CONFOCAL LASER SCANNING MICROSCOPY (CLSM): A NEW TOOL FOR THE VISUALIZATION OF THE TISSUE IMPLANT INTERFACE.

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INTRODUCTION: Microscopic inspection of failed implants are an unique source of direct histological evidence for implant performance eventually leading to improved implant design and to treatment changes beneficial to implant success (1, 2). Investigated are either ground sections of methyl-metacrylate-embedded implants: At the cellular level by histologic analysis of the peri-implant tissue by light microscopy, or at the supramolecular level by transmission electron microscopy (TEM). Alternatively, the tissue/ implant interface is visualized by scanning electron microscopy (SEM). The two approaches exclude each other because of the invasiveness of sample preparation.
In complementation, we elaborated conditions for the non-invasive subcellular analysis of the tissue/ implant interface by confocal laser scanning microscopy (CLSM), and for subsequent analysis by SEM.

METHODS: *In vitro*: Human fibroblasts were seeded onto Ti-disks (Ø 1 cm) with machine polished (MP), sandblasted/ acid etched (SA), titanium plasma-sputtered (TPS), hydroxyl apa-tite-coated (HA) surfaces, and grown to half-confluency. Cells were permeabilized, glutaral-dehyde-fixed and labeled for F-actin by TRITC-phalloidin. *Ex vivo*: Ti-step cylinders were retrieved from patients due to loosening upon othodontic loading, Ti-step screws for infection-related fibrosis. Retrieved implants were stored in 10% formaldehyde. For labeling they were transferred to modified Hanks' buffer pH 6.5 (MHB) permeabilized and fixed essentially as above, and subsequently labeled.
CLSM: In vitro samples were viewed as mounted specimen, ex vivo specimen in buffer using water immersible objectives.
SEM: Fluorescently labeled specimen were subsequently dehydrated in graded alcohol and critical point dried for SEM. The plain
metal surface was visualized after removing adhering tissue and the gold layer by sonication /sodium hypochlorite. For details see 3, 4.

RESULTS: Least-invasive methods were elaborated for labeling the tissue-implant interface by immuno-fluorescence. CLSM in solution was used to identify subcellular structures and to document its architecture. Subsequent analysis by SEM revealed the surface of the same tissue and upon tissue removal that of the respective metal surface.

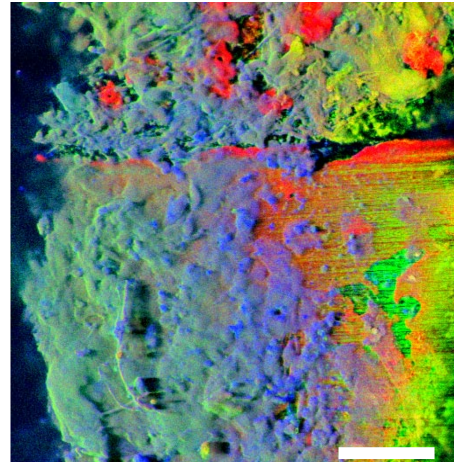

Fig. 1: Fluorescence CLSM image of a Ti-step cylinder, retrieved upon loosening due to orthodontic loading. The smooth part protruded into the oral cavity, the rough TPS-region was anchored in the palatal bone. Triple-labeled for filamentous actin (red); for fibronectin (blue) and for smooth muscle (sm)-alpha-actin (red). Overlay of composite images (75 x 1 µm optical sections). Bar: 100µm.

DISCUSSION & CONCLUSIONS: CLSM can be used for documenting the tissue/ implant interface at a subcellular level. The restriction of processing time for labeling and microscopy to 15-18h proved important to control fluorescence fading. Visualization in buffer using water immersion objective lenses was essential for subsequent analysis by SEM.

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