Process development for *Galleria mellonella* derived gloverin with insect cells

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For the production of biologically active recombinant proteins the baculovirus-insect cell expression is considered to be one of the most common systems. Insect cells are eukaryotic cells and therefore highly interesting for the production of complex proteins due to their ability of posttranslational modifications such as glycosylation, fatty acid acetylation, disulfide bond formation, and phosphorylation. Baculovirus expression vectors are commonly used in combination with lepidopteran species such as *Spodoptera frugiperda* (Sf9 and its parental line Sf21). However, infection of Sf9 cells by the baculovirus leads to cell lysis and a consequent dying of the culture. Moreover, protein production in Sf9 cells is restricted by the decreasing productivity of the cells with increasing cell densities. Here, the use of stably transfected *Drosophila melanogaster* S2 cells can be a promising alternative. In contrast to the baculovirus induced protein production in Sf9 cells, the S2 cell system provides protein production at high cell densities for an extended period of time.

For the development of a production process for *Galleria mellonella* derived gloverin, various bioprocessing aspects need to be considered. In order to achieve an economically viable production of Gm-gloverin in the insect cell systems mentioned above, an efficient mass transfer is required. Mass transfer capabilities of a bioreactor system like the commonly used stirred tank reactor (STR) are thereby defined by the agitation and aeration configuration. In turn, the agitation and aeration configuration also determines shear stress to which the cells are exposed to during cultivation. The objective of this study is the examination of hydrodynamic stress responses of S2 and Sf9 insect cells with respect to growth, productivity, cell size distribution and morphology of suspended cells in STR's. Hydrodynamic stress is thereby quantified by the energy dissipation rate (EDR). Moreover, a novel in-situ microscope is implemented to support the characterization of cell responses. This is in line with the PAT (*Process Analytical Technology*) - initiative of the FDA (*Food and Drug Administration*) which demands for better understanding and control of manufacturing processes to ensure acceptable and reproducible product quality.