Edith Groenendijk
Jan J. K. Dominicus
Wilfred R. Moorer
Irene H. A. Aartman
Marinus A. J. van Waas

Microbiological and clinical effects of chlorhexidine enclosed in fixtures of 3I-Titamed® implants

Authors' affiliations:
Edith Groenendijk, Jan J. K. Dominicus, Clinic of Oral Implantology, Academic Center for Dentistry Amsterdam (ACTA), the Netherlands
Wilfred R. Moorer, Department of Cariology, Endodontology and Pedodontology, ACTA, the Netherlands
Irene H. A. Aartman, Department of Orthodontics and Social Dentistry, ACTA, the Netherlands
Marinus A. J. van Waas, Department of Oral Function, ACTA, the Netherlands

Correspondence to:
Wilfred R. Moorer
ACTA
Louwesweg 1
1066 EA Amsterdam
The Netherlands
Tel.: +31 20 5188436
Fax: +31 20 6692881
e-mail: w.moorer@acta.nl

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Abstract: This double-blind study used a split-mouth design to investigate the microbiological and clinical effects of 0.2% chlorhexidine enclosed in fixtures. Twelve patients had 46 fixtures implanted. At second-stage surgery, a microbiological sample (baseline sample) of the inner parts of the fixtures was taken. Then, a 0.2% chlorhexidine solution was applied into the inner space of 23 fixtures (test group), and in 23 fixtures saline was applied (control group). Abutments were installed and gingival index, plaque index and crevicular fluid flow were monitored weekly. After 6 weeks, a second microbiological sample of the inner part of the fixtures was taken.

At baseline, viable bacteria were detected within 46% of the fixtures. After 6 weeks, bacteria were found in 87% of the fixtures. The numbers of bacteria in the control group were significantly higher than those in the test group. The results indicate that, after first-stage surgery, contamination of the inner spaces of the fixtures is commonplace. Application of a 0.2% chlorhexidine solution at second-stage surgery inhibits growth or acquisition of bacteria in the fixtures. In both test and control groups, the crevicular fluid flow as well as the gingival index decreased during the experimental period. At 4, 5 and 6 weeks after chlorhexidine application, these values in the test group appeared lower, but did not attain statistical significance.

Persistent infection is certainly one of the factors that interferes with clinical implant success. Except for microorganisms localized at the outer surfaces of implant systems that cause peri-implantitis, bacteria present *within* the implant system could trigger inflammation at the level of the abutment–fixture contact. Bacteria enclosed within the implant space, not unlike those in infected root canals, are more or less shielded from host defense activities. They may persist for long periods of time.

Ericsson et al. (1995, 1996) observed, in studies on dogs, that an inflammatory cell infiltrate was consistently present at the borderline between abutment and fixture. This infiltrate occurred both at sites that had not been exposed to plaque and at sites that had been exposed to plaque for 9 months. It was suggested that this inflammatory infiltrate represents the efforts by the host to close off bacteria present within the implant system and that this may explain the 1-mm bone loss observed during the first year after abutment connection.

In a clinical study by Quirynen and van Steenberghe (1993), it was suggested that the microorganisms present inside the fixture 3 months after abutment connection may be the result of leakage at the abutment–fixture interface. From a later

in vitro study, Quirynen et al. (1994) concluded that the gap between the abutment and the fixture might act as a reservoir from which microorganisms may leak from the fixture into the peri-implant sulcus and *vice versa*. This was in line with the findings of Traversy and Birek (1991). Jansen et al. (1997) reported that the very precise fit of the components of several implant systems could not prevent organisms from passing through the interface gap. This was confirmed in a similar study by Gross et al. (1999). Persson et al. (1996) examined the microbiota on the internal surfaces of 28 Brånemark implants that had been in function for 1–8 years. They suggested that the presence of microorganisms at the inner surfaces of the fixtures and abutments is the result of contamination during the first and/or second stage of implant installation, and/or an ingress of microorganisms from the oral cavity during functioning after bridge installation. In the study of Keller et al. (1998), it was stated that the microbial leakage through the gap between the suprastructure and the abutment plays an important role in the bacterial colonization of the internal parts of screw-retained crowns and bridges. Piattelli et al. (2001) studied dye penetration and bacterial penetration through the implant–abutment interface. All of their screw-retained abutments allowed bacteria (and dye) to penetrate into the inner cavity of the fixture. No penetration occurred with cement-retained abutments.

