# A MODULAR APPROACH FOR EFFICIENT PRODUCTION OF MULTI-HA INFLUENZA VLP-BASED VACCINES

António Roldão, iBET, Instituto de Biologia Experimental e Tecnológica ; Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal
aroldao@itqb.unl.pt
Daniela Sequeira, iBET, Instituto de Biologia Experimental e Tecnológica ; Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal
Ricardo Correia, iBET, Instituto de Biologia Experimental e Tecnológica, Portugal
Manuel JT Carrondo, iBET, Instituto de Biologia Experimental e Tecnológica ; Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal
Ana P Teixeira, iBET, Instituto de Biologia Experimental e Tecnológica ; Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal
Paula M Alves, iBET, Instituto de Biologia Experimental e Tecnológica ; Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal

Key words: Virus-like particles; baculovirus-expression vector system; universal influenza vaccine; stable insect cell line.

Safer and broadly protective vaccines are needed to cope with the continuous evolution of circulating influenza virus strains. Promising approaches based on the expression of multiple hemagglutinins (HA) (alone or in combination with neuraminidase and *matrix M1* proteins), in a single vector or virus-like particle (VLP) have been proposed. However, expression of multiple genes in the same vector can be an issue due to tandem repetition of promoter sequences leading to its instability. By combining stable with transient expression we can rationally distribute the number of genes to be expressed by each system and thus mitigate this risk. Therefore, we developed a modular system using stable and baculovirus-mediated expression of HA in insect High Five cells for production of multi-HA influenza enveloped VLPs.

First, a stable pool of High Five cells expressing two HA was established by random integration and intracellular HA expression confirmed by immunofluorescence microscopy. This cell pool was then infected at CCI of 2 or 3×106 cells/mL with M1-encoding baculovirus to evaluate the incorporation of stable expressed HA in the M1 core, thus generating Influenza VLPs. Similar levels of Influenza VLPs could be detected in culture medium by hemagglutination assay regardless of the CCI used. Aiming to increase HA production, infections at a higher CCI were attempted by implementing a feeding strategy designed based on the exhaustion of key nutrients, analyzed by 1H-NMR spectroscopy. Noteworthy, the shake flask cultures that were supplemented and infected at a CCI of 4×106 cells/mL showed a 8-fold increase in HA levels when compared to above tested conditions. The robustness of our modular system was then challenged by infecting the stable High Five cell pool with a baculovirus encoding M1 plus three HA proteins. Results obtained at CCI of 4×106 cells/mL with supplementation showed a 4-fold increase in HA levels when compared to standard infection conditions (CCI of 2 and 3×106 cells/mL). Finally, to demonstrate the scalability of the strategy herein designed, cultures in fully controlled 2L stirred tank bioreactors were performed, and a 1.5-fold improvement in HA levels was obtained when compared to shake flask cultures.

Overall, this work demonstrates the suitability of combining a stable insect cell line with baculovirus-mediated expression as a faster platform for production of multi-HA Influenza VLPs surpassing standard methods such as coinfections or the use of larger, unstable vectors.

Acknowledgements
This work was supported by EU-funded project EDUFLUVAC (FP7-HEALTH-2013-INNOVATION).