OVERCOMING DOWNSTREAM BOTTLENECKS IN CELL & GENE THERAPY MANUFACTURING

SPOTLIGHT

INTERVIEW

Advances in the development of bioprocesses for vaccines and viral vectors for gene therapy

Manuel Carrondo has been a Professor of Chemical and Biochemical Engineering at the University Nova de Lisboa for over 20 years. His key areas of research, from which over 230 papers have been published, include animal cell technology and its application for human and animal health; therapeutic and diagnostic recombinant proteins, including fusion proteins; virus like particles and pseudo viruses as vaccination agents and viruses as deliverables for gene therapy. Carrondo's expertise has been tapped by advisory board roles at a number of companies and institutes, most recently the Fraunhofer Institute for Biomedical Engineering, iNOVA4Health Portugal and the OECD working party on Biotechnology, Nanotechnology and Converging Technologies. Earlier in his career he founded the Portuguese Academy of Engineering as well as iBET – Institute for Experimental and Technological Biology of which he is currently vice president. He has served as visiting professor at Carnegie- Mellon University and MIT, among others. Manuel Carrondo graduated in Chemical Engineering from Universidade do Porto in Portugal, followed by an MSc and PhD at Imperial College London in Environmental Engineering, and finally a Habilitation in Biochemical Engineering at his present university.

Q Can you tell us about the work your group has led on developing in-process quantification methods for viral vectors and virus-like particles?

We at the Animal Cell Technology Unit of iBET integrate upstream and downstream processes with physico-mathematical tools for the development of bioprocesses for complex biopharmaceuticals, in particular vaccines and viral vectors for gene therapy applications.

Quoting Peter Drucker's maxim, "If you can't measure it, you can't manage it". From basic research on emerging viral diseases to clinical applications of viral vectors for gene therapy, accurate quantification of the product and process parameters is critical. We have been developing a wide spectrum of analytical tools for measuring virus-like particles and viral vectors. These processes include titration methods such as TCID50 and flow cytometry, RT-qPCR and ELISA, nanoparticle tracking analysis, HPLC, capillary zone electrophoresis, mass spectrometry, immunofluorescence and electron microscopy, dynamic light scattering, surface plasma resonance, tunable resistive pulse sensing, which is called q-nano very often. And then in particular for the 'universal' candidate vaccine for flu, classical methods like hemagglutination, neuraminidase and single radial immunodiffusion assays, and new methods like biolayer interferometry, often called octet, which is a high throughput set up for measuring biomolecular interactions, click chemistry and Isotope dilution mass spectrometry.

So these diverse methods are fully established, we've been putting them together over the years. This means we almost know everything that is happening, and hopefully now we can predict the future of processes we're developing. That's the key point.

Q Your group has developed mathematical models and computational tools to assess viral vector production and purification. How useful are these methods and are they easy to apply to other viruses?

We are coming from an engineering or technology background, so models for us are not gospel, but are ways to understand the reality and predict what happens next.

In terms of viral vector development, we've developed models for both upstream and downstream processes. Some of these models are deterministic, some stochastic and some of them will be hybrids and these are essentially based on mass transfer balances and thermodynamics.

The tools we have developed are moderately easy to implement for other vectors. They're very useful for the daily control and monitoring of bioprocesses, as well as for high throughput screening of process parameters, including the typical parameters like the rate of stirring, oxygen concentration and temperature. These tools not only allow for scale-up but also to establish scale down models for designing experiments to fine tune culture conditions.

The upstream part is more usual. There are many types of models that we apply here, which are I would say not intrinsically novel, the hybrid model is relatively novel in our hands but we've been developing it for 15 years. The downstream part is less common and obvious, and again we use mechanistic models to allow us to increase the efficiency of both productivity and recovery.

Models also add robustness to the process. This means that you can be sure 99% of the time that you will be operating above your requirements, so these models are very important in transferring the technologies

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to industry and minimizing the effect of uncertainty for some of those critical process parameters.

We started about 15 years ago with our own racks and platforms produced at home for continuous testing. But now companies like GE Healthcare, PALL and Novasep have ways and equipment that allow the design of a continuous chromatography purification process with a minimal set of column breakthrough experiments. More people can come into this field, now that walls have been broken by us and others.

Q How useful do you think a tagged enveloped virus-like particles could be as a strategy for downstream processing? What benefits can they offer? And are there any downsides?

Tagging doesn't impact the physicochemical properties of the virus-like particles or viruses and therefore they are excellent research tools for visualizing and controlling what's happening. Product monitoring is performed across multiple stages of production and purification and tagging allows to have a model molecule that is easy to follow and can be used to design and validate our upstream and downstream processes. Essentially, tagging is a tool for process development, model development and prediction. The disadvantage is that tagged viral virus-like particles cannot be used as a final pharmaceutical product. Eventually they could, but it's out of the question at the moment. So, unless you can remove the tag, and often you can in the final product, tagged particles cannot not be used as final pharmaceuticals.

