

## Micro-carriers for cultivation of adherent fish cells in suspension

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Outbreaks of the Koi Herpesvirus Disease, caused by the *Cyprinid herpesvirus 3* (CyHV-3, also *koi herpesvirus*, KHV) affects carp farming around the world. Efficient *in vitro* propagation of the virus is required for further research to enable the development of new vaccination concepts. However, to facilitate experiments on *in vitro* virus replication, efficient cell propagation is required. Available cells for KHV replication, e.g. common carp brain cells (CCB), are adherent and cultivated in tissue culture flasks. This cultivation system is limited regarding nutrient and oxygen supply and cultivation area. Therefore, cultivation of adherent CCB on micro-carriers (e.g. Cytodex-3, Cytopore) in suspension was examined to be able to scale up KHV expansion in a suspension bioreactor approach. The cell density was determined by 3D fluorescence microscopy of cells stained with Hoechst 33342 to observe the attachment of cells to the carriers and cell growth. Multiple parameters for cell growth were examined, e.g. different vessels (T25 culture flasks, spinner flasks) and incubation with various shaking or stirring rates, applied micro-carrier and cell concentrations, and culture media. A maximum cell density of >300 cells/Cytodex-3 (>3.5\*10<sup>6</sup> cells/mL culture) was achieved nine days after seeding in spinner flasks incubated at 25 °C with a stirring rate of 75 rpm and applied concentrations of 12,000 Cytodex-3/mL and 30

cells/Cytodex-3 at seeding. In comparison, regular cultivation of CCB in flasks for adherent cells resulted in a maximum cell density of 4.6\*10<sup>4</sup> cells/mL, indicating improved cell cultivation in suspension.

The presented work provides new insights into an alternative cultivation system for fish cells. Further examination and optimization of this system might facilitate further studies on efficient *in vitro* KHV replication.

This work was financially supported by the German Federal Ministry of Food and Agriculture (BMEL) through the Federal Office of Agriculture and Food (BLE), grant number 2815HS010.

**Keywords**—*Cyprinid herpesvirus 3, koi herpesvirus, micro-carrier, suspension cell culture*