Aber knowledge base

In conversation with **Dr Sven Ansorge**

Dr Aditya Bhat (VP Technology, Aber Instruments) and Dr Sven Ansorge (Director of Manufacturing, ExCellThera) had an in-depth conversation regarding capacitance technology, how it is perceived with respect to PAT, the importance of capacitance technology to be manufacturing ready and finally, how the technology can benefit the viral vector/ vaccine production processes. Dr Ansorge is an expert on capacitance measurement and on viral vector manufacturing processes. Hence, his perspective on the subject is unique and very much appreciated!
Here is an excerpt of their conversation.

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Q Can you tell us a little more about how you were first introduced to the technology, how you'd come across it, and your initial impressions in the early days?

SA: I was exposed to the technology a bit more than 15 years ago. By coincidence, one of the projects that I was able to work on was evaluating a tool for online monitoring using capacitance, or the online monitoring of cell culture processes.

What was interesting there was that I was able to, very early on, work with different expression systems, and particularly the use of capacitance in further characterizing these expression systems.

This was in 2003, so at the time we're only using glass and stainless-steel bioreactors. A totally different time. We didn't have these high throughput process development devices available yet. Through that, capacitance caught my interest.

And I was then reaching out to Amine Kamen who was later on my PhD supervisor. And they had done work in parallel, and I thought it was exciting work, where they were using capacitance to monitor insect cell cultures, Baculovirus expression systems.

I was reading through the papers; when you have Baculoviruses, and when you have insect cells, they increase in size when they get infected with the Baculoviruses. And you can really pick up interesting signals there.

I graduated with my PhD in 2010 and the project was process development for lentiviral vectors. It wasn't the right time, because at the time gene therapy and cell therapy was far away from where it is now. But already then, we were using capacitance to monitor these cultures.

Q When it comes to PAT, everyone who is running a bioreactor is measuring pH, dissolved oxygen and temperature. And these are done online, or inline. Everyone is also measuring their cells in some way. But most people are still doing this offline. Trypan blue can probably be considered the gold standard in our industry, even today.

I wonder why there hasn't been a more active adoption of not just capacitance, but other inline technologies that are out there that are equally promising?

SA: Yes. That's a very good question. As an industry, we're not exactly fast. It's not an industry which turns around quickly. So, it takes more than a decade to introduce technologies which are not, in my mind, that risky to implement. But there are reasons for that.

And one of the reasons is when you're in a process development setting and you're moving from, for example, shake flasks into bioreactors and all of sudden you add capacitance to it. You have a fed batch process based on Trypan blue counts. How do you translate that into something which all of a sudden works on capacitance? So this is one of the challenges.

The other challenge is that, for a couple of years, we didn't know enough about what's in there and what's hidden in the signal. I think we know enough now. You don't want to have a probe fail either.

Biogen have done excellent work on this. There's a paper on how can we validate these probes. What we use as a standard, to test that any probe is still working.

AB: Yes, for any relatively new technology, you've got to go through the transition of being used in the lab, understanding what the technology is actually measuring. And then once you've got the confidence in that technology, transfer it to manufacturing. And that is quite a big transition for a technology. And Aber went through that transition about 15 years ago. Where the industry really warmed up to using capacitance in manufacturing.

It's also about the offering; how reliable the system actually is. How robust is the system? Does it come with validation packages? Does it come with system check procedures? Because obviously, manufacturing environments, as they should be, are risk averse. So you've got to think about the worst case scenario, and then see whether you've got a plan for that. So it is not an overnight success when it comes to technology being used in manufacturing. But this certainly happened for Aber.

> "So, the debate around cells per millilitre and cell counts, versus capacitance is a good debate. But I think we're now at a point where we're almost beyond that debate."

Q **Capacitance technology can be defined as pre-PAT and post-PAT initiative from the FDA. Because our technology, of course, was invented back in 1988.**

The fact that the FDA was saying that your bioreactor cannot be a black box anymore, and you need to monitor your critical process parameters more strictly, especially those that contribute to the critical quality attributes of your process, really got the technology the attention that it deserved.

A lot of our customers convert capacitance, which is measured in pF/cm, to offline methods of measurement that could be cells per millilitre, optical density, dry weight, etc. But we can also increasingly see a number of our customers, such as Biogen and Corteva, being more comfortable trusting raw capacitance and they base all of their control strategies on raw capacitance.

And I just wanted to get your perspective on how capacitance used to be perceived in comparison to offline measurements, and where it is today.

SA: People are getting more comfortable, that's true, a lot more comfortable I'd have to say. Because at the beginning, obviously, you really had to convince people, first of all, is this reliable? And also, that you can measure whatever it is you're measuring, but what does that tell you? What do I do with that?