Chlorhexidine is the most effective and thoroughly tested antiplaque agent. It has the ability to reduce the amount of a wide spectrum of bacteria around natural teeth (Lang & Brecx 1986). Bacterial colonization of implants and teeth follow similar patterns (Lekholm et al. 1986; Mombelli et al. 1987; Quirynen & Listgarten 1990; Rams et al. 1991, Leonhardt et al. 1992; Danser et al. 1997). Note the excellent review by Quirynen et al. (2002).

Therefore, we undertook an effort to reduce the number of microorganisms in fixtures by enclosing chlorhexidine within the fixtures. The aim of this experimental study was to compare the microbiological and clinical effects of a 0.2% chlorhexidine solution applied to the inner space of fixtures, with the effects of a physiologic saline solution only.

Material and methods

Subjects

Twelve edentulous patients, four male and eight female, of the Academic Center for Dentistry Amsterdam (ACTA) were informed about and selected for the study. In all except one patient, an overdenture on four implants in the mandible was indicated. In one patient an overdenture on two implants was planned. All patients had been edentulous and full denture wearers for many years and had an ASA classification of 1. They had not taken antibiotics 3 months prior to inclusion in the study.

Procedure

In this double-blind experimental study with a split-mouth design, two implants per patient were randomly allocated to the test group and two to the control group (one implant in each group for the patient for whom only two implants were planned). The surgeon started the experiment during second-stage surgery. In the test group, a 0.2% chlorhexidine solution (Corsodyl®) was injected into the inner part of the 3I-Titamed® fixtures. In the control group the inner parts were rinsed with physiologic saline solution only. The clinical measurements were performed by the prosthodontist. Neither the prosthodontist, the patient nor the microbiologist was informed about which fixture was in the test or control group.

Two-stage fixtures were placed according to protocol. After 3 months, second-stage surgery was performed. Prior to surgery, the patient was instructed to rinse with 0.2% chlorhexidine solution for 2 min. In order to prevent contamination of the operation site with saliva, sterile gauze was inserted around the operation site and the area was kept dry using suction. The operation site was dried with sterile gauze and a ridge incision was made. Immediately after the cover screws were removed, a microbiologic sample of the inner part of each fixture was taken (for details see the Microbiology section). After sampling the inner parts, fixtures and their environment were rinsed with physiologic saline solution. The internal parts of fixtures in the test group were dried by means of sterile paper points no. 140 and then 0.2% chlorhexidine solution was injected into the inner space of the fixture (internal volume approximately 7 μl). The abutment was immediately connected and fastened at the manufacturers recommended torque of 20 Nm by means of a torque controller. The abutments in the control group were connected and fastened immediately after rinsing with physiologic saline solution. After the gauze was removed, the operation site was rinsed with physiologic saline solution in order to remove the remains of chlorhexidine solution in the operation site and sutures were made. The patient was instructed to rinse with 0.2% chlorhexidine solution twice daily for 2 weeks. After this period, the prosthetic treatment started. The four implants were connected with a U-shaped Dolder bar. In the case with two implants, we used an egg-shaped Dolder bar.

Clinical indices

Three different kinds of measurements were taken each week for a period of 6 weeks after abutment connection. Crevicular fluid flow rate (CFFR) was measured at the buccal surface of each implant by means of a sterile paper strip (TearFlo® distributed by Rose Stone Enterprises) and counted in mm/10 s (Apse et al. 1989). First, the site was kept free from saliva using gauze. The peri-implant tissue was carefully dried with a cotton swab. The strip was bent in order to fit around the abutment and was inserted in the peri-implant sulcus without damaging the tissue. Per patient, the mean CFFR of the two implants per group (test and control) was calculated.

