

An inorganic antimicrobial surface modification for orthopaedic implants

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INTRODUCTION: The rising number of periprosthetic infections has led to the development of various strategies for bactericidal implant coatings. [1] We investigated the approach of an inorganic calcium hydroxide $\text{Ca}(\text{OH})_2$ coating that expresses an initial antibacterial effect before it transforms into hydroxyapatite. [2] While safety of this electrochemically deposited antimicrobial coating was previously shown preclinically [3], we here verified the bactericidal effect in-vitro.

METHODS: $\varnothing 15\text{mm}$ titanium disks were coated by titanium plasma spray (TPS) before a $20\ \mu\text{m}$ thick $\text{Ca}(\text{OH})_2$ coating was electrochemically deposited. The surface was inspected by SEM (Zeiss Supra). $\text{Ca}(\text{OH})_2$ pellets (Fluka) with a calcium hydrogen phosphate dihydrate filler (Emcompress Premium JRS Pharma) were manufactured ($\text{Ca}(\text{OH})_2/\text{CaHPO}_4 \cdot 2\ \text{H}_2\text{O} = 50\% : 50\%$) and served as standardized positive control.

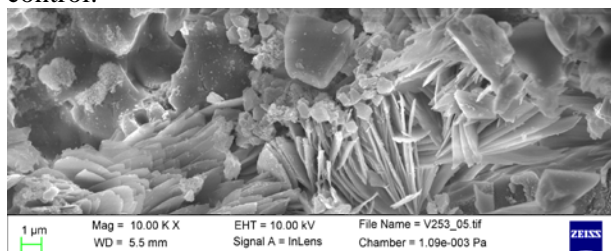


Fig. 1: SEM image of TPS surface electrochemically coated with a lamellar $\text{Ca}(\text{OH})_2$ -layer.

S.aureus ATCC[®] 35556[™] was prepared in 10 ml Luria Broth (LB) media (Invitrogen) and cultivated over night at 37°C . OD600 measurements (Jenway 6320D spectrophotometer) and dilutions were performed to provide 10^4 cfu in $20\ \mu\text{l}$ LB media. The bacteria suspension was plated on LB-agar (Invitrogen) after mixing with additional $480\ \mu\text{l}$ of LB media. After 10 minutes of soaking time, the Ti-disks or pellets were placed and incubated at 37°C overnight. The inhibition and diffusion zones around the disk/pellets were then determined along three orientations.

RESULTS: The morphology of the coating consists of fine lamellae on top of the TPS coating, see Fig. 1. Bacterial growth is inhibited in a region around the $\text{Ca}(\text{OH})_2$ pellet due to increased pH, see Fig. 2 left. Whereas unaffected colonies are found around the uncoated Ti-disks, an inhibition zone and a diffusion zone is observed around the $\text{Ca}(\text{OH})_2$ -coated disks, see Fig. 2 right and Table 1.

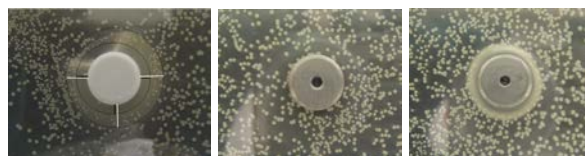


Fig. 2: Growth of *S.aureus*. a) Standardized $\text{Ca}(\text{OH})_2$ -loaded tablets are used as positive control: growth is repressed in the inner ring. A diffusion ring extends to the outer ring, b) uncoated Ti as a negative control: No impact to bacteria growth. c) $\text{Ca}(\text{OH})_2$ -coated Ti: Complete inhibition in the close proximity, reduced bacteria growth in the diffusion zone.

Table 1. Inhibition (I) and diffusion (D) zones.

	I [mm]	D [mm]
$\text{Ca}(\text{OH})_2$ pellet	3.0 ± 0.2	4.9 ± 0.2
Ti control	0 ± 0	0 ± 0
disk 1	0.7 ± 0.3	2.1 ± 0.5
disk 2	0.5 ± 0.4	1.8 ± 0.5
disk 3	0.6 ± 0.3	2.6 ± 0.6
disk 4	0.4 ± 0.2	1.0 ± 0.2

DISCUSSION & CONCLUSIONS: The antibacterial effect of the electrochemically deposited $\text{Ca}(\text{OH})_2$ -coating in LB agar without additional buffer is confirmed.

REFERENCES: ¹ J. Gallo, et al (2014) *Int J Mol Sci* 15, 13849-13880. ² O. Braissant, et al (2015) *JBMR B* 103 6: 1161-7. ³ Harrasser, et al (2016) *J Appl Biomater Funct Mater* 14 4: e441-e448.

ACKNOWLEDGEMENTS: This project with project number 120331-06 was supported by the Forschungsfonds Aargau.