So, the debate around cells per millilitre and cell counts, versus capacitance is a good debate. But I think we're now at a point where we're almost beyond that debate.

So let me just speak about viable cell density for a second. Because we just accept that this is the gold standard on how we characterize our cell cultures. I think it makes sense from a certain perspective because it's important to know how fast my cells are doubling. So doubling time is an important parameter, and it will always be one.

Now, counting cells per millilitre, and particularly when these cells change in size, it's probably not

the best approach. In almost any process you will see at a certain point in time, you will see that your cells change in size for whatever reason. It could be nutrient limitations, too many nutrients being added, change in osmolality. In the case of viral vectors it could be changes in size, etc. You're not picking this up when you're only counting cells per millilitre. You will pick this up when you measure capacitance.

When you go down to these examples and show data on processes that you've characterized using capacitance, where you also have good and reliable cell size data, then you realize, oh, maybe it makes sense, and it's what people have realized over the last couple of years, to use the capacitance value as it comes out of the system and take it as it is.

AB: True. Having said all of that, there is still a good reason why a lot of people want to convert capacitance to cells per millilitre. Maybe their process development work has been done with offline measurements, and capacitance comes in later in the process, and so you cannot, just overnight switch your process strategies from offline-based methods to capacitance.

So there's a legitimate reason, and so many of our customers, can get a really good correlation between capacitance and viable cell density.

SA: As effective as it is early on in the culture, because it perfectly correlates in almost all cases with viable cell density, it gets complex later on because it measures much, much more than only the membrane-enclosed volume fraction or the bio-volume.

AB: That's a really good point, there are a couple of reasons why capacitance can deviate from the traditional conventional offline cell counts. Take Trypan Blue. As you mentioned correctly, capacitance is the viable bio-volume of the suspension, and not just the cell density or cell number. And there could be changes in cell size during your process which are captured in capacitance but are not captured in offline cell density.

For Trypan blue measurement, the cell is dead when the membrane is so ruptured that the stain enters the cell and colours it blue. Trypan blue is a relatively large molecule. So the membrane needs to be significantly ruptured.

For capacitance measurement, a cell is dead when it cannot hold charge around itself, and for that to happen, the membrane doesn't need to be all that ruptured. And this was proved by the likes of Professor Mike Butler, when he was at the University of Manitoba, where they compared different forms of cell density measurement, and discovered that capacitance was actually more sensitive to the onset of apoptosis as compared to Trypan blue.

So, the hypothesis was that using capacitance could help you reverse apoptosis. And Biogen, published a paper where they empirically proved this. They induced apoptosis in culture, and they measured it using capacitance and Trypan blue. And they could, using real time capacitance information, reverse apoptosis, but could not do that with Trypan blue measurements, because it only corresponded with mid to late apoptotic stages.

SA: Let me build on that. Because I think you brought forward a very interesting example, which is the reversal of apoptosis through online monitoring. There's another example which is in my mind which is also from Biogen. An Zhang, and his group, have really done excellent work. The technology is used not only in process development, but also in GMP manufacturing. And in GMP manufacturing we see and demonstrate the value of it.

So, the second example that I wanted to bring forward is the control of feed additions that they are triggering through capacitance, and they are

showing that this leads to a better outcome than when you do it through measuring viable cell density or through other approaches.

We can use these signals for process control. And we should, because it's a really good PAT tool.

I think we're at a tipping point when it comes to implementation of PAT's, and PAT technologies. There's capacitance, which, for me personally, this is a must-do. The limitation that I see, though, is that we do not have the ability yet to measure capacitance early on in process development.

AB: That is a very relevant point. The way our industry is evolving, bio-reactors are getting smaller, high throughput screening is a very useful thing to do. So, it is really crucial for a technology like capacitance to also be available in smaller volumes.

A couple of years ago, we launched the Futura Pico which is by far the smallest diameter capacitance probe on the market. It's a 7.5 millimetre diameter probe. And that was initially designed for the smaller bioreactors like the Applikon Mini's for instance. But we now have empirical evidence that it also works in much smaller platforms like shake flasks and even in centrifuge tubes in some cases.

But I don't think that this is the end goal. You mentioned high throughput screening earlier as well. And certainly the effort would be to make our technology accessible on these platforms. That's certainly where things are going.

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Q Could you summarize the work that you have done? You mentioned viral vectors earlier and I am sure everyone would love to hear a little more about that.

SA: Sure. I mentioned earlier how I got in touch initially with the technology. Working with different expression systems. There was work that we did on insect cell cultures, and with Baculovirus systems. What you have is quite a dramatic increase in cell size when these get infected. This makes for really nice signals, because at a certain level of infection, your cell density will level off but capacitance will continue to increase because of that increase in cell size.