Next, the modified plaque index (mPlI; Mombelli et al. 1987) was used to assess the amount of plaque on a scale ranging from 0 to 3 (0 = no detection of plaque; 1 = plaque only recognized by running a probe across the smooth marginal surface of the implant; 2 = plaque seen by the naked eye; 3 = abundance of soft matter). The scores of the two implants in each group were averaged per patient. Finally, although peri-implant tissue is not completely comparable with gingival tissue (Berglundh et al. 1994), we used the gingival index (GI) by Löe & Silness (1963) to describe the health of the peri-implant mucosa. Per implant, a score ranging from 0 to 3 was given (0 = absence of inflammation; 1 = mild inflammation, slight change in

color, little change in texture; 2 = moderate inflammation, redness, edema and hypertrophy, bleeding on pressure; 3 = severe inflammation, marked redness and hypertrophy, tendency for spontaneous bleeding, ulceration.). Scores were averaged for the two implants in each group per patient.

Microbiology

Microbiological samples of the inner spaces of the fixtures were taken just before abutment connection (week 0) and 6 weeks after surgery (week 6) in order to measure the number of viable bacteria. At week 6, the microbial sample was taken after the clinical measurements. Patients were instructed to rinse with 0.2% chlorhexidine solution for 2 min before sampling. To prevent contamination of the site with saliva, sterile gauze was inserted around the abutment and the area was kept dry using suction. The implants and the oral mucosa were dried with sterile gauze. The external surfaces of the implants were disinfected with 70% ethanol. The abutments were held in place by an abutment clamp (Nobel Biocare®), and the abutment screws were removed aseptically. Samples of the fluid contents of the inner parts of the fixtures were taken by means of two sterile paper points no. 140. The paper points containing the sample were immediately transferred to a tube containing 0.5 ml of reduced transport fluid (RTF; Syed & Loesche 1972). Within 2 h, the tubes were transported to the laboratory, the samples dispersed, serially diluted in RTF, plated onto blood agar and incubated in an anaerobic glove box. Blood agar was made from tryptic soy agar (Difco®, Detroit, MI, USA), 5% defibrinated horse blood, 5 mg/l hemin and 1 mg/l menadion. The atmosphere in the anaerobic glove box consisted of 80% N_2, 10% H_2 and 10% CO_2. After 1 week at 37°C, total colony forming units (CFU) were counted. Numbers of viable microorganisms were calculated per fixture. CFUs $\geq 10^4$ per 7 µl internal volume (i.e. $\geq 1.4 \times 10^6$/ml) were considered as potential pathological amounts of bacteria (Sundqvist 1994). No attempt was made to identify the bacteria. For statistical purposes, the numbers of CFUs per fixture space were divided into three categories: (a) $< 10^3$; (b) $\geq 10^3$ and $< 10^4$; and (c) $\geq 10^4$. The scores of the two implants in each group were then averaged per patient.

Statistical analysis

Data were analyzed using the Wilcoxon signed rank test for two related samples when differences were assessed between test and control group and between weeks 0 and 6. The Friedman test was used to assess differences among weeks 0–6, and *post hoc* Wilcoxon signed rank tests were conducted when necessary. Differences were considered statistically significant when $P < 0.05$. However, as a result of the high number of comparisons between the test and control group with respect to the clinical indices, a significance level of 0.01 was chosen.

Results

Microbiology

At baseline, 3 months after fixture installation, we found viable bacteria in 21 of the 46 implants. The amounts of CFUs at weeks 0 and 6 for the test and control site are listed in Table 1. At week 0, the amounts of CFU in the control and test groups did not differ significantly ($P = 0.581$). However, at week 6, 48% of the microbial samples in the control group and 17% of the implants of the test group had total CFU $\geq 10^4$. The categorized counts in the control group were significantly higher than in the test group ($P = 0.018$).

