The development of cultivation processes can be significantly more efficient and cost effective when performed in parallel screening systems operated at small scale. Although perfusion systems at screening scale have been proposed, none of them enables a real mimic of very high cell density perfusion bioreactors. The latter is however important to maximize the performance of processes at 80, 100 x 10^6 cells/mL, or higher cell densities, in particular to optimize the nutrient supply and feeding regime. The purpose of the present work was to develop such a system and to apply it to perfusion optimization. Chinese Hamster Ovary cells (CHO) and Human Embryonic Kidney cells, HEK293, both producing biopharmaceuticals (monoclonal antibody and non-antibody), were used for this development. Although CHO cells are the established workhorses of the biopharmaceutical industry, human HEK293 cells can be advantageous compared to rodent cells for the secretion of some biopharmaceuticals. However, HEK293 cells are more sensitive than CHO cells to the culture conditions and their culture at high cell density is more challenging.

We evaluated two different stirred tank bioreactors of ≤ 250 mL with cell separation carried out either by Alternating Flow Filtration (ATF) or classical Tangential Flow Filtration (TFF). With this equipment, we developed perfusion processes for HEK293 cells and for CHO cells, stably achieving ≈ 80 to 100 x 10^6 cells/mL with very high viability. These systems were used to minimize the cell specific perfusion rate and the feeding of glucose and glutamine by a specific approach, reducing the generation of the by-products. The influence of the feeding regimes on the glycosylation patterns of the recombinant proteins was investigated. The effect of shear stress generated by the ATF and by the TFF was studied from a theoretical point of view indicating that ATF generates a lower shear stress, independently from the effect of the pump used for the recirculation in the TFF loop. In the experimental study supporting this theoretical result, the effect of shear stress on the cells was investigated by transcriptomics analysis.