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Interaction of Osteoblasts with the Surface Structure of Different Oxide Layers on Titanium Substrate

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INTRODUCTION: Titanium is the material of choice for many biomedical applications. Its potential as a biomaterial and especially its bio-compatibility can be explained by its native surface oxide layer of TiO_2 with a thickness of 2-6 nm.[1] In our recent works, investigations have been carried out to determine to what extent a reinforcement or a change in the chemical composition of the native oxide layer could even improve the biocompatibility of cp-Ti.

METHODS: Oxide layers consisting of TiO_2, Nb_2O_5, SiO_2 and TiO_2-SiO_2 have been produced by the Sol-Gel process and have been deposited on mirror-like polished substrates of cp-Ti by spin-coating. The physical and chemical properties have been characterized by spectroscopic ellipsometry, by atomic force microscopy (AFM) for topography, by scanning electron microscopy (SEM) and by contact angle measurements (sessile drop method).

MC3T3-E1 osteoblasts have been used for biological tests: cell proliferation by cell counting, cell vitality with Alamar blue dye, cell morphology by SEM observations and cytochemical immune-labelling of actin and vinculin.

RESULTS: Oxide layers with a thickness of 100-130 nm had been produced. The morphology investigations revealed a nanoporous structure of the TiO_2 and Nb_2O_5 (Fig. 1) layers whereas the SiO_2 and TiO_2-SiO_2 layers appeared compact and very smooth.

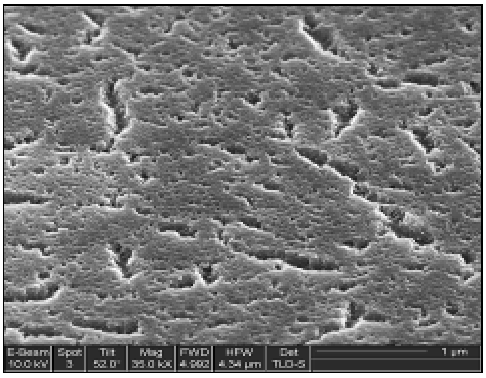

Fig. 1: SEM micrograph of the nanoporous surface of a Nb_2O_5 layer on a cp-Ti substrate with a subsequent drying and annealing at 450 °C for 1 hour.

The roughness values Ra, determined by AFM, ranged from 0.36 nm to 3.46 nm. The contact angle measurements indicate that the SiO_2 layer surfaces are very hydrophilic with contact angles of about 22° towards water whereas TiO_2, Nb_2O_5, SiO_2 and TiO_2-SiO_2 layers are more hydrophobic with contact angles between 52° and 56°.

The proliferation rates for all samples tested were higher than for the polystyrene control. The results for the TiO_2, TiO_2-SiO_2 and cp-Ti layers were about the same, the results for the Nb_2O_5 layer were slightly worse. The SiO_2 layer revealed the poorest results.

Cell vitality tests proved a good biocompatible behavior of all layers tested. For Nb_2O_5 and cp-Ti a significantly increased cell activation has been noted.

DISCUSSION & CONCLUSIONS: The SiO_2 layer showed the poorest cell proliferation and vitality results. This may be caused by its high hydrophility. The TiO_2 layer did not improve the cells response compared to the native oxide layer on cp-Ti. The TiO_2-SiO_2 layer showed a better proliferation than the Nb_2O_5 layer whereas both revealed a good vitality and cell spreading. Both layers showed about the same contact angle whereby the TiO_2-SiO_2 layer has a smooth appearance whereas the Nb_2O_5 layer appears porous and rough-textured. A special interest is given to the Nb_2O_5 layer that induced the most favourable cell spreading with normal actin stress fibres and a well developed network of focal adhesion contacts as revealed by SEM and cytochemical labelling, respectively.

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