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APPLICATION NOTE REVIEW:

THE APPLICATION OF RADIO-FREQUENCY IMPEDANCE (RFI) IN CELL CULTURE

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The application of Radio-Frequency Impedance (RFI) in cell culture - a review

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NEED FOR THE ABER RFI MEASUREMENT IN CELL CULTURE

There is an increased focus on in-line techniques for bioprocess monitoring driven primarily by the initiative of the FDA regarding "Process Analytical Technology" (PAT) www.fda.gov/cder/OPS/PAT.htm. Traditionally, parameters such as pH, dissolved oxygen and temperature are routinely monitored in a bioreactor. However, to gain better process knowledge and control, it is important to measure biological or biochemical parameters. One of the most important, yet also the most challenging components to monitor on-line and real time is biomass, a critical process parameter that significantly impacts the critical quality attributes of the process/product. Most of the cGMP processes at the production scale using mammalian cells lack appropriate probes to evaluate biomass content in the bioreactor on-line, in real time, non-invasively and reliably.

Of the available on-line biomass assays, the radio-frequency impedance (RFI) method has a clear advantage for process development and manufacturing because it is an unambiguous reflection of viable cell biovolume rather than the total number of cells (Carvell & Dowd, 2006). The viable cell concentration is of prime importance in metabolic studies and those relating to the efficiency of target protein production.

In this series of cases studies, we show a range of applications in cell culture, where RF impedance has not only been used to monitor and control cell concentration, but also control critical events during the process. The examples are taken from a wide range of papers and posters, and some unpublished data between 2003 and 2017.

THEORY OF RFI AND THE β-DISPERSION

Before illustrating the applications, it is useful to understand the principle behind RFI. For modelling purposes, a suspension of cells can be thought to be composed of three separate parts: the cytoplasm, the outer plasma membrane and the suspension medium. The cytoplasm is a highly complicated and structured mixture of salts, proteins, nucleic acids and smaller molecules. In addition, in eukaryotes various internal, membrane-bound structures are also present which can affect a cell's dielectric properties. Surrounding the cell's conducting core is a non-conducting plasma membrane. The suspension medium is

generally aqueous and ionic. Thus, electrically speaking, a cell suspension can be considered as a suspension of spherical capacitors, each containing a conducting matrix (cytoplasm) and surrounded by a conducting suspension medium.

When an infinitesimal alternating electric field is applied to a suspension of cells in an aqueous ionic solution, the ions in that solution are induced to move. The positively charged ions travel in the direction of the field, whilst the negatively charged ones travel in the opposite direction (Figure 1a). The ions both inside and outside the cells can only move so far, before they encounter the intact plasma membranes, which act as an insulating physical barrier, thus preventing further ion movement. This results in the development of a charge separation or polarisation at the poles of the cells (see figure 1a). The magnitude of the suspension's field-induced separations is measured by its capacitance (C) in Farads (F). However, as a Farad is a very large capacitance, the capacitance for cell suspensions is expressed in pico-Farads (pF). Thus by measuring the capacitance of the suspension at one or more appropriate frequencies, its biomass can be estimated because, as the volume fraction of the cells increases, it results in more polarized membranes and hence, a higher measured capacitance. Dead cells and non-biomass solids do not possess intact plasma membranes and therefore, are not polarised. This means that they do not contribute significantly to the capacitance of the cell suspension.

The frequency, or the rate of change of the direction of the alternating electric field per second, is of significance, and is measured in Hertz (Hz). The greater the rate of change, the higher the frequency. Frequency has a marked effect upon the capacitance of a cell suspension as the ions moving up to, and polarizing the plasma membranes, take a finite time to reach them and cause the polarizations.