Compared to week 0, there was a large and significant increase in numbers of bacteria after 6 weeks ($P = 0.003$) in the control group, but not in the chlorhexidine group ($P = 0.070$).

Clinical indices

The mean scores and standard deviations of the clinical indices are given in Table 2. In both the test and control groups, the CFFR and the GI decreased during the observation period ($P < 0.001$). There were no significant differences in CFFR, mPII and GI between the control and the test groups during the 6-week observation ($P > 0.01$).

Discussion

Three months after fixture implantation, viable bacteria were detected in 46% of the implants. This could mean that contamination of the inner part of the fixture occurred at first-stage surgery (and that

Table 1. **Amounts of bacteria in CFU per fixture per site at weeks 0 and 6**

Patient	Test site		Control site	
	Week 0	Week 6	Week 0	Week 6
1	0	0	0	0
1	0	0	0	11,500
2	39,000	0	55	6000
2	40	105	100,000	15,000
3*	0	7000	0	2450
4	1850	0	195	5500
4	25	6000	25	650
5	0	50,000	0	10,000
5	0	50,000	330	7000
6	0	2200	25	150,000
6	0	800	0	10,000
7	0	400	600	2000
7	0	1000	13,000	6000
8	0	2200	0	200
8	0	0	0	3650
9	60	405	10,500	3150
9	0	16,000	0	60,000
10	0	100,000	0	500,000
10	55	9500	70	500,000
11	1400	4050	6000	3950
11	17,000	3750	0	12,000
12	0	3350	9150	26,000
12	400	2250	0	30,000

*Two instead of four implants in patient 3.

Table 2. **Mean, SD and *P*-value of the clinical indices per group per week**

Week	Test site	Control site	*P*
CFFR in mm/10 s			
1	4.1±1.1	4.1±1.5	0.952
2	2.5±1.2	2.7±1.4	0.666
3	1.8±1.1	2.1±1.1	0.161
4	1.2±0.9	1.7±1.3	0.258
5	0.9±0.7	1.5±0.9	0.139
6	0.6±0.6	1.4±1.0	0.073
mPII			
1	0.1±0.2	0.1±0.1	0.564
2	1.0±0.9	0.9±1.1	0.783
3	1.3±1.0	1.5±1.1	0.257
4	1.3±0.8	1.5±0.8	0.157
5	1.2±0.6	1.2±0.6	0.564
6	1.2±0.6	1.2±0.6	0.655
GI			
1	1.8±0.5	1.8±0.4	0.317
2	1.4±0.5	1.6±0.5	0.194
3	1.0±0.4	1.3±0.5	0.107
4	0.5±0.5	0.9±0.4	0.026
5	0.3±0.4	0.7±0.3	0.021
6	0.3±0.4	0.5±0.5	0.248

Significance level $\alpha = 0.01$.

these bacteria survived for 3 months in the fixture) or that, after surgery, bacteria gained access by leakage. Six weeks after abutment connection, microorganisms could be detected in the inner part of the fixtures in 87% of the implants. This means that another 41% of the fixtures were contaminated during abutment connection or in the 6 weeks thereafter. This is in line with the findings of Quirynen & van Steenberghe (1993) and Persson et al. (1996), who found microorganisms in the inner part of the fixture 3 months after abutment connection and after 1–8 years of function, respectively. These authors suggested that the presence of microorganisms might be the result of contamination during the first stage and/or during the second stage of implant surgery and/or by leakage of microorganisms into the inner part of the fixture after installation of the suprastructure. In the present investigation, we took efforts to avoid contamination of the fixtures during surgery. Patients were instructed to rinse with chlorhexidine for 2 min before surgery and sampling, the operation site was isolated and dried by sterile gauze, and careful suction was used. Therefore, contamination of the inner part of the fixture during surgery seems possible but unlikely. Access of bacteria into the inner part of the fixture during first-stage surgery as well as during function, as suggested by Persson et al. (1996), seems probable indeed.

