Prevention of Bacterial Leakage into and from Prefabricated Screw-Retained Crowns on Implants in Vitro

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Previous in vitro studies have shown that a mean gap of less than 4 µm between prefabricated crowns and implants of the Ha-Ti implant system is not a barrier to infiltration by *Staphylococcus aureus*. These studies confirmed earlier in vivo work showing that a multitude of oral microorganisms could colonize and infiltrate these gaps. In the present investigation, 30 Ha-Ti implant-crown assemblies were tested for bacterial leakage after the gaps were sealed with the chlorhexidine-containing varnish Cervitec. *S. aureus* leakage into the totally submerged test specimens was detected in 1 of 5 samples incubated for 4 weeks, while no leakage was detected in specimens incubated for 3, 5, 6, 7, and 8 weeks. When the sealed test specimens were partially submerged (that is, excluding the screw hole of the crown) and incubated for 3 to 11 weeks, none of the internal surfaces of the 30 test specimens manifested contamination. The clinical relevance of gap sealing in maintaining inflammation-free marginal mucosa and in achieving clinically successful treatment of peri-implantitis has yet to be determined.
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Key words: bacterial leakage, dental implants, prefabricated crowns

Various studies have shown that when conventional metal casting techniques are used, a mean marginal fit of cast restorations of 20 µm is achievable under optimal clinical and laboratory conditions.[1–3] On the other hand, clinical investigations have demonstrated that restorations fabricated by metal casting commonly exhibit average marginal gaps exceeding 80 µm.[4,5] Furthermore, the reported high standard deviations indicated that around cast crowns, marginal gaps vary considerably in magnitude.[6] The close relationship between iatrogenic irritation caused by ill-fitting margins of fixed restorations and inflammatory reactions in the marginal gingiva has been demonstrated in dentulous patients.[7,8] Epidemiologic investigations[9,10] as well as experimental studies[11,12] have supported this relationship. Given the similarity of soft tissue attachment to natural teeth and to implants[13–17] and the similarity of periimplant microbial colonization to that around natural teeth,[18–20] the relationship between ill-fitting margins and bacterial irritation must also be acknowledged as a potential clinical problem with implant-supported restorations.

It has been postulated that suprastructures, especially of 2-stage implant systems in which crown margins are routinely located submuco-

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sally, should exhibit a minimal circumferential marginal gap.[21] There is little evidence in the literature that attributes peri-implantitis solely to bacterial accumulation or mechanical irritation. It still remains to be determined which of these variables plays a more significant role in the process of peri-implant inflammation.

In an in vitro investigation, the marginal fit of micromechanically prefabricated crowns of the Ha-Ti implant system (Mathys Corporation, Bettlach, Switzerland) was evaluated using scanning electron microscopy.[22] Measurements of gaps were made continuously along the entire crown margin before and after laboratory procedures, and again after 1 million cycles of continuous loading. The mean gap between crown margin and implant was less than 4 µm for all test series. Laboratory procedures and functional loading caused no statistically significant greater values. These results permitted the conclusion that significantly improved marginal fit of restorations upon dental implants can be achieved with micromechanically prefabricated crowns, as compared to conventional cast crowns.[23]

In a further study, bacterial leakage in and out of Ha-Ti implants and their micromechanically prefabricated crowns was determined through either the marginal gap or the screw hole both in vivo and in vitro.[24,25] It could be demonstrated that even implant systems with a high degree of precision fit of the suprastructure components, as it was described for the Ha-Ti implant system, do not guarantee sealing against micro-organisms at the marginal gap. Similar results were obtained in in vivo and in vitro studies showing bacterial colonization on internal surfaces of Brånemark[26,27] (Nobel Biocare, Göteborg, Sweden) and IMZ restorations[28,29] (Interpore International, Irvine, CA). Comparable bacterial floras were described in vivo for these implant systems. Some of the bacteria identified could be associated with peri-implantitis.[19,30] Penetration of oral micro-organisms through gaps of implant-supported suprastructures may constitute a certain risk for soft tissue inflammation[31] and for successful treatment of peri-implantitis with or without guided tissue regeneration.[32] Therefore, sterilization of suprastructure components and disinfection of internal surfaces of implants was recommended for the treatment of peri-implantitis.[31,33] It is proposed that sealing the marginal gaps and screw holes with a chlorhexidine varnish be considered as another means for preventing bacterial penetration into implant-crown assemblies. The aim of the present study was to determine the efficacy of sealing in the prevention of in vitro bacterial leakage in and out of Ha-Ti implants and their micromechanically prefabricated crowns.