Q Could you tell us about the new purification strategies for virus-like particles and viral vectors, particularly the development of flow through platforms for purification?

Field flow fractionation is a downstream process, developed essentially for the separation and characterization of macromolecules. It is a liquid chromatography technique where sample separation occurs in a laminar flow channel and particles are eluted in order of increasing size, and separation of the sample is rapid and gentle. Flow through purification is a very powerful tool in our hands, essentially because if you select the process parameters correctly, for example key issues like ionic strength or residence time, you can have access to a platform-like process for products that possess very small variation in their properties (i.e., different strains of viruses or virus like particles or monoclonal antibodies). Once you know the key parameters to control it, changing it slightly for a different virus or virus-like particles, should mean very similar approaches or much less experimental work. Therefore, it is again a way of intensification with less experimental work. We are now using this technique more recently for purification of cells in cell therapy, which comes from the knowledge we've gained already with virus.

Q What are the advantages of using a baculovirus insect cell system as production platform in developing a virus-like particle vaccine?

The earliest advantage considered for use of the baculovirus insect cell expression system was the fact that baculovirus are non-pathogenic to mammalian cells. This together with their inherent inability to replicate in mammalian cells make baculoviruses potentially safe candidates for therapeutic gene delivery. Another attractive feature is that baculoviruses are easy to manipulate, they can carry large and multiple DNA inserts, and can be readily produced, scaled up and purified at high titers. Insect cells are very versatile in that they are reasonably easy to culture at 27°C, in serum free media and without the need for CO2. The costs involved are therefore lower when compared to mammalian cells cultures. And finally, unlike microbial expression systems, proteins generated in insect cells can undergo post-translational modifications similar to mammalian cells, be it folding, glycosylation, phosphorylation, acetylation or acylation.

Q You gained funding for a project on the incorporation of nanobiotechnologies for downstream processing of vaccines. Can you tell us about your aims for this research?

This work is included in a European co-funded project called DiViNe, which is coordinated by iBET and partnered by Affilogic (France), Aquaporin (Denmark), Merck KGaA (Germany), GenIbet Biopharmaceuticals (Portugal) and GSK (Italy).

The project aims to purify vaccines to obtain high yields at affordable cost, with a sustainable approach of water recycling at low energy consumption. The DiViNe partners will use a new generation of affinity chromatography ligands, Nanofitins®, with capture features being at least similar to the expensive Protein A when used to purified antibodies, but whose applications could extend far beyond the sole field of antibodies. Nanofitins® are developed by Affilogic, a start-up biotech company.

The general concept of the project consists in using affinity chromatography to the highly demanding field of vaccine production. The partners will combine two major nanotechnology innovations (Nanofitin® ligands for affinity capture and Aquaporin Inside™ membranes for fluid recycling) to develop an integrated purification platform applicable to three different type of vaccines: glycoconjugates, protein antigens and viruses. All together these three types represent above three quarters of the vaccines currently being produced.

Aquaporin has very special types of membranes for purification of industrial wastes and we will implement its technology for water recycling and reusability, so that we could recover all the liquids used for fermentation and buffers in chromatography and in principle we could substantially reduce waste water in the process.

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If this system works we will be getting vaccines that will be much cheaper and allow broader access for emerging countries. It will eventually also permit vaccines to have platform approaches. Currently vaccines are very different from monoclonal antibodies in that more or less each vaccine has a specific production process; they don't really have platforms for vaccines. This could be one step in the right direction to allow vaccines to be able to be produced with a platform-like process.

The aims at this stage are quite ambitious. We are just at the end of the second year currently, things are going quite well, but the proof of the pudding is in the eating and we're still a couple of years away from that.

Q What developments are on the horizon for refining production processes for viral vectors and virus-like particles that will have the most impact on translation to the clinic?

I think some of the issues I will refer to are general. The biopharmaceutical arena, where 20 years ago monoclonal antibody were new, is enlarging in size, and we have no doubt that we'll follow process intensifications by continuous integrated biomanufacturing for both upstream and downstream bioprocesses. The use of single-use bioreactors and purification systems and application of omics technologies to allow systems level understanding are typical areas of work across the board.

Some of the work in which we've been involved is producing or designing models for the tool manufacturers, for instance Sartorius, Merck Millipore and GE Healthcare, to produce for us new purification materials targeted to virus production and purification.

As I've already mentioned, we'll need to push for intensification of processes, improvement on cell lines, and again that has already happened in other areas before. So, we'll have to try to move adherent cells to suspension, from low cell density to high cell density, develop stable and transient insect cell platforms for vaccines.

The virus-like particles themselves can be used as immunogens or scaffolds for the display of antigens, including membrane proteins that are difficult to produce, and that are needed for antibody, drug screening, and preclinical research in pharmaceutical, or eventually as nanocarriers in gene therapy applications.

This is a huge field of research at the moment, very lively, interesting and hot. We're very pleased to have started early on, to be in the position to somehow help define the trends of future work.

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