At week 0, the CFU counts in the control and test groups appeared mostly low, but in five out of 46 the numbers of CFU were high ($\geq 10^4/7\,\mu l$). In one of these cases, there was a dehiscence of the cover screw through the mucosa. In the other four cases, the cover screws were found loosened, allowing easy ingress of bacteria.

In the control group, a large and significant increase in the amounts of viable bacteria 6 weeks after abutment connection had occurred. This was not only due to an increase in the total amount of contaminated fixtures, but also the amount of CFU per fixture had increased in most of the cases (Table 1). We suppose that leakage through the abutment–implant interface and/or through the abutment screw component is a significant factor in the contamination of the inner parts.

In a healthy situation, the peri-implant mucosa forms a firm seal around the implant, protecting the fixture against microorganisms from the oral environment. As soon as implants come into contact with the oral cavity, bacteria colonize them (Mombelli et al. 1987, 1988; Keller et al. 1998). Reduction of microbial accumulation by an optimal oral hygiene is a very important factor in the prevention of peri-implantitis (Leonhardt et al. 1992; Pontoriero et al. 1994). However, the peri-implant tissues appear less effective than the gingiva in the defense against microbial invasion. Lindhe et al. (1992), using dogs, found that lesions caused by bacterial infections in peri-implant mucosa involve the alveolar bone earlier than a periodontal infection does around natural teeth. Any factor that compromises the defense against microorganisms should be taken into account.

Such a factor is the presence of bacteria in the internal spaces of implants. Although it is not known what role these bacteria play in the propagation of peri-implantitis and marginal bone loss, the existence of a reservoir of bacteria from which leakage out of the implant into the surrounding tissues could occur is not unlike the situation in infected natural root canals (Wu & Wesselink 1993). Similarities of root-filled teeth with contaminated spaces between root-filling and crown spring to mind (Fogel 1995). In endodontics, antibacterial treatment of infected spaces within teeth is the most important characteristic of the therapy indeed.

Chlorhexidine within the fixture could kill bacteria or prevent bacterial growth. We have shown here that this is a significant effect indeed. Chlorhexidine could also diffuse out, exert an antibacterial effect, and in this way might reinforce the host defense protecting the peri-implant tissues.

Conclusion

At second-stage surgery (abutment connection), bacteria are either absent or their numbers in the fixtures are generally small, except in those cases with loose cover screws. Six weeks after abutment connection, the number of contaminated implants as well as the amounts of bacteria within the inner parts of the fixtures had increased. Contamination at surgery or leakage through the abutment–fixture interface appears to be the cause. A 0.2% chlorhexidine solution, compared to saline, applied within the inner implant space of fixtures at second-stage surgery, resulted in significantly lower numbers of bacteria. Within the limits of this study, we could not prove a significant effect of the enclosed chlorhexidine on crevicular fluid flow, plaque index and GI.

Résumé

Cette étude en double aveugle a utilisé un modèle de bouche divisée pour étudier les effets cliniques et microbiologiques de la chlorhexidine placée dans les implants. Douze patients ont reçu 46 implants. Lors de la seconde chirurgie, un échantillon microbien (échantillon de base) des parties internes de l'implant a été prélevé. Une solution de 0,2% de chlorhexidine a ensuite été placée dans la partie interne de 23 implants (groupe test) et du sérum physiologique dans 23 implants servant de contrôles. Les piliers ont été placés et l'indice gingival, de plaque dentaireainsi que le flux du fluide créviculaire ont été enregistrés toutes les semaines. Après six semaines, un second échantillon microbiologique a été prélevé de la partie interne des implants. Lors de l'examen de départ, des bactéries viables étaient détectées dans 46% des implants. Après six semaines, des bactéries étaient trouvées dans 87% des implants. Le nombre de bactéries dans le groupe contrôle étaient significativement plus important que dans le test. Les résultats indiquent qu'après une chirurgie de première étape, la contamination des espaces intérieures de l'implant est fréquente. L'application d'une solution de 0,2% de chlorhexidine lors de la seconde chirurgie inhibe la

croissance ou l'acquisition de bactéries dans les implants. Tant dans les groupes tests que contrôles, le flux du fluide créviculaire ainsi que l'indice gingival diminuaient durant la période expérimentale. Quatre, cinq et six semaines après l'application de la chlorhexidine, ces valeurs dans le groupe test apparaissaient inférieures, mais ne l'étaient pas statistiquement.