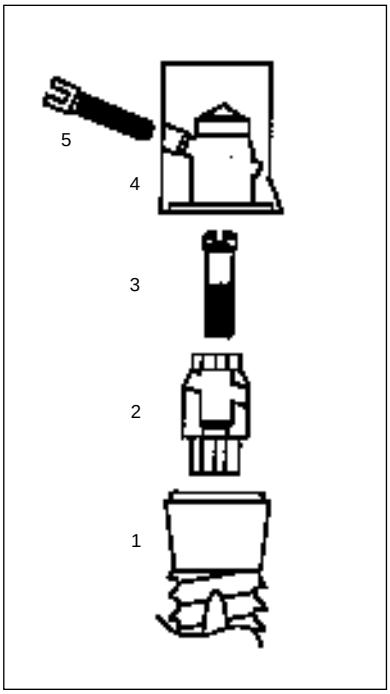


Fig 1 Prefabricated crown of the Ha-Ti implant system: implant (1), abutment (2), abutment screw (3), prefabricated crown blank (4), and transverse screw (5).

Materials and Methods

Ha-Ti implants with prefabricated screw-retained crowns were used for the in vitro testing of bacterial leakage. The implants are made of pure titanium and exhibit the shape of a step-screw with a self-tapping design. The surface is machined and blasted with pure crystalline aluminum oxide. The Ha-Ti implant uses a titanium transmucosal abutment connected to a prefabricated crown made of a high noble alloy. The crown is secured in place through the use of a lingual setscrew (Fig 1). To meet functional and esthetic requirements, the blank is individually milled and veneered in the laboratory. The crown margin, which is precisely mated to the implant base in the manufacturing process, remains untouched.

A series of 30 prefabricated crowns for Ha-Ti implants with a diameter of 4.5 mm were used for testing the bacterial leakage into and out from the

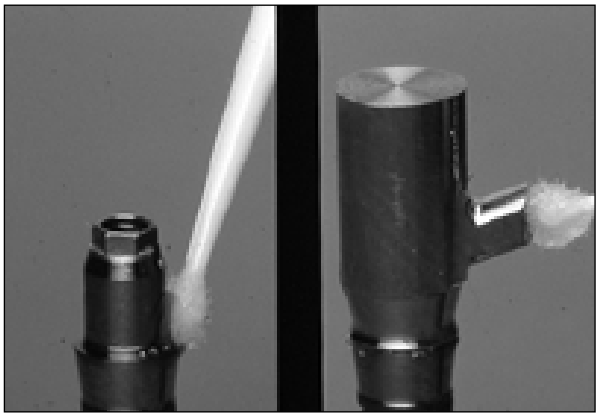


Fig 2 (*Left*) Application of Cervitec to the implant shoulder and (*right*) to the transverse screw hole of the prefabricated crown.

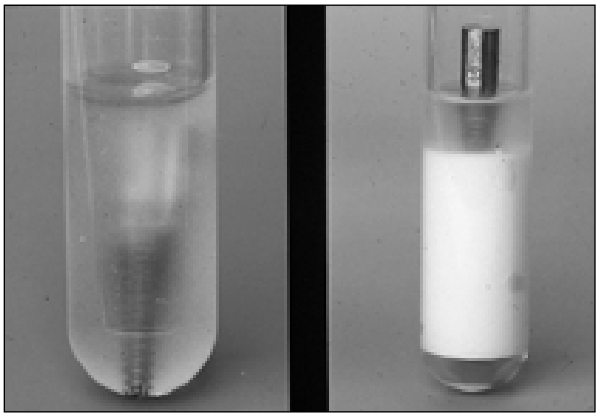


Fig 3 (*Left*) Complete immersion of marginal gap and transverse screw hole of the prefabricated crown and (*right*) partial immersion of only the marginal gap in *S. aureus* culture.

