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GOING VIRAL

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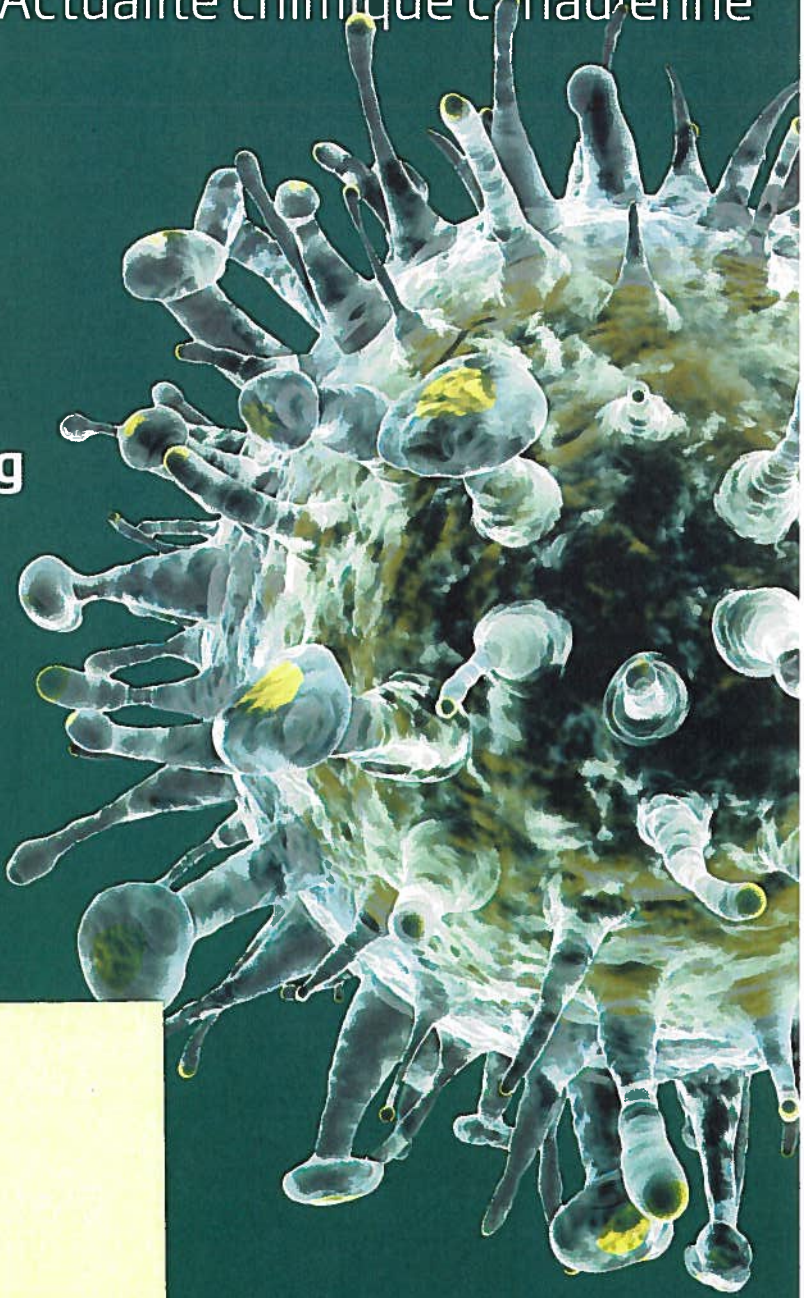

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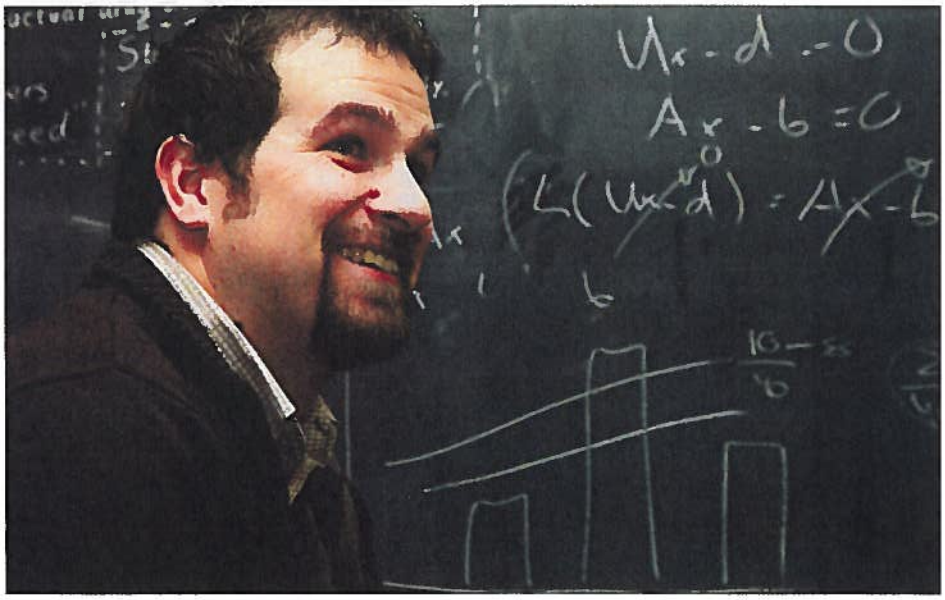
Q&A

GOING Viral

Marc Aucoin is perfecting processes for large-scale virus production. But don't worry, it's a good thing.