Zusammenfassung

Mikrobiologische und klinische Auswirkungen von Chlorhexidin, welches in Implantate des 3I-Titamed Systems eingebracht wurde

In dieser Doppelblindstudie wurde eine Versuchsanordnung mit unterschiedlich behandelten Seiten angewendet, um die mikrobiologischen und klinischen Auswirkungen von 0.2% Chlorhexidin, welches in die Implantate eingebracht wurde, zu untersuchen. Bei 12 Patienten wurde 46 Implantate eingesetzt. Bei der Zweitoperation wurden mikrobiologische Proben (Ausgangsuntersuchung) vom inneren Teil der Implantate entnommen. Danach wurde im inneren Teil von 23 Implantaten eine 0.2% Chlorhexidinlösung appliziert (Testgruppe), während bei 23 weiteren Implantaten Kochsalzlösung verwendet wurde (Kontrollgruppe). Die Prothetikteile wurden eingesetzt, danach wurden wöchentlich Gingivalindizes, Plaqueindizes und Sulkusflüssigkeitsmessungen durchgeführt. Nach 6 Wochen wurde vom inneren Teil der Implantate einen zweite mikrobiologische Probe entnommen.

Bei der Ausgangsuntersuchung konnten bei 46% der Implantate lebendige Mikroorganismen gefunden werden. Nach 6 Wochen konnten bei 87% der Implantate Bakterien nachgewiesen werden. Die Anzahl der Bakterien war in der Kontrollgruppe signifikant grösser als in der Testgruppe. Die Resultate zeigen, dass eine Kontamination der Innenräume der Implantate beim ersten chirurgischen Eingriff häufig vorkommt. Die Applikation von 0.2% Chlorhexidinlösung bei der Zweitoperation verhindert das Wachstum oder die Ansiedelung von Bakterien in den Implantaten. Sowohl in der Test- als auch in der Kontrollgruppe nahmen die Sulkusflüssigkeit und der Gingivalindex im Laufe der Beobachtungszeit ab. Vier, fünf und sechs Wochen nach Applikation von Chlorhexidin schienen die Werte in der Testgruppe tiefer zu sein, erreichten aber keine statistische Signifikanz.

Resumen

Este estudio a doble ciego usó un diseño de boca dividida para investigar los efectos microbiológicos y clínicos de clorhexidina al 0.2% incluida en las fijaciones. Se implantaron 46 fijaciones en doce pacientes. En la segunda fase quirúrgica se tomó una muestra microbiológica (muestra inicial) de las partes internas de las fijaciones. Entonces se aplicó una solución de clorhexidina al 0.2% en el espacio interior de 23 fijaciones (grupo de prueba), y de suero salino en 23 fijaciones (grupo de control). Se colocaron los pilares y se monitorizaron semanalmente el índice gingival, índice de placa y flujo de fluido gingival. Tras 6 semanas se tomó una segunda muestra microbiológica de las partes internas de las fijaciones.

Al inicio, se detectaron bacterias viables en el 46% de las fijaciones. Tras seis semanas se encontraron bacterias en el 87% de las fijaciones. El número de bacterias en el grupo de control fue significativamente mas alto que en el grupo de prueba. Los resultados indican que, tras la cirugía de primera fase, es común la contaminación de los espacios internos de las fijaciones. La aplicación de una solución de clorhexidina al 0.2% en la segunda fase quirúrgica inhibe el crecimiento de o la adquisición de bacterias en las fijaciones. Tanto en los grupos de prueba como de control descendieron el flujo de fluido gingival al igual que el índice gingival durante el periodo experimental. A las cuatro, cinco y seis semanas de la aplicación de la clorhexidina, estos valores aparecieron mas bajos en el grupo de prueba, pero no mostraron significancia estadística.