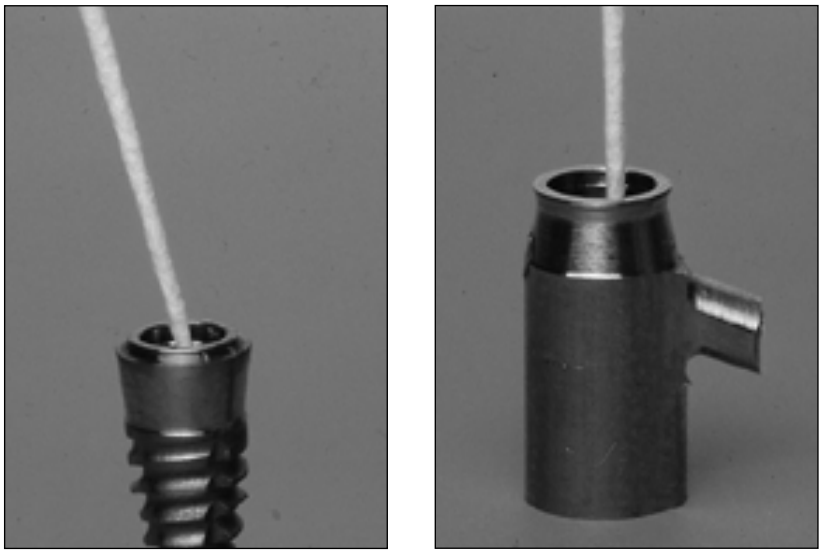
inner chamber of the implant. *Staphylococcus aureus* ZIB 6901 cultures grown in trypticase soy broth (TSB) (Becton Dickinson Microbiology System, Cockville, MD) were used as test bacteria. *S. aureus* was chosen over other known periodontal pathogens because of its small size (about 1 µm) and because it is easy to culture. Cervitec varnish (Vivadent, Schaan, Fürstentum, Liechtenstein), containing 1% (wt/wt) chlorhexidine diacetate, was used to seal the gaps.

Leakage into the Test Specimens. Test specimens were autoclaved and assembled in a sterile laminar flow hood. The gaps of the sterile test specimens were sealed during the assembly process. This was achieved by applying Cervitec varnish to all contact surfaces of the implant assembly components. First, a thin layer of Cervitec was applied to the implant shoulder, then to the upper rim of the abutment before affixing the prefabricated crown. Both the transverse screw and transverse screw hole were also coated with varnish. Finally, Cervitec was applied to the head of the transverse screw after the prefabricated crown had been firmly affixed onto the implant (Fig 2).

