

# The in-vitro effect of a titanium implant on oral microflora: comparison with other metallic compounds

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**Summary.** Dental implant research has been mostly concerned with the biocompatibility of materials for implantation. In this study the effects of titanium dioxide and other metallic salts on seven bacterial species commonly found in dental plaque, two which are uncommon, and a yeast, were determined by agar incorporation and diffusion techniques, and compared with the effects of a titanium implant abutment. Neither the titanium dioxide nor the implant abutment demonstrated any inhibitory activity, although other compounds such as cobalt used in dental alloys had some effects.

## Introduction

Extensive research has been carried out on various materials, including metals and alloys, which could be used as implants to replace lost teeth (Williams, 1986). One such implant described by Branemark *et al.* (1977) and composed of commercially pure titanium has had considerable clinical success. A major concern is the biocompatibility of these implants but there has been little research into the effect of implant materials on the peri-implant microflora. A previous in-vitro investigation (Bundy *et al.*, 1980) showed some antibacterial activity of titanium against *Streptococcus mutans*. Other studies on the influence of metals on the growth of oral bacteria have been of limited value because they have also been performed on a single bacterial species (Nunez *et al.*, 1976).

In the present study, the effect of a titanium implant on seven species of bacteria commonly found in dental plaque, two which are uncommon, and a yeast, was examined and the activity of titanium dioxide was compared with that of other compounds which have also been used in various alloys for dental implants. A passivating layer of titanium dioxide has been shown to form on titanium implants immediately after manufacture (Kasemo, 1983). Our choice of metallic compounds for comparison was based on previous implant studies demonstrating corrosion products in the peri-implant tissues (Van Orden, 1985).

## Materials and methods

### Bacteria

*Escherichia coli* NCTC 10418, *Streptococcus sanguis* NCTC 7863, *Bacteroides gingivalis* NCTC 11834, *B. intermedius* NCTC 9338 and *Fusobacterium nucleatum* NCTC 10562 were used as reference strains, together with *B. gingivalis* W50 (kindly provided by Dr H. N. Shah, The London Hospital) and *B. intermedius* 212 which was identified in this Department (Duerden *et al.*, 1980). Other strains were kindly made available to us as follows:

*Candida albicans* strains 55 and 56, *Enterobacter cloacae* strains R14756 and F1441, *F. nucleatum* M2216 and a *Veillonella* sp. (Department of Bacteriology, Royal Hallamshire Hospital, Sheffield), *S. mutans* strains B13 and INGBRITT, *S. sanguis* G9B, *Actinomyces odontolyticus* and *Actinomyces* sp. strain 66 CX (C.W.I. Douglas, Department of Oral Pathology, University of Sheffield) and *V. parvula* 216 (J. Brazier, Luton Public Health Laboratory).

The streptococci were grown in a CO<sub>2</sub>-enriched atmosphere and obligate anaerobes were cultured in an anaerobic cabinet (Don Whitley Scientific Ltd, Shipley, West Yorkshire) in an atmosphere of N<sub>2</sub> 80%, CO<sub>2</sub> 10%, H<sub>2</sub> 10%. The remaining organisms were grown aerobically. All cultures were incubated at 37°C.

### Metal salts

The following chemicals were obtained from Sigma and stock solutions were made at the concentrations indicated: HgCl<sub>2</sub> (0.1M), NiCl<sub>2</sub> (0.1M), CoCl<sub>2</sub>·6H<sub>2</sub>O (0.1M), CrCl<sub>3</sub>·6H<sub>2</sub>O (0.1M) and TiO<sub>2</sub> (0.1M). These solutions were sterilised by filtration through 0.22-µm membrane filters (Millipore).

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### Titanium implant

A titanium abutment cylinder, height 5.5 mm, (Nobel-pharma Implant System Art. No. SDCA 005, Gothenberg, Sweden) was tested.

### Culture media

Brain Heart Infusion Broth (Oxoid) supplemented with yeast extract 5 mg/ml, haemin 5 µg/ml and menadi-one 1 µg/ml (BHIS) was used throughout. Agar 1.0% was added for both the agar-dilution and well-diffusion plate experiments. Lysed blood 5% was also added for the growth of *B. gingivalis* and *B. intermedius* in all susceptibility experiments.

