

Long-Term 3D-Culture of HEP G2 Cell Line on Macroporous Ceramic Carriers

Oscar Platas, Vivien Lutz, Richard Getto, Ralf Pörtner¹
Hamburg University of Technology, Institute of Bioprocess and Biosystems Engineering

Introduction

The fact that human hepatocellular carcinoma cell lines like Hep-G2 secrete the major plasma proteins and present hepatocytes surface antigens, has directed the attention towards these cell lines for research purposes. They have a potential to be used as screening systems, where cultures of these cells are put under the influence of different substances to quantify toxic and other effects of new drugs, cosmetics, food additives, chemicals etc. It is recognized that cells growing three-dimensional embedded in their cell-typical extracellular matrix behave far more similar to natural tissues compared to cells in monolayers. In vitro tests with 3D cell-matrix-complexes ought to be the better models to replace animal tests. For cultivation in a tissue like structure a suitable macroporous carrier is required. Sponceram[®], a doped ceramic material, developed by Zellwerk GmbH, Germany, has outstanding characteristics with respect to adherent growing cells (tested successfully for more than 100 cell lines, among them CHO, CHO DHFR-, BHK, HEK, COS, many tumor cell lines, hybridomas, hepatocytes, fibroblasts, chondrocytes, keratinocytes, osteoblasts, adult and embryonic stem cells). Here Sponceram[®] was used either as cubic particle in a fixed bed reactor or as thin porous discs in a rotating bed bioreactor (these data kindly provided by Zellwerk). In both cases long-term, protein-free cultures of Hep-G2 cells were performed and evaluated.

Bioreactor systems and carriers

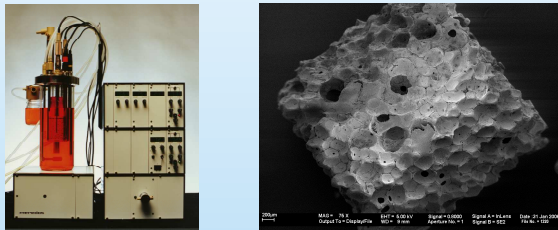


Fig. 1: Bioreactor system and carrier for fixed-bed culture
Left: Fixed bed reactor (medorex, Germany), fixed bed volume 90 mL, fixed bed with axial-flow integrated in the conditioning vessel (1 L)
Right: Sponceram[®]-particle (size 3x3 mm, Zellwerk)

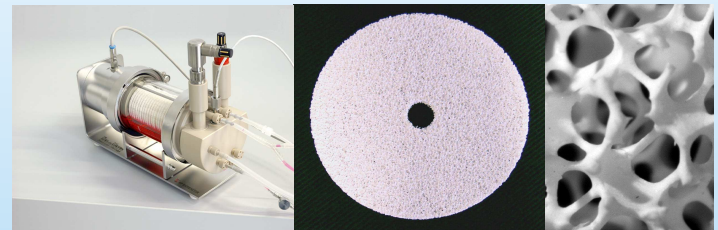


Fig. 2: Bioreactor system and carrier for rotating-bed culture
Left: Z[®] RP Bioreactor (Zellwerk), working volume 400 mL
Middle: Sponceram[®]-disc (diameter 65 mm, thickness 3.5 mm, Zellwerk)
Right: SEM of Sponceram[®]-disc

Results

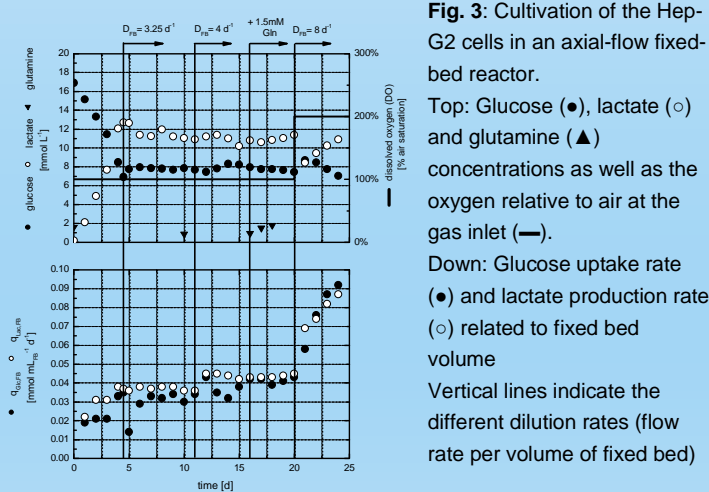


Fig. 3: Cultivation of the Hep-G2 cells in an axial-flow fixed-bed reactor.
Top: Glucose (●), lactate (○) and glutamine (▲) concentrations as well as the oxygen relative to air at the gas inlet (—).
Down: Glucose uptake rate (●) and lactate production rate (○) related to fixed bed volume
Vertical lines indicate the different dilution rates (flow rate per volume of fixed bed)

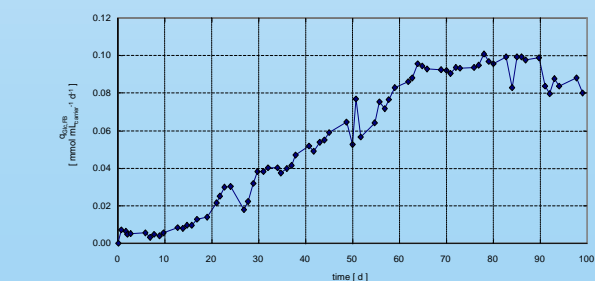


Fig. 4: Cultivation of the Hep-G2 cells in a rotating bed bioreactor. Glucose uptake rate related to total disc volume (approx. 200 mL)

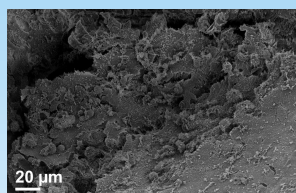


Fig. 5: SEM of 3D cell growth of Hep-G2 cells on Sponceram[®]-particles in a fixed bed bioreactor

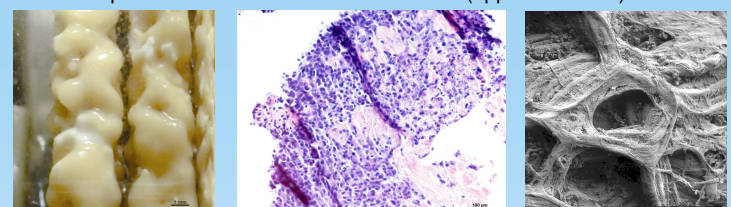


Fig. 6: Images of 3D cell growth in the rotating-bed bioreactor
Left: Hep-G2 cells embedded in ECM grown on Sponceram[®]-disc bar approx 5 mm)
Middle: Hematoxylin-Eosin stained thin-section of ECM-embedded Hep-G2
Right: SEM of self-organized ECM with embedded Hep-G2 cells (bar 200 μm)

Conclusion

In both bioreactor systems long-term cultures were successfully performed up to several months on Sponceram[®]. Final volume specific glucose uptake rates were in a similar range. SEM-images underlined the 3D-growth of cells. For the rotating bed bioreactor tissue like structures could be detected.