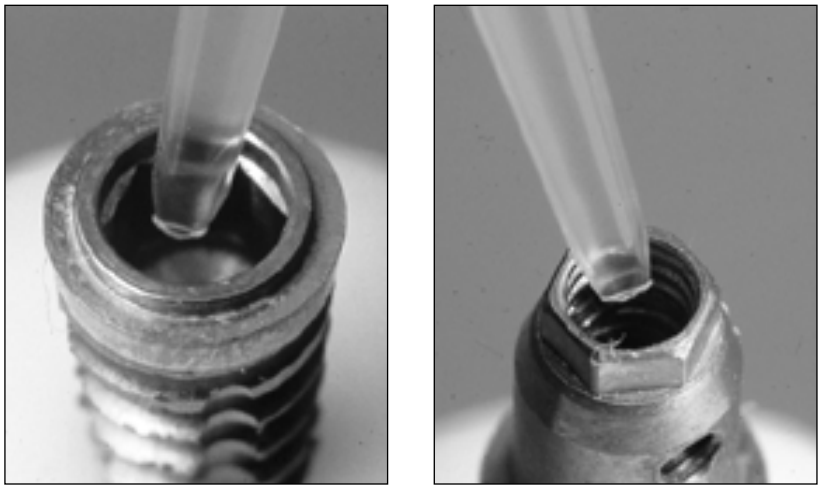
The assembled test specimens were immersed in 70% ethanol for about 30 seconds to minimize the possibility of contamination with other bacteria during handling and were then air dried. The implant assembly was then submerged in 4 mL *S. aureus* culture in Falcon plastic tubes (Becton Dickinson Labware, Lincoln Park, NJ) and incubated at 37°C. To ensure bacterial viability, the bacterial cultures were replaced biweekly. During this period, the bacterial counts varied between about 10^7 and 8×10^8 colony-forming units/mL. The entire procedure was performed twice from beginning to end. The implant-crown assemblies were first completely immersed (including the marginal gap and transverse screw hole of the prefabricated crowns) for 8 weeks; then, the implant-crown assemblies were partially immersed (to only the marginal gap of the prefabricated crowns) for 11 weeks (Fig 3).

To test for bacterial leakage, 5 test specimens were removed from the culture tubes at weekly intervals, immersed for 3 minutes in 70% ethanol, and air dried. This procedure ensured the sterility of the outer surface without affecting bacterial viability on the inside.[25] Then the specimens were carefully disassembled, and the inner surface of the crowns, as well as the internal hexagon of the implants, were sampled for bacterial contamination with sterile paper points (Fig 4). Paper points were streaked on blood agar plates and incubated in 4 mL TSB at 37°C for 24 hours. Growth of *S. aureus* on the blood agar and/or in the TSB medium was recorded. If a single colony of *S. aureus* was detected on the blood agar plates from direct plating of any of the paper points, or from indirect plating via in the TSB medium, that constituted a confirmation of leakage. The sensitivity of the method was 1 to 10 cfu in TSB and 10 to 100 cfu per paper point by direct plating.

Leakage from the Test Specimens. The 30 test specimens were assembled and sealed as described above with the following modifications. Before affixing the abutment, 2 µL *S. aureus* culture were carefully pipetted into the internal hexagon. The abutment was then screwed onto the implant and



Figs 4a and 4b (*Left*) Sampling of the internal hexagon and (*right*) the inner surface of the crown with sterile paper points.



Figs 5a and 5b (*Left*) Introduction of 2 mL *S. aureus* culture into the internal hexagon of the Ha-Ti implant and (*right*) into the axial screw hole of the abutment.

another 2 µL of the same culture were pipetted into the upper cavity of the abutment (Fig 5). The completely assembled test specimens were then shortly immersed in 70% ethanol to disinfect the outer surface. The test specimens were totally submerged in 4 mL sterile TSB and incubated at 37°C for a duration of 1 week. Growth of *S. aureus* in the culture medium was recorded. This procedure was repeated; in the second series, samples from the contaminated inner surfaces of each implant assembly were taken at the end of the 1-week incubation to determine whether viable *S. aureus* were present.

Sensitivity of *S. aureus* to Chlorhexidine. The sensitivity of *S. aureus* strain ZIB 6901 to chlorhexidine was evaluated by determining the minimal inhibitory concentration (MIC) with a macrodilution test.[34] A 10% solution of chlorhexidine digluconate (pharmacy of University Hospital Basel) was diluted to 0.2% in sterile water and used to make serial dilutions in TSB. Constant inocula of *S. aureus* were added. After 16 to 24 hours of incubation at 36.5°C, the tubes were inspected for bacterial growth.