### Susceptibility tests

All tests were done in duplicate and each experiment was repeated once, i.e., each organism was tested with each metallic compound four times.

**Agar incorporation.** Growth was harvested from culture plates and suspended in saline to a concentration of c.  $10^9$  cfu/ml by comparison with Wellcome opacity tubes; 0.001 ml of suspension ( $10^4$  cfu) was then delivered by a Multipoint Inoculator (Denley Instruments Ltd, Billingham, Sussex) from a 1 in 100 dilution of the suspension of each strain on to the surface of the agar plates. Metal MICs were estimated by an agar dilution technique in which serial ten-fold dilutions of metallic compounds from 0.00001M to 0.1M were prepared in BHIS agar. The MIC was the lowest concentration of metal salts to prevent growth. A strain of *E. coli* (NCTC 10418) known to be susceptible to the above concentrations of metal ions (except titanium), and an agar plate containing no metal ions were used as controls.

**Agar diffusion.** All organisms were cultured on BHIS agar with and without lysed blood 5%. The inoculum was 0.1 ml of a suspension containing  $10^4$  cfu/ml; it was spread over the surface with a glass spreader. The sterile titanium implant was then carefully placed into the agar plate with the top protruding into the atmosphere. The plate was then incubated upright. Any zone of inhibition around the implant was considered a sign of susceptibility.

## Results

### Agar incorporation

The susceptibility of all test organisms was remarkably consistent with only two minor exceptions (table). The chloride salt of mercury was the most active compound tested and was ten times more active than either cobalt or nickel; these compounds were in turn ten times more active than chromium. Titanium dioxide was the least active metallic compound tested. Mercuric chloride was

**Table.** Antimicrobial activity of metallic compounds

Metallic compound	MIC (M) of compound against	
	Test strains	<i>E. coli</i> NCTC 10418
HgCl <sub>2</sub>	0.001*	0.0001
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.01	0.001
NiCl <sub>2</sub>	0.01†	0.001
CrCl <sub>3</sub> ·6H <sub>2</sub> O	0.1	0.01
TiO <sub>2</sub>	> 0.1	> 0.1

\* *S. mutans* INGBRITT was inhibited by 0.0001M HgCl<sub>2</sub>.

† *E. cloacae* R14756 was inhibited by 0.1M NiCl<sub>2</sub>.

>100 times more active than titanium dioxide. There were no differences in MIC results on repeated testing.

### Agar diffusion

No zone of inhibition was observed around the implant with any of the test organisms on BHIS agar with and without blood.

## Discussion

Comparative activities of mercuric, cobalt, nickel and chromium chlorides when tested against all organisms were almost identical to those found by Riley and Mee (1982) who examined the activity of the same metals against *Bacteroides* spp. We found no difference in the susceptibility of the various test species to each compound. The most active was the chloride salt of mercury, then cobalt and nickel, and chromium was the least active of the four. Unexpectedly, the similarity of results obtained with gram-positive and gram-negative bacteria and a eukaryotic organism suggested a non-specific inhibitory action of the metallic compounds on metabolic activity and cell growth. Titanium dioxide did not inhibit growth of any of the organisms studied by agar incorporation. This finding would be expected because the titanium implant did not inhibit any of the organisms studied by agar diffusion. These results contrasted with the findings of Bundy *et al.* (1980), although these workers only tested one strain of *S. mutans*.

As the colonisation of the peri-implant region by oral microflora is of particular clinical significance (Stallard, 1985), our in-vitro studies may be of importance as they show that the titanium implant will probably have little inhibitory effect on the microflora. A 3-year longitudinal prospective in-

vivo study (Adell *et al.*, 1986) showed that the microflora on titanium abutments appeared different from that on teeth. They suggested that surface energy characteristics of the titanium might have influenced the accumulation of the microflora. We cannot confirm this directly but our results suggest

that the discrepancy is unlikely to be due to the antimicrobial activity of the titanium.

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