要約

この二重盲検は口腔分割デザインにおいて、フィクスチャーに含ませた０．２%クロルヘキシジンの細菌学的および臨床的効果を調べた。１２名の患者に４６本のフィクスチャーを埋入した。二次手術時にフィクスチャー内部の細菌標本（ベースラインの標本）を採取した。それから０．２%クロルヘキシジン溶液を２３本のフィクスチャーの内腔に入れ（試験群）、残り２３本のフィクスチャーには食塩水を入れた（対照群）。アバットメントを連結し、歯肉インデックス、プラーク・インデックスと歯肉溝液流量を毎週モニターした。６週間後にフィクスチャー内部の二度目の細菌標本を採取した。

ベースライン時に生細菌はフィクスチャーの４８%以内に検出された。６週間後にはフィクスチャーの８７%に細菌がみとめられた。対照群の細菌数は、試験群より有意に高かった。この結果は一次手術後にフィクスチャー内部の汚染がよく起こることを示唆している。二次手術時の０．２%クロルヘキシジン溶液の使用は、フィクスチャー内の細菌の増殖や取得を抑制する。試験群も対照群も歯肉溝液流量と歯肉インデックスは実験期間中に減少した。試験群においてこれらの値はクロルヘキシジン塗布４、５、６週間後に減少したようだったが、統計学的有意差ではなかった。

References

Apse, P., Ellen, R.P., Overall, M. & Zarb, G.A. (1989) Microbiota and crevicular fluid collagenase activity in the osseointegrated dental implant sulcus: a comparison of sites in edentulous and partially edentulous patients. *Journal of Periodontal Research* **24**: 96–105.

Berglundh, T., Lindhe, J., Jonsson, K. & Ericsson, I. (1994) The topography of the vascular systems in the periodontal and peri-implant tissues in the dog. *Journal of Clinical Periodontology* **21**: 189–193.

Danser, M., van Winkelhoff, A.J. & van der Velden, U. (1997) Periodontal bacteria colonizing oral mucous membranes in edentulous patients wearing dental implants. *Journal of Periodontology* **68**: 209–216.

Ericsson, I., Persson, L.G., Berglundh, T., Marinello, C.P., Lindhe, J. & Klinge, B. (1995) Different types of inflammatory reactions in peri-implant soft tissues. *Journal of Clinical Periodontology* **22**: 255–261.

Ericsson, I., Nilner, K., Klinge, B. & Glantz, P.-O. (1996) Radiographical and histological characteristics of submerged and nonsubmerged titanium implants. An experimental study in the Labrador dog. *Clinical Oral Implants Research* **7**: 20–26.

Fogel, H.M. (1995) Microleakage of posts used to restore endodontically treated teeth. *Journal of Endodontics* **21**: 376–379.

Gross, M., Abramovich, I. & Weiss, E.I. (1999) Microleakage at the abutment–implant interface of osseointegrated implants: a comparative study. *International Journal of Oral & Maxillofacial Implants* **14**: 94–100.

Jansen, V.K., Conrads, G. & Richter, E.-J. (1997) Microbial leakage and marginal fit of the implant–abutment interface. *International Journal of Oral & Maxillofacial Implants* **12**: 527–540.

Keller, W., Brägger, U. & Mombelli, A. (1998) Peri-implant microflora of implants with cemented and screw retained suprastructures. *Clinical Oral Implants Research* **9**: 209–217.

Lang, N.P. & Brecx, M.C. (1986) Chlorhexidine digluconate – an agent for chemical plaque control and prevention of gingival inflammation. *Journal of Periodontal Research* **21**: 74–89.