By **Tyler Irving**

Chemical engineering is all about optimizing production. But what if your production plant isn't a collection of pipes and reactor vessels, but rather a living cell? What if your product isn't a molecule, but a virus or virus-like particle? That's the world in which Marc Aucoin works. The professor of chemical engineering at the University of Waterloo views viruses as both tools to tinker with cellular machinery and as valuable products for vaccines or gene therapy. *ACCN* spoke with him to find out how chemical engineering applies to viral systems.



Marc Aucoin engineers the production systems of viruses and virus-like particles for vaccines and gene therapy.

ACCN Most people think of viruses as disease agents; how can they be useful?

MA Viruses have evolved over millions of years to be very effective delivery agents of genetic material. That's really how they can be useful. For example, the idea behind gene therapy is to use viruses to transfer genetic material to a host cell in order to cure a genetic defect or disease.

Another aspect of viruses that we can use to our advantage is their specificity. In a lot of cases, viruses ultimately kill cells they infect, but not all cells have the same susceptibility to infection. If a virus was found to target cancer cells, this virus could then be used as a treatment. And that's obviously very beneficial and something that we would need to produce a lot of.

From another point of view, viruses are used as vectors to introduce genes into cell lines that are grown in suspended culture. Those transformed cells would then produce whatever protein you want them to. Much of my work focuses on using viruses to transform insect cells.

ACCN Why would you want to do that?

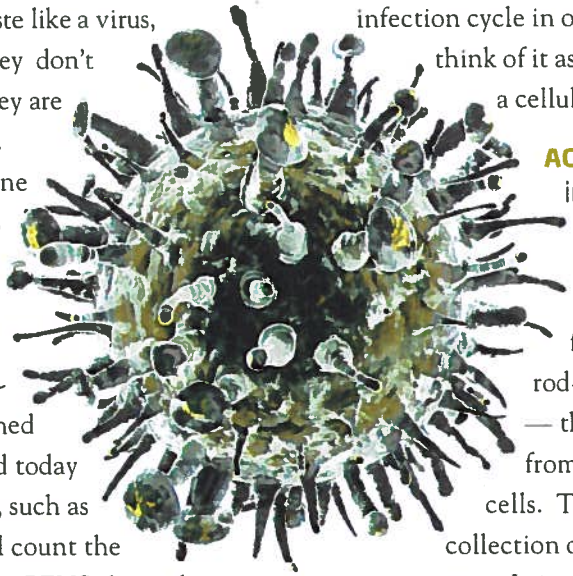
MA Historically, engineered *E. coli* cells have been the main vehicle for producing industrial quantities of recombinant proteins, such as synthetic human insulin. But bacteria are such simple organisms that they don't always have the right processing abilities to replicate human proteins exactly. For example, they can't

do post-translational modifications, like adding sugar molecules onto the outside of the proteins — something that we now know greatly influences the efficacy of the protein as a therapeutic.

Insect cells make proteins that are much more similar to what humans would produce. In addition, insect cells grow readily in suspension culture, so we can make lots of them using bioreactors. On top of that, there is a class of viruses — the baculoviruses — that readily infect only insect cells. It turns out to be relatively easy to manipulate a baculovirus to carry a gene of interest. So we have a great mechanism to get insect cells to produce as much of a specific protein as we want. This is called the baculovirus expression vector system, or BEVS.

ACCN Can you give us some examples of products made using the BEVS?

MA One common product is virus-like particles. I usually say that these particles smell, feel and taste like a virus, but they're not a virus because they don't contain any genetic information. They are used as vaccines against actual viruses.



Three years ago, GlaxoSmithKline got approval from the United States Food and Drug Administration to use the BEVS to produce Cervarix, which is a virus-like particle used as a vaccine against the human papilloma virus. This essentially opened the field for others to use BEVS and today there are other products on the way, such as influenza vaccines. But you can still count the number of commercially available, BEVS-derived products on your fingers.

If we look a bit further ahead, there is an opportunity with future research to use baculoviruses as gene therapy vectors.

ACCN You mean they can infect human cells?

MA Not exactly. They are able to enter mammalian cells, but genes can only be expressed if the promoter sequences in front of them are recognized by the host. Because baculoviruses evolved to infect insect cells, human cells don't recognize the promoter sequences within their genome, so those genes are not expressed and the virus can't replicate. But what you can do is alter the baculovirus to carry a gene of interest with a promoter that is recognized by human cells, and that's what people have started doing.

