

ANTIMICROBIAL EFFECTS OF TITANIUM SURFACES WITH INCORPORATED COPPER

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Introduction

The market of dental implant showed a steadily growth over the last two decades. Simultaneously implant failure caused by bacterial infections and poor implant ingrowth gained significance [Mombelli, 2000]. The main cause for infection related implant failures is the affection by bacteria, mainly by *S. aureus*. Therefore implant surfaces are designed to show antimicrobial effect [Brunette et al., 2001].

Here we present results from a newly developed porous, antimicrobial titanium surface with incorporated copper. The antimicrobial effect and the required amount of copper were studied on *E. coli* and on *S. aureus*.

Methods

Titanium discs (cpTi, grade 4) were cleaned, spark-assisted anodized and rinsed with deionized water in an ultrasonic bath. The spark-assisted anodizing process produces multiple layers [Jung, 2010]. The copper was incorporated in the tightly adhered fine-porous layer by an electrochemical process. The discs were characterised by SEM and EDX. Nitric acid was used to dissolve the complete copper from the surface layer for quantifying by Atom Absorption Spectrometry (AAS). Dilution series with *E. coli* and *S. aureus* and live/dead staining were performed to assess the antimicrobial activity of the modified surfaces. The release kinetics was studied by mimicking the physiological situation in simulated body fluid (SBF).

Results

The electrochemical process time was varied to generate specific amounts of copper incorporation in titanium discs. The copper quantity on the discs determined by AAS and correlated with EDX measurements (figure 1).

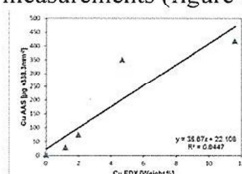


Figure 1: Correlation between EDX [weight %] and AAS [µg per disc surface] measurements.

Both bacteria strains can be marked with DNA specific fluorescent stains: We used syto 61 to stain all bacteria in red-whereas dead bacteria are stained in green (sytox green), see figure 2.



Figure 2: *S. aureus*, confocal laser scanning microscope (all bacteria=red; dead=green).

Preliminary experiments showed that $70 \mu\text{g} \cdot \text{mL}^{-1}$ of a copper salt concentration devitalize *E. coli* and that $35 \mu\text{g} \cdot \text{mL}^{-1}$ is the bactericidal concentration for *S. aureus*.

It was furthermore discovered that the copper release from the discs at 37°C (blue line) showed another effect than the accelerated aging at 80°C (red line) (figure 3).

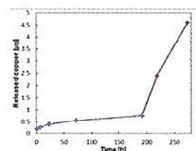


Figure 3: Copper release in SBF at 37°C (blue line) and accelerated aging at 80°C (red line).

Discussion

We showed that copper has an antimicrobial effect on *E. coli* as well as *S. aureus*. The copper release from the discs is relatively slow (e.g. after 63 simulated days only 9% of copper was in solution (data not showed)) which indicates a long-term antimicrobial activity of the titanium implant surface.

Our next step is to establish the inhibiting copper salt concentration for both bacterial strains and the antimicrobial effect of the modified titanium surfaces in direct contact with the bacteria. Furthermore the release kinetics for different copper surface concentrations will be studied.

References

- Mombelli, Periodontology, 28:177-189, 2000.
- Brunette et al, Titanium in Medicine, Springer-Verlag, 2001.
- Jung, European Cells and Materials, 19(Suppl 2):4, 2010.