Leonhardt, A., Berglundh, T., Ericsson, I. & Dahlén, G. (1992) Putative periodontal pathogens on titanium implants and teeth in experimental gingivitis and periodontitis in beagle dogs. *Clinical Oral Implants Research* **3**: 112–119.

Lekholm, U., Ericsson, I., Adell, R. & Slots, J. (1986) The condition of the soft tissues at tooth and fixture abutments supporting fixed bridges. A microbiological and histological study. *Journal of Clinical Periodontology* **13**: 558–562.

Lindhe, J., Berglundh, T., Ericsson, I., Liljenberg, B. & Marinello, C. (1992) Experimental breakdown of peri-implant and periodontal tissues. A study in beagle dog. *Clinical Oral Implants Research* **3**: 9–16.

Löe, H. & Silness, J. (1963) Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontologica Scandinavica* **21**: 533–551.

Mombelli, A., van Oosten, M.A.C., Schürch, E. Jr & Lang, N.P. (1987) The microbiota associated with successful or failing osseointegrated titanium implants. *Oral Microbiology and Immunology* **2**: 145–151.

Mombelli, A., Buser, D. & Lang, N.P. (1988) Colonization of osseointegrated titanium implants in edentulous patients. Early results. *Oral Microbiology and Immunology* **3**: 113–120.

Persson, L.G., Lekholm, U., Leonhardt, Å., Dahlén, G. & Lindhe, J. (1996) Bacterial colonization on internal surfaces of Brånemark system implant

components. *Clinical Oral Implants Research* **7**: 90–95.

Piattelli, A., Scarano, A., Paolantoni, M., Assenza, B., Leghissa, G.C., Di Bonaventura, G., Catamo, G. & Piccolomine, R. (2001) Fluids and microbial penetration in the internal part of cement-retained versus screw-retained implant–abutment connections. *Journal of Periodontology* **72**: 1146–1150.

Pontoriero, R., Tonelli, M.P., Carnevale, G., Mombelli, A., Nyman, S.R. & Lang, N.P. (1994) Experimentally induced peri-implant mucositis. A clinical study in humans. *Clinical Oral Implants Research* **5**: 254–259.

Quirynen, M. & Listgarten, M.A. (1990) The distribution of bacterial morphotypes around natural teeth and titanium implants *ad modum* Brånemark. *Clinical Oral Implants Research* **1**: 8–12.

Quirynen, M. & van Steenberghe, D. (1993) Bacterial colonization of the internal part of two-stage implants. An *in vivo* study. *Clinical Oral Implants Research* **4**: 158–161.

Quirynen, M., Bollen, C.M.L., Eyssen, H. & van Steenberghe, D. (1994) Microbial penetration along the implant components of the Brånemark system. An *in vitro* study. *Clinical Oral Implants Research* **5**: 239–244.

Quirynen, M., De Soete M, . & van Steenberghe, D. (2002) Infectious risks for oral implants: a review of the literature. *Clinical Oral Implants Research* **13**: 1–19.

Rams, T.E., Roberts, T.W., Feik, D., Molzan, A.K. & Slots, J. (1991) Clinical and microbiological findings on newly inserted hydroxyapatite-coated and pure titanium human dental implants. *Clinical Oral Implants Research* **2**: 121–127.

Sundqvist, G. (1994) Taxonomy, ecology and pathogenicity of the root canal flora. *Oral Surgery, Oral Medicine and Oral Pathology* **78**: 522–530.

Syed, S.A. & Loesche, W.J. (1972) Survival of dental plaque flora in various transport media. *Applied Microbiology* **24**: 638–644.

Traversy, M.C. & Birek, P. (1991) Fluid and microbial leakage of implant–abutment assembly *in vitro*. *Journal of Dental Research* **71**: 754 (Abstract).

Wu, M.-K. & Wesselink, P.R. (1993) Endodontic leakage studies reconsidered. Part I. Methodology, application and relevance. *International Endodontic Journal* **26**: 37–43.