Table 1 Leakage into Totally Submerged Test Specimens

	Incubation (weeks)						
	3	4	5	6	7	8	Total
No. of test specimens analyzed	5	5	5	5	5	5	30
No. of leaking test specimens	0	1	0	0	0	0	1

Table 2 Leakage into Partially Submerged Test Specimens

	Incubation (weeks)						
	3	4	5	7	9	11	Total
No. of test specimens analyzed	5	5	5	5	5	5	30
No. of leaking test specimens	0	0	0	0	0	0	0

Results

Leakage into the Test Specimens. *S. aureus* leakage into the totally submerged, sealed test specimens was detected in 1 of the 5 samples incubated for 4 weeks, while none was detected in the samples incubated for 3, 5, 6, 7, or 8 weeks (Table 1). When the sealed test specimens were partially submerged (that is, the transverse screw hole was excluded from any contact with the *S. aureus* broth) for 3 to 11 weeks, none of the internal surfaces of the 30 implant specimens manifested contamination (Table 2).

Leakage from the Test Specimens. All implant test specimens showed no bacterial leakage within 1 week, when both the transverse screw hole and marginal gap were immersed. In the repeat series, samples taken from the implant and abutment chambers did not reveal any viable *S. aureus* after 1 week.

Sensitivity of *S. aureus* to Chlorhexidine. The minimal inhibitory concentration was 0.001% chlorhexidine.

Discussion

Earlier studies demonstrated that implant systems with a high degree of precision fit of suprastructure components, eg, the Ha-Ti implant system,[22] do not guarantee protection in vivo against microorganisms at the marginal gap.[24] An assessment of the sealing capacity at the marginal gap, as well as the transverse screw hole of the same prefabricated crowns on Ha-Ti implants, was developed in vitro.[25] Experimental errors, including premature leakage and/or subsequent intrusion of bacteria during the assembling and disassembling of the suprastructure components, were demonstrably shown to be avoided. The results were compatible with other in vivo and in vitro findings, which showed bacterial colonization on internal surfaces of Brånemark[26,27] and IMZ restorations.[28,29]

Bacterial colonization may occur even more rapidly during functional loading of suprastructures.[31] Micromovements between individual components of implant-supported restorations will eventually cause local enlargement of the marginal gap, as well as a pumping effect.[35] Several authors[27,36,37] concluded from the analysis of available clinical data that, after second-stage implant surgery of submerged implants and loading, an ecological balance may become established between internal implant microbiota and the host. Nevertheless, penetration of oral microorganisms through gaps of implant-supported suprastructures may add to the risk of soft tissue inflammation,[33] or the failure of the treatment of peri-implantitis with or without guided tissue regeneration after inflammatory loss of supporting bone.[32]

It has been shown that sealing the gaps with the chlorhexidine-containing varnish Cervitec maintained the implant seal and prevented bacterial penetration into the test specimens for up to 11 weeks under unloaded conditions in vitro. The protective effect of sealing the gaps appears obvious. But how much could the bactericidal activity of chlorhexidine contribute to protection? The chlorhexidine concentration in the varnish of 1 wt% was far above the MIC of 0.001% of the *S. aureus* strain used in the study, and it would likely

increase during evaporation of the solvent. Colonization of the varnish could thus be effectively prevented. Assuming that the chlorhexidine would diffuse totally into the 4 mL of incubation medium, the MIC clearly was not reached, since a fair proportion of *S. aureus* survived for 2 weeks before the culture was replaced. On the other hand, no viable *S. aureus* were recovered from the inside of the assemblies 1 week after 1 to 2×10^6 cells had been deposited. This effect could be the result of diffusion of chlorhexidine into the small volume of 2 mL bacterial culture applied.

Summary

Application of a bactericidal varnish is simple. If similar results can be obtained under clinical conditions, bacterial colonization could be prevented during a critical period of implant survival. This procedure would complement sterilization of suprastructure components and disinfection of internal surfaces of implants as recommended for treatment of peri-implantitis.[31,33] How much longer the bacteria-free status could have been maintained is unknown. In addition, loading of suprastructures may change the situation and limit the indications for gap sealing. These indications have not yet been determined, in anticipation of the results of in vivo and in vitro tests under loaded conditions.

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