Another future possibility is that you could alter the baculovirus to express proteins found on the outside of other viruses. In that case, the baculovirus itself would elicit an immune response from humans in much the same way virus-like particles do.

ACCN What does all this have to do with chemical engineering?

MA I see the BEVS as a chemical production system, with viruses and virus-like particles as the product. As a chemical engineer, I'm interested in the overall conditions of the bioreactor, but I'm also interested in what's happening at the microscopic level. For me, that means studying how individual cells are infected and transformed by the virus. Part of my program involves trying to alter aspects of the infection cycle in order to maximize the yield. I like to think of it as trying to create an assembly line at a cellular level.

ACCN How do you alter the infection cycle?

MA One interesting thing about baculoviruses is that they have two forms. The budded form is a small, rod-shaped virus that leaves the cell — that's where the term budded comes from — and is then able to infect other cells. The occluded form is essentially a collection of multiple unbudded viruses within a protein matrix. It is quite stable in the environment. In nature, the occluded form is produced late in the cycle, as the infected insect larva is dying. It remains on the leaf to infect other insects. In cell culture, we don't need the occluded form, so the first researchers in this field essentially hijacked the promoters for the genes that make the protein matrix of the occluded form. They used those promoters to produce their gene of interest.

But again, those occluded form genes are only expressed in the very late stages of infection. Since then, a number of different promoters have been identified that are expressed at earlier stages of the infection cycle. By using these, you can actually control both the extent of

expression and the timing of when your protein products are produced.

ACCN Why is it important to be able to do that?

MA If you have multiple proteins in your virus-like particle, and they're all being produced late in the cycle, you're essentially competing for cell resources to produce them. You might get a better overall production by staggering when proteins are made, thereby allowing the cell to focus all its resources on producing proteins one at a time, as opposed to all at once.

For example, one of the systems that we're currently studying is an influenza virus-like particle, which consists of three proteins: hemagglutinin, neuraminidase, and a matrix protein. Generally, the hemagglutinin and neuraminidase end up in the cell membrane, and the matrix protein gets coated with these on its way out of the cell. We believe that if we express the hemagglutinin or the neuraminidase early, we can have the cell ready for expression of the matrix protein later on. By staggering the expression of these proteins in time, we believe we will get a better yield.

ACCN Are there other ways you can optimize yields?

MA Another focus of our lab is to study what metabolites the cells are using either when they are growing or at various stages of the infection cycle. If we understood exactly what they needed and when, we could tailor the nutrients available to the cells at different periods of time. It's a bit like sport drinks with different formulations for both before and after your activity. By gaining a better understanding of the overall system, we should be able to both improve yields and reduce costs.

Another improvement could be made in downstream processing, that is, recovering the product from the broth. This area is not necessarily treated with as much respect as it should be, but it accounts for a significant portion of the cost of any bioproduct. Typically, after you finish the culture you do a centrifugation step to get rid of the cells and recover your product in the liquid supernatant. Then you pass that through a series of chromatography columns to bind your product and separate it from any impurities.

Still, each additional step adds an additional expense to your process. One thing we're investigating is the use of membrane chromatography. The idea is that instead of pushing material through a filter or packed bed, you're flowing across a membrane. In that case, you don't necessarily have to remove the cells ahead of time, because the charged membrane can selectively pick up only the product — in this case, the virus-like particle you want.

ACCN Do people get confused about whether you're a biologist or a chemical engineer?

MA All the time. But the truth is, you cannot do one without the other. If your goal is to produce a lot of virus or virus-like particles, you can't exclude the fundamental concepts that chemical engineers know. You also need to fully appreciate what you're working with in terms of the biology. They are intimately linked, and there is a true need to understand both sides.

In my lab, I have a 50-50 mix of biologists and chemical engineers, and when you come out of my lab you are well versed in both. It's this mix that actually makes a perfect marriage.

ACCN Is there a breakthrough that would allow for significant advances in your field?

MA What would be extraordinary is if we could actually measure everything at the single cell level. There are single cell measurements we can do now, but a lot of our techniques are destructive so we can't, for example, follow a single cell through the infection cycle. If we could do that, I think we would find out a lot more.

ACCN What drives you to study this field?

MA First, viruses are cool. Second, I truly believe one of the reasons why we live so much longer these days is because of medical intervention — either through diagnosis or prevention — and vaccination is one of these interventions. But I was at a conference in South America just recently, and the problems surrounding access to therapeutics because of cost was front and centre. We sometimes forget that as Canadians we probably have the best access to medical treatments. If there's anything that we can do as chemical engineers that would help make these therapies and treatments available to as many people as possible, then I want to be involved in that work. I'm not going to create a new therapy or a new drug, but we can make a big difference in how these products are made.