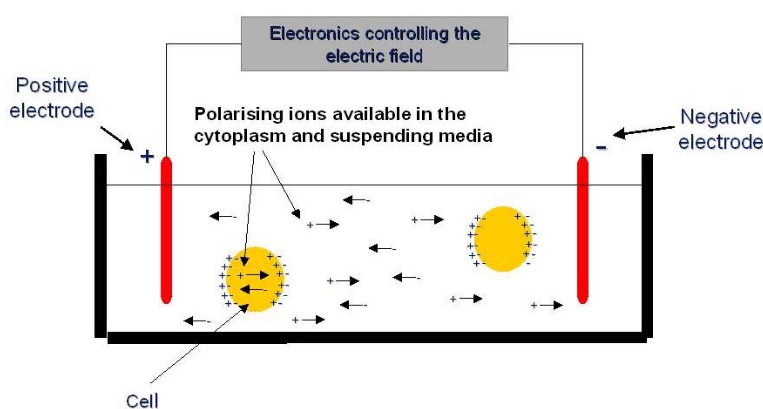


Figure 1. (a) Principle of radio-frequency impedance. When the electric field is applied to a suspension of the cells in an aqueous ionic solution, the positive ions are pushed in the direction of the field and the negative ions in the counter direction. The ions can only move so far until they encounter the cells' plasma membranes, which prevent further movement. This results in a charge separation or polarization at the poles of the cells.

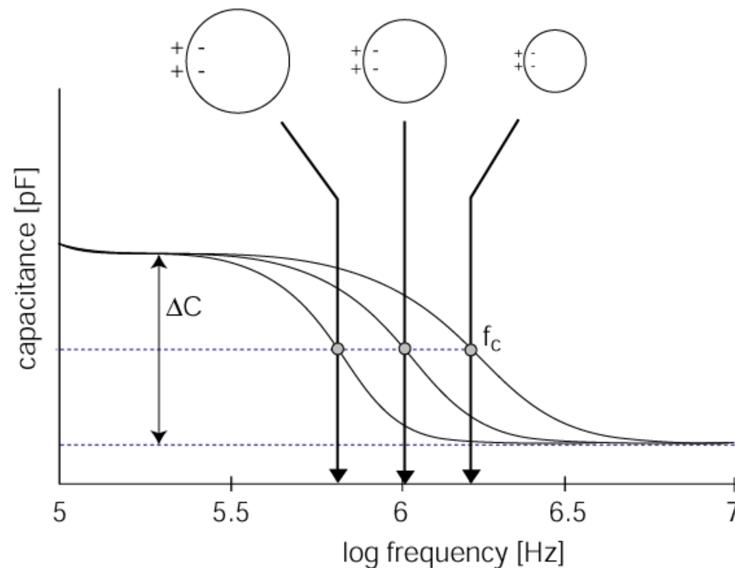


Figure 1(b) The capacitance of a cell suspension as a function of frequency shown along with the equivalent polarizations of the cell. At approximately 10^5 Hz many ions have time to reach the cells' plasma membrane before the electric filed is reversed. At very high frequencies typically over 10^7 Hz, few ions have time to polarize the cell membranes before the field is reversed, this results in a negligible contribution towards the capacitance. When the fall in capacitance is half completed, we have the characteristic (critical) frequency (fc).

Figure 1b illustrates the polarizations induced across the cells within a suspension as the frequency of the electric field is increased. At low frequencies, below approximately 0.1 MHz , many ions have time to reach the cells' plasma membranes before the field is reversed, driving the ions in the opposite direction. In this case, the induced polarizations are large, and hence the capacitance of the cell suspension is high. As the frequency is increased over 1 MHz , fewer ions have time to reach the plasma membranes before the field is reversed and, therefore, the extent of the polarization is less, and hence the capacitance of the suspension is lower. At very high frequencies, typically 10 MHz and above, even fewer ions have time to polarize the membranes and so the resulting membrane polarization is small, giving a negligible contribution to the overall measured capacitance. What remains is a background capacitance due largely to the dipoles of the water in the suspending medium.

From Figure 1b one can also see that, as the frequency is increased, the capacitance of the suspension falls from a high low-frequency capacitance plateau (maximal cell polarization) to a low high-frequency plateau (minimal cell polarization). This fall in capacitance, due to the loss of induced charging of the cells' plasma membranes as frequency is increased, is called β-dispersion, and for most cells it is centred between 0.5 and 3 MHz . When performing RF impedance measurements for cell culture, it is typical to measure capacitance at one (rarely two) frequency, and plot this capacitance over time to get the growth profile of the culture in real time.

