

On the way to novel nanostructured bone replacement materials:**First cytological investigations of mesoporous solids**

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INTRODUCTION: Bone is an organic-inorganic composite material that combines high strength with special elastic properties. Materials currently in use as bone replacement materials include inorganic phosphates, ceramics, glasses, metals and polymers, none of which has the characteristics of a composite. The surface structure of an implant material can affect cell attachment, growth and differentiation. Specifically, it has recently been shown that nanostructured surfaces can influence these types of cell behaviour.

Materials for the replacement of the middle-ear ossicular chain must satisfy certain specific requirements. In particular, the audiological and vibrational properties should preferably be similar to those of bone in order to generate a similar sound transmission behaviour. The bioactivity of the material must be adjusted so that formation of a thin layer of cells on the surface of the implant is favoured. Extensive ossification must be suppressed in order to avoid fixation of the implant.

In our approach we engineer novel bone replacement materials in a biomimetic approach which is based on nanostructured solids. Synthetic organic-inorganic composite structures should possess similar sound transmission behaviour as natural bone. Nanostructure engineering will be used to control the attachment and proliferation of cells. The interactions of cells with the engineered materials are investigated in cell culture assays. The acoustic properties are tested using Laser Doppler vibrometry. As a first step, we have synthesized mesoporous silica films and have investigated the interaction of cells with these materials.

METHODS: To prepare samples for structural analysis and cell culture testing, thin films of nanostructured silica materials on standard glass slides were prepared by dip-coating. Non-ionic surfactants (octaethyleneglycolmonodocecylether, C₁₂EO₈, or amphiphilic triblock copolymers as Pluronic P-123®, EO₂₀PO₇₀EO₂₀ or F-127® EO₁₀₆PO₇₀EO₁₀₆, EO: ethylene oxide, PO:

propylene oxide) served as structure-directing agents. Together with a silica precursor, these structure-directing agents form organic-inorganic hybrid nanostructures. The organic part of the hybrid structure can then be removed by calcination to yield silica nanostructures. A control sample of amorphous silica was obtained by applying a similar synthesis procedure, but omitting the organic material.

RESULTS: X-ray diffraction shows that the as-synthesized film obtained with C₁₂EO₈ possessed a nanostructure with a characteristic repeat of ca. 7 nm, decreasing to ca. 5.2 nm after calcination. The corresponding values for the films obtained with Pluronic P-123 were 8 nm before and 5.7 nm after calcination. In case of Pluronic F-127 the corresponding values were 8 nm before and 7 nm after calcination.

First results in cell culture assays using the C3H10T1/2 human mesenchymal precursor cell line indicated a reduced cell adhesion on the as-synthesized composite materials. However, after removal of the organic matrix by calcination, highly efficient cell adhesion was observed that was indistinguishable from cell adhesion to standard cell culture plastic material. The same results were obtained when using the human kidney carcinoma cell line 293. After initial attachment, the cells showed continued adherent cell proliferation until confluency was reached. The results were similar for the unstructured sample, i.e., the nanostructures did not influence the cell behaviour. In conclusion, the mesoporous materials exhibited an excellent in vitro biocompatibility after calcination.

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