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Influence of UV-light and non-thermal plasma on rough titanium surfaces *in vitro*

Introduction and Purpose

Various studies described positive effects of **ultraviolet (UV) irradiation** or **non-thermal plasma (NTP) treatment** on **titanium and zirconia surfaces**. The aim of this study was to determine and compare the effects of UV-light and non-thermal plasma treatment on rough titanium surfaces regarding the changes in wettability, surface chemistry as well as cell attachment and proliferation of murine osteoblast-like cells *in vitro*.

Methods

- **Sandblasted and acid-etched titanium disks** (grade 4, Fig. 1) were divided into a non-treated control group and two experimental groups either treated by UV-light (0.05 mW/cm2 at λ = 360 nm and 2 mW/cm2 at λ = 250 nm) or by NTP of argon (24W; -0.5 mbar) for 12 minutes each
- Wettability was assessed using **dynamic contact angle measurement** (Surtens Universal, OEG, Germany)
- **X-ray** photoelectron spectroscopy (XPS) analysis was performed (Kratos Axis Nova, Kratos Analytical, UK)
- Murine osteoblast-like cells (MC3T3-E1, Sigma Aldrich, Germany) were used for *in vitro* experiments
- Cell attachment was assessed using **fluorescin diacetate / propium iodide staining** (live-dead-staining) after 2, 24 and 72 hours and cytotoxicity assay (**LDH**)
- Proliferation was determined using an **XTT assay**

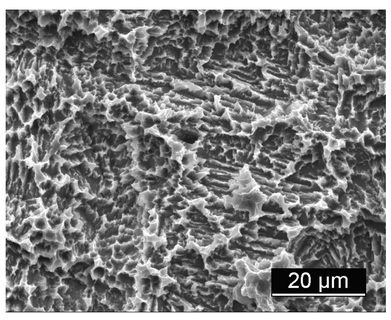

Fig. 1: Electron micrograph of a non-treated titanium disk

Results

- UV-light and NTP treatment **did not alter** the **surface structure or roughness** parameters
- UV light and NTP **significantly increased wettability** on the titanium surfaces ($P < 0.001$, Fig. 2)
- UV-light and NTP **significantly decreased carbon** remnants ($P < 0.002$, Fig. 3)
- NTP was even **more effective** in carbon removal than UV light ($P = 0.03$, Fig. 3)
- UV light and NTP **significantly increased cell attachment** compared to the non-treated disks ($P < 0.001$, Fig. 4)
- NTP **significantly increased cell proliferation** ($P = 0.002$, Fig. 5) compared to the non-treated as well as to the UV-treated disks
- Neither NTP nor UV-light treatment resulted in cytotoxic effects