PROBE OFFERING AND DEVELOPMENTS

Up until 2017, side entry probes in diameters of 25 mm (to fit standard Ingold type housings) and top entry probes of 12 mm, in lengths of up to 600 mm were available (Figure 2). In September 2017, a 7.5 mm Pico probe became available with an innovative design for dealing with restrictive space within small bioreactors. At the time of writing this case study, the 7.5 mm probe is the smallest diameter reusable capacitance sensor available in the market. All of the probe offerings are designed to endure multiple autoclave or SIP cycles. The 25 mm probe design is available in flush and annular designs. The annular ring design has been shown to be more sensitive in applications with highly aerated fermentations, but the main advantage in cell culture is that this ring design can be used with the 12 mm diameter probes commonly used on small bioreactors. In addition, single use options are available with both a disc style and insertion style probes that can be gamma irradiated or ebeamed.

Figure 2 Futura system overview

APPLICATIONS - RFI CASE STUDIES IN CELL CULTURE

The following section describes the use of Aber RFI for different applications in cell culture.

MONITORING CHO CELL CULTURE PROCESSES

CHO cells are the most common mammalian cell line used for mass production of therapeutic proteins. RFI probes are commonly used to both monitor and control these processes.

A study was carried out by Millipore to assess the performance of both the multiuse annular probe and the disc style probe with flush platinum electrodes in a 3L rigid plastic bioreactor (Baggio *et al*, 2010). The disposable probe was connected to a light weight "Mini-Remote Futura" pre-amplifier and the bio-capacitance was converted into cells/ml by using a simple linear correlation factor. The calculated cell concentrations were compared with off-line measurements from the Vi-cell (Beckman Coulter, USA).

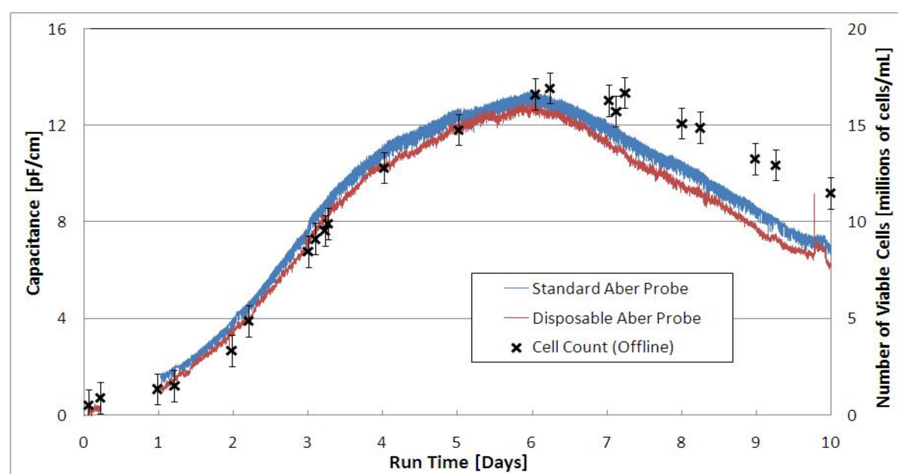


Figure 3 Comparison of live biomass using on-line reusable and disposable probes and off-line viable cell concentration measurements

The results shown in Fig 3 show that the capacitance measurements from both probes trended very closely to one another and to the off-line measurements up to the peak cell concentration on day 6. In the death phase there was a small divergence between the on-line and off-line data. This divergence has been studied in more detail by a number of groups (Braasch *et al*, 2013, Lee *et al,* 2014) and the reasons for the differences in the death phase are now understood and can relate to changes in bio-volume or the bio-capacitance method picking up changes due to apoptosis at a much earlier phase than the trypan blue method. When looking at the online capacitance and offline measurements closely, there could be a strong argument that the RFI measurement provides a signal that is more closely related to the metabolic activity of the cells (Braasch *et al* , 2013).

The Mini-Remote Futura electronics have since been branded under the Sartorius Stedim Biotech (SSB) BioPAT ViaMass trade name. After design, manufacturability, and material sourcing amendments, SSB now

produces the SU biomass sensor disc under license (Figure 4) The company integrates this sensor into its SU Flexsafe bioreactors and markets these products in a dual-branding arrangement with Aber Instruments.

