two implant systems with different connection types, including the influence of mechanical loading on microleakage. They concluded that *E.coli* is able to pass the microgap between implant and abutment, even without loading, and this might be related to chewing and the associated pumping effect.[20,22]

Moreover, some studies on microleakage have not employed bacteria. One possibility is to use dye tracing, so that microleakage analysis can be performed photometrically.[10] Another approach is to use an endotoxin of bacteria, as these are of low molecular weight (50-100 kDA) and can therefore more easily pass through gaps than bacteria can.[1,33]

However, the size of the microgap in two-part implant systems is influenced by both biological and mechanical factors. Microgaps in implant-supported bridges are caused by divergence between the positions of the connecting surfaces of the individual implants and the corresponding connecting surface at the bridge base. These inaccuracies are multifactorial.[34] Sources of error can be the implant direction, the impression technique, the impression material, the fabrication process, the configuration and the clinical/technical experience of the dentist/technician.[35,36] Forces

between implant and superstructure may be generated by inaccurate fitting, can be transferred to the implant bearing and result in fractures of the bone bearing or implant, and may lead to tension, fractures and loosened screws in the restoration.[3,37,38] With current fabrication methods, absolute passive fit of implant-supported bridges is not possible and microgap between implant and superstructure may be inevitable.

However, the extent of the microgaps is variable and should be reduced to the lowest possible value – for both technical and biological reasons. There are no generally accepted minimum values, but current studies indicate that the minimum size of microgaps may be 150 µm.[39-41]

The results of the present study show that the microleakage of implant-supported bridges at the abutment level is significantly greater than at the implant level. The bacterial penetration on day 0 in one of the implants of group_1_il (No.2.6, bridge No.2) was presumably not due to migration, but rather due to the pumping effect when the components were being screwed together. The differences in bacterial penetration in the two study groups may be related to the configuration of the contact of the surfaces of the implant, abutment and superstructure. The bridges at the implant level have a flat connector with clearance fit, so that presumably a slight displacement is possible in the horizontal plane between the implants and the bridge. A horizontal misfit can be compensated through the parallel implant axes which were used for the whole experimental set-up. The abutments of test specimens at abutment level have a cone in the oral direction, which is picked up in the base of the bridge construction. If there is a vertical or horizontal misfit, the edge of the bridge cannot assume the terminal position. By tightening the prosthetic screws with torque, strong axial forces and bending moments can act on the abutment and the screw.[42] It has already been proven in several studies that mechanical loading of implants can initiate changes in microgap size.[8,9] With this background, it can be confirmed that there is an increase in microgap formation in test specimens at the abutment level.

But more studies are necessary to evaluate fixed complete arch prostheses with implant support. In addition, it would also be interesting to perform microscopic studies to measure the size of the microgaps, as these could show how microleakage is influenced by poorly fitting prostheses.

CONCLUSION

Within the limitations of the present study, it has been concluded that:

1. Both types of implant bridges showed microleakage.
2. For the implant-supported fixed bridges, bacterial penetration was significantly lower at the implant level than at the abutment level.
3. Further studies, especially in vivo studies, are necessary to simulate exact the conditions of the oral environment. But it should be noted that the reproducibility is limited due to the interindividual differences.

MANUFACTURERS DETAILS

- Implant model analogs, Camlog, Winsheim, Germany
- Screwed copings, Camlog, Winsheim, Germany
- Fujirock Plaster, GC, Leuven, Belgium
- Impression copings, Camlog, Winsheim, Germany
- Silicone Adisil, Siladent, Goslar, Germany
- Implants Screw line, Camlog, Winsheim, Germany
- AlphaDie Polyurethane, Schütz Dental, Rosbach, Germany
- Coping cylinders, Camlog, Winsheim, Germany
- CoCr-alloy, Heraeus Kulzer, Hanau, Germany
- Vario SR abutments, Camlog, Winsheim, Germany
- Silicone, Aerotrim, Overpelt, Belgium
- Workbench Herasafe HS, Heraeus Kulzer, Hanau, Germany
- Nutrient solution LB Broth, BD, New Jersey, USA
- TSA plates, BD, New Jersey, USA

COMPETING INTERESTS

The authors declare that they have no conflict of interest in this research. The authors are not affiliated or receive benefits from the companies mentioned in this research.

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