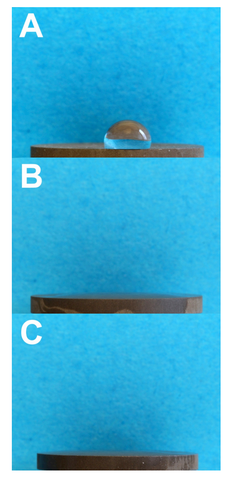

Fig. 2: Drop shape analysis
A) non-treated
B) UV-light
C) NTP

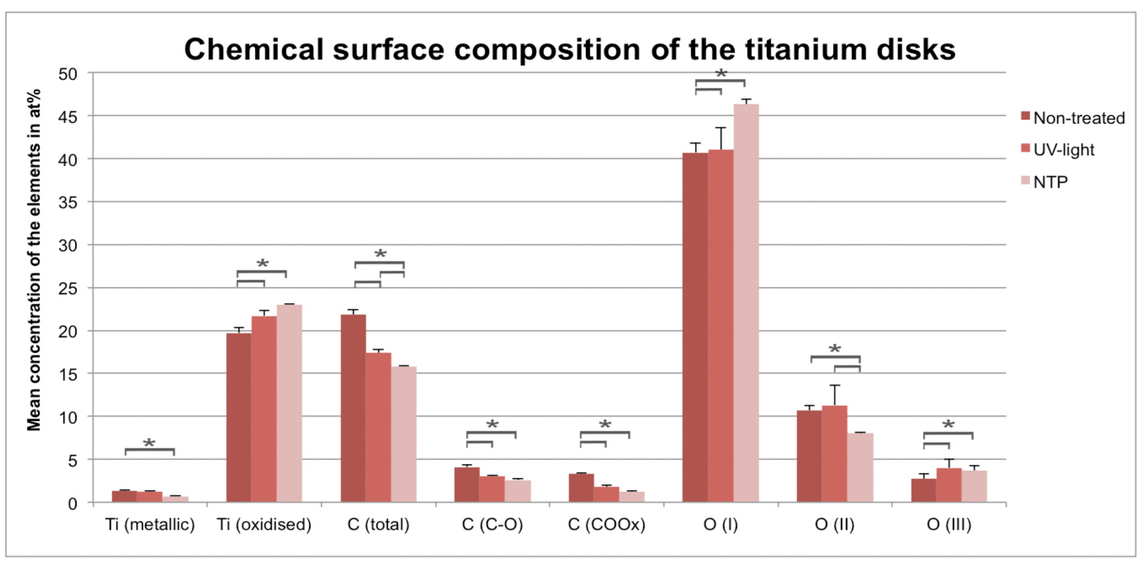

Fig. 3: Surface composition of the disks. Mean concentration of the elements in at% and standard deviation. Ti: titanium; C: carbon; C_{C-O}: carbon bound to oxygen; C_{COOX}: ester, carboxylic or carbonate groups, O_I: oxides; O_{II}: OH-groups; O_{III}: adsorbed water.
* statistically significant differences

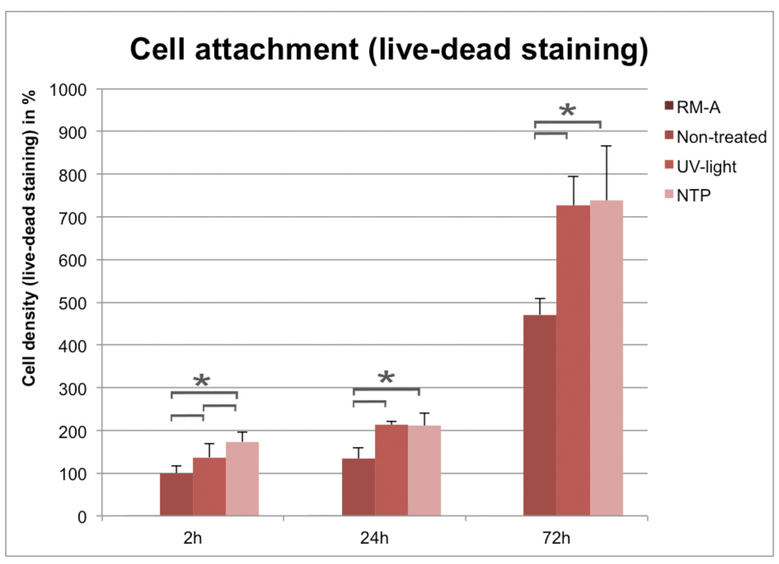

Fig. 4: Cell attachment of MC3T3-E1 cells after 2, 24 and 72 hours of incubation. * statistically significant differences

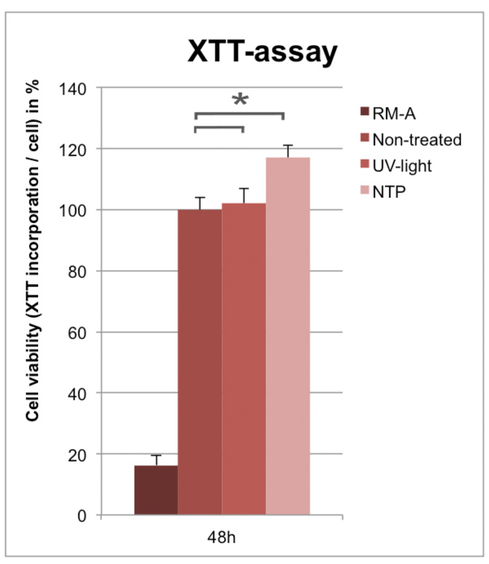

Fig. 5: Proliferation assay (XTT) after 48 hours of incubation. * statistically significant differences

Conclusions

Surface treatment by UV-light or NTP led to a **significant reduction of carbon remnants** and a significant **increase in wettability on rough titanium surfaces**. Both methods are able to **increase the bioactive capacity of titanium surfaces *in vitro*** with slight advantages for NTP in carbon removal and cell proliferation compared to UV-light. However, further studies are needed to confirm the identified effects as well as the determined advantage of NTP *in vitro* and *in vivo*.

Disclosure

This research project was granted by the Oral Reconstruction Foundation (CF11501). The UV and NTP devices were provided free of charge by the manufacturers. Titanium disks were provided by Camlog Biotechnologies AG. The authors declare no conflict of interest.

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