Figure 4 BioPAT® ViaMass electronics supplied by Sartorius Stedim Biotech

Figure 5 shows the results of a CHO cultivation in a Sartorius Flexsafe® RM 50L bag fitted with a single use RFI disc sensor. The capacitance signal is compared with the viable cell density of the offline Cedex HiRes measurement as a reference. In addition, the Cedex measurement principle reveals the average viable cell diameter (µm). This was multiplied with the viable cell density for each sample. The result is the viable cell volume (cm^3/ml) or the viable cell volume as percentage of the total viable volume (%). Additionally, the wet cell weight is plotted with the data relating to cell viability (from the Cedex). Focusing on the result shown in figure 5, in the first 8 days representing the exponential phase of growth, excellent correlation was observed between the capacitance signal of the online biomass measurement and the viable cell density, the viable cell volume and wet cell weight. Subsequently, the cell diameter begins to increase rapidly and the viability drops. From now on, the deviation between capacitance and cell density increases with increasing cell diameter. However, the calculated viable cell volume and capacitance correlate well up to the end of the cultivation. It is evident that the online measurement of the biomass (as percentage of the volume) shows every small effect of biomass change during the cultivation process, e.g. each dilution due to additional feed medium and growth rate changes in the stationary and dying phase of the process.

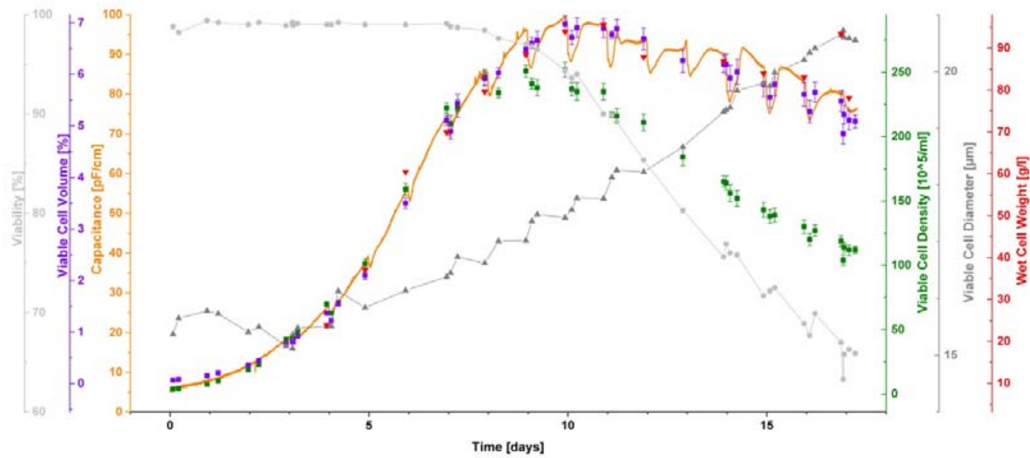


Figure 5 Online Biomass measurement and offline reference for CHO fed-batch cultivation with single use BioPAT® ViaMass at the 50L scale(Courtesy S. Ruhl, & J. Scholz. Sartorius Stedim Biotech, Germany).

The final example in this section shows how two different designs of a RFI probe perform when used in to measure a batch cell culture. Figure 6 shows a comparison study between the 12 mm Annular probe and the newly launched 7.5 mm Pico for measuring a CHO cell culture process. Both probes were placed in a Dasgip benchtop reactor. The performance of both probes was highly comparable and the cell concentration trends followed each other nicely. In addition, both probes correlated well with offline viable cell density measurement. The performance of the small probes is particularly important if small bioreactors are being used in scale up studies and it is important to have the same pF/cell biovolume for an individual scale for the small design compared with the probe designs used in production cGMP vessels. Not only can the capacitance probes be used to measure cell concentration in different scale up platforms, but the capacitance trends obtained can be used to determine the success of a scale up process or strategy.