We had also applied capacitance to CHO cell cultures, where we were working with a system where we saw really nice changes in the signals when one specific nutrient became limiting. And then also when we added feed, we saw responses in the signal to these additions.

Most of the work that was done after that, other than the CHO work, which is still continuing with the 293 cell line, where we're mainly looking at lentiviral vectors, the process development and production of, for example, a live influenza virus. So, these are systems where you pick up changes more at the membrane level. That's capacitance per membrane area, which we think is at play. When the virus gets out of the cell and the particular viruses that are enveloped, such as the coronavirus. Capacitance should also be a tool that can be used there for that reason. Because when you look at enveloped viruses, they bud out of the cell membrane. We see this in certain systems, in particular, antiviral vectors, and also to a certain extent, influenza.

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But regardless, in all of these processes, what they have in common is that you get very characteristic patterns from your capacitance signal. And you can relate them back to changes in cell size, or changes at the membrane level when the virus gets out.

So this should not only provide you with good information early in process development and you want characterize your process, but also, for example, when you want to look at or confirm that one batch is behaving such as the previous batch, for example.

AB: Like a fingerprint.

SA: Yes. Batch variability, reproducibility, etc. These are the main areas that we covered. Now, another area where we used capacitance was also in the production of viruses, but a different type of virus. And I categorize them as the non-enveloped viruses. So these could be gene therapy vectors such as AAV. They could also be Adenoviruses, which are still used as gene therapy vectors, but also as vaccines, and also on Oncolytic viruses.

So, in all these cases, these viruses, at certain stages in the process lead to cell death. And as you explained really nicely early on, since capacitance picks that up when a cell is really dead, and when the cells basically explode because the virus gets out of the cell, you get very nice correlations of the capacitance signal, with viability or cell death.

In most of these cases or processes, the harvest criterion is related back to viability. And initially, this was done by Trypan blue. But we have good data showing that when you determine when to harvest through an online tool such as capacitance, then this is a clear advantage.

AB: So, am I right in thinking that, when the viruses are released from the cell, and they cause cell lysis, there is a narrow window where you should be harvesting those viruses? Otherwise it impacts viral titre negatively?

SA: Yeah. So it might impact viral titre and might also impact downstream processing, this might make a major difference.

When you have systems which undergo cell lysis, the longer you wait, typically, the more difficult it will be to purify the virus out.

Q You mentioned a little bit about vaccine production. Of course, this is something that everyone in the world is talking about right now. Could you talk specifically about the COVID-19 situation, and the vaccines that are being developed by multiple groups, across the world. Where do you think capacitance measurement can benefit this specific process?

SA: When you look at Coronavirus, and obviously there are several groups now and companies working on manufacturing a vaccine and they have different approaches. But approaches which use vaccines that are produced, or that will resemble the virus in a way, this could be a virus-like particle, it could be an inactivated vaccine; you use cell culture to produce these vaccines, chances are high that there will be at least one candidate which will manufacture it in that way. Then, I think one should look at capacitance as one tool to speed up process development. In particular, to look at the interface of upstream and downstream processing. To determine when to harvest your bioreactor more easily than through offline sampling.

This is also something that I wanted to bring to the discussion. There are different concepts that are out there. One concept -- and this is what's mostly used now in industry -- offline sampling and having several equipments connected to the bioreactor. Some of them directly connected to the bioreactor, which is something that, you know, when you think about GMP manufacturing, probably people will not like too much, because you have plenty of sterile loops coming out of your bioreactor going directly to analyze this.

And the other approach which I've always been in favour of, which is using probes that will provide you with a signal from inside your bioreactor so you don't even have to take a sample out of the bioreactor. Now, we will never get away from sampling. But the more information we can collect directly through in-situ probes, the better. I think [capacitance] can be a good tool to use in vaccine manufacturing and vaccine process development.

Q Regarding offline sampling, I agree that it's very difficult to completely eliminate offline sampling. But the question that needs to be asked is, if you were to take an offline cell density measurement sample, you take it once every 24 hours, what can you do with that information? And what can you do with cell density or viable bio-volume information that's taken once every four seconds using a capacitance tool?

I think this is one of the first points that you mentioned during our conversation. The question is, what does capacitance measure and what can you get out of the measurement? And I think the real difference truly is not just that it's measuring live cells and it's measuring the viable bio-volume, but also that it's real-time measurement from inside the bioreactor, a more realistic representation.

SA: You're mentioning real time monitoring and the value of having information available rapidly. One thing that has changed over the years, it took us weeks to analyze a single run at the time.

Now, when we talk about combining capacitance with other online monitoring and real time monitoring techniques, typically, when we want to use these in combination for process control, I think there lies a great opportunity. Because if we bring in data analysis, we have better tools now available to us to analyze huge data sets.

There, I think we can still make good progress in that field.

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