Cell viability on titanium implant surfaces modified with antibacterial copper

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INTRODUCTION: Implant associated infection is a burden for patients and a cost problem for the health care system. Infection preventing measures would significantly contribute to cost reduction. We have recently developed an electrochemical method to deposit antibacterial copper on titanium implants. In the present *in vitro* study, the biocompatibility of copper (Cu) functionalized surfaces on 3 different human cell types was tested. The viability of human primary gingival fibroblasts (HFIB-G), of immortalized gingival keratinocytes (IHGK) and of an osteosarcoma cell line (SaOs-2), seeded onto Cu-doped titanium discs was investigated.

METHODS: Discs of cpTi grade 4 (∅12 mm, 2 mm) were anodized and Cu-deposited using the spark-assisted anodizing method run in a combined deposition-anodisation process using proprietary electrolyte and proprietary process parameters (KKS TioCel^{TM}) [1]. The amount of deposited Cu was determined by dissolving the Cu in 65% HNO_3 at 50 °C over night and analyzing by atom absorption spectroscopy (Perkin Elmer, AAnalyst 800). Cu amounts between 1-50 µg/disc (5-164 ng/mm^2) were obtained. HFIB-G cells were cultured in monolayer using DMEM/F12 supplemented with 10% fetal bovine serum (FBS). SaOs-2 cells were cultured in DMEM/F12 supplemented with 10% FBS and 2 mM L-glutamine, while IHGK were expanded in EpiLife medium complete of growth supplements. Dried, ethanol and UV sterilized, discs at 6 different Cu concentrations were transferred into pre-coated poly-(2-hydroxyethyl methacrylate) 12-well plates and seeded at the following cell concentrations: 5'000 cells/cm^2 (HFIB-G) and 10'000 cells/cm^2 (SaOs-2 and IHGK). Samples (n=4) were cultured for 3 days. Cell viability was determined using the WST-1 assay. IC_{20} and IC_{50} were calculated with a four-parameter fit using the software GraphPad Prism® (confidence interval 95%).

RESULTS: Cu deposits are homogeneously distributed over the disc surface (Fig. 1). Inhibition curves and IC_{20} and IC_{50} values resulting from the viability tests are shown in Fig. 2 and Tab. 1, respectively. Fibroblasts are more sensible to Cu, showing an IC_{20} of 7.4 µg/disc, while the keratinocytes are the more resistant cells (IC_{20}: 8.6 µg/disc).

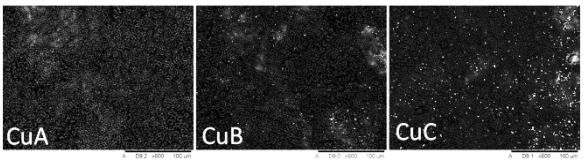

Fig. 1: Scanning electron microscope images of titanium discs with Cu deposits (white spots); CuA: 1.6 ± 0.5 µg/disc; CuB: 18.7 ± 3.1 µg/disc, CuC: 49.7 ± 5.5 µg/disc (x600; TM3000 Hitachi; 15 kV; backscattered electrons).

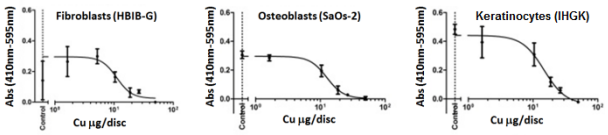

Fig. 2: Inhibition curves for determination of IC_{20} and IC_{50} for the three cell types (n=4).

Table 1. Inhibition values IC_{20} and IC_{50} for the different cell types given in µg/disc and ng/mm^2.

Cell type	µg Cu/disc		ng Cu/mm^2	
	IC_{20}	IC_{50}	IC_{20}	IC_{50}
Fibroblasts	7.4	11.1	24.4	36.7
Osteoblasts	8.3	12.5	27.5	41.5
Keratinocytes	8.6	14.8	28.5	48.8

DISCUSSION & CONCLUSIONS: The cells presented slightly different resistance against Cu (fibroblasts < osteoblasts < keratinocytes). A mean Cu amount of 8 µg/disc (27 ng/mm^2) for 80% cell viability (IC_{20}) and 13 µg/disc (43 ng/mm^2) for 50% cell viability (IC_{50}) could be used as future benchmark for implant functionalization. All Cu was released in the medium during the culture period as seen from the absence of Cu on the disc surface after the viability analysis. A tolerated Cu concentration of 2.7 µg/ml = 42.5 µM for 80% and of 4.3 µg/ml = 67.8 µM for 50% cell viability is calculated. The 50% value is in the range of the lethal Cu concentration for *Staph. aureus* (~5 µg/ml [2]) indicating a good antibacterial effect with an acceptable cell viability of 50%.

REFERENCES: [1] C. Jung (2010) *eCM*, **19** (Suppl 2):4. [2] C. Jung, N. Ryter, J. Köser, W. Hoffmann, L. Straumann, N. Balimann, F. Meier, M. de Wild, F. Schlottig, I. Martin, U. Pieles (2012) *eCM*, **23** (Suppl 1):16.