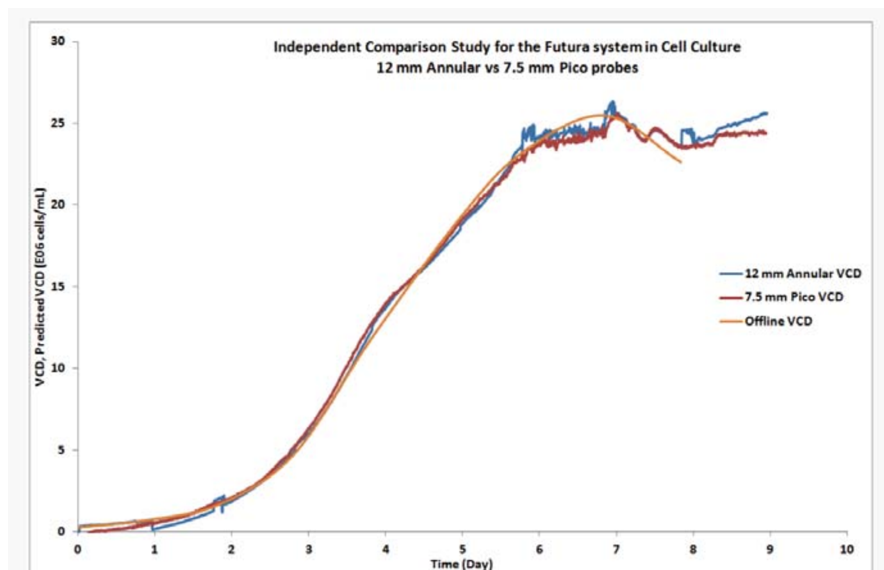


Figure 6. Comparison study between the 12 mm diameter Annular probe and the 7.5 mm Pico for measuring a CHO cell culture process.

ONLINE CELL CULTURE CONTROL IN CGMP

RFI has been used successfully in production scale processes for monitoring and controlling animal cell processes. Different options for transmitters are available for such cGMP applications. A transmitter with a touch screen interface (the Aber V350) can be installed next to the bioreactor (Figure 7).

Figure 7 Aber v-350 transmitter

Alternatively, the single or multichannel 'Connect Hub' , without any screen can also be setup. The availability of single use RFI probes has opened up many more options for future applications.

In many cell culture manufacturing processes it is traditional to define a fixed-volume feed strategy for nutrient feeds, based on historical cell demand. However, one major drawback of this strategy is that once the feed volumes are defined, they are inflexible to batch-to-batch variations in cell growth and physiology. This can lead to inconsistent productivity and product quality. An auto feedback system using online Aber RFI measurements makes it possible to automatically control the complex feed rates (Zhang *et al* 2015). The nutrient feed amount can be determined by calculating the *integrated biomass* which is derived by determining the area under the biocapacitance (BC) curve across a predetermined time interval (typically 24 h).

The original approach in the study by Zhang *et al* (2014) was to convert the BC reading to VCD and then feed based on the estimated cumulative of estimated cell growth (cICG). However, the capacitance reading was found to directly correlate with the cICG-based feed amount which made it feasible to directly use the online BC reading to control the feed rate. This also eliminates the need to use models based on either the Cole-Cole equation-based approach or multivariate analysis (Lee *et al* , 2014, Carvell *et al*, 2017) to adjust capacitance to match the offline viable cell density (VCD) during the later stages of cell growth, where there may be a divergence observed between the measurements.

PERFUSION MONITORING AND CONTROL

Many cGMP cell culture processes are based on a perfusion process. Control of the feed or addition rates to maintain pseudo-steady-state conditions in these bioreactors can be especially challenging due to high and fluctuating cell concentrations that can rapidly change environmental conditions. With infrequent manual daily sampling based on trypan blue exclusion haemocytomer cell counting, the control system can have too little information on which to base an appropriate decision to manipulate the process, and hence will lead to large process deviations. Tight control of the perfusion or concentrate addition rate allows the bioreactor to be operated under the optimum conditions for maximum recombinant protein production.

A robust automatic perfusion rate control system based on the RFI probe is now being used in cell culture manufacturing processes. The system operates in a completely closed loop i.e. no samples need to be taken to obtain process information. In the control algorithm, a cell specific perfusion rate is specified and the RFI signal is converted into a perfusion flow rate through calculation and implementation with a variable speed controlled pump (Figure 8). An example of the actual time-dependent