

INFLUENCE OF MICROSTRUCTURAL CHANGES OF TITANIA CELL CARRIERS ON HEPATOCYTE PERFORMANCE

S. Buchloh¹, B. Stieger², J. Mayer¹

¹ETH Zürich, *Inst. for Biocompatible Biomaterials Science and Engineering*, Schlieren, Switzerland

²Div. of Clin. Pharmacol. and Toxicol., Dep. of Medicine, University Hospital, Zürich, Switzerland

INTRODUCTION: For an Extracorporeal Liver Support Device a suitable cell carrier material has to be found. Titanium dioxide ceramics, a biocompatible material, have been demonstrated to be useful as porous cell substrate materials whose properties serve to enhance cell vitality¹. While other cell types are sensitive to subtle differences in surface roughness and surface chemistry² this has not been investigated for hepatocytes. On collagen foams of pore size ranges around 80 µm, hepatocytes exhibited higher albumin secretory activity than on foams in the 18 µm range³. In this work the influence of the porosity of a Titania cell carrier material on hepatocyte performance was characterized. Hepatocyte cell number, vitality and activity were determined by protein mass, MTT and NR assays.

METHODS: Rutile powders with different grain size distribution have been produced by thermal treatment of a Titaniumdioxid powder (Kronos 1171) and subsequent sieving. These powders were mixed with an organic Binder (PVA 22000, Fluka), uniaxially pressed to disks of 20 mm diameter. After sintering the greenbodies up to 1650°C the final porous disks were grinded to their end shape of approximately 15 x 1 mm and polished. Samples were cleaned and steam sterilized at 121°C, 2 bar for 30 min.

Ceramic samples, polished titania single crystals and as positive control poly-vinyl-chlorite (PVC) plates were placed in 24 well plates and seeded with freshly isolated rat hepatocytes⁴, using 1 ml Williams Medium E per well, with a cell density of $1 \cdot 10^6$ cells/ml. For control the tissue culture plates poly-styrene (TCPS) was used uncoated and collagen coated (CC TCPS). Samples were incubated at 37 °C for 5 days in a humidified atmosphere with 5 % CO₂. Medium (0.5 ml) was exchanged first after 3 h and then every 24 h. After 5 d, protein mass, MTT (at 560 nm) and NR (at 540 nm) were measured.

RESULTS: Measured cell mass for all flat and dense surfaces (Titania ceramic, Titania single crystal, TCPS and PVC) show less total protein than the porous ceramics (Fig. 2). Comparing the dense ceramic (0 µm) and the ceramic with pores around 200 µm, protein mass is doubled on the

porous material. In SEM (Fig. 1) it can be seen that the hepatocytes form large agglomerates on, respectively in the pores.

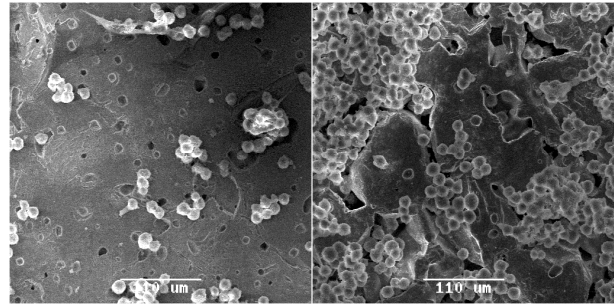


Fig. 1: SEMicrograph of hepatocytes on Titania ceramics after 5 d. Right: Dense ceramic (0 µm). Left: Porous ceramic (pore size ≈ 200 µm)

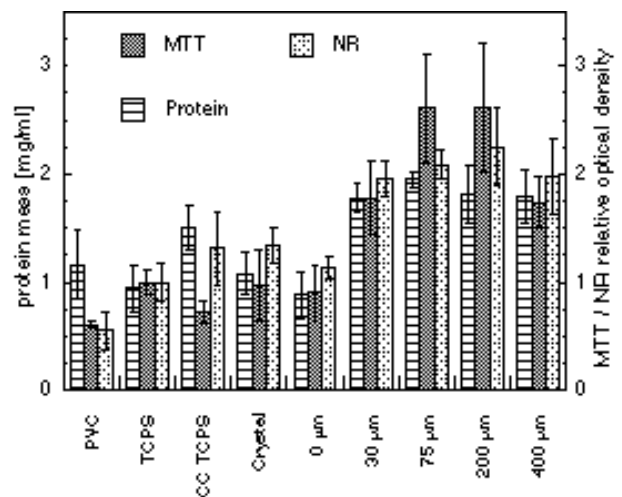


Fig. 2: Hepatocytes on Titania ceramic after 5 days. Numbers shown give average pore size. (n=6)

DISCUSSION & CONCLUSIONS: For the investigated range of porosities an influence of the pore size on hepatocyte attachment and performance can be found. Possible reasons for this different performance on porous materials of the same composition can be the induced agglomeration as described by Ranucci³, or a better diffusion of media to the cells. A third possibility is, that the higher available surface leads to higher attachment of hepatocytes.

Hence a possible hepatocyte cell carrier has to be structurally optimized to enhance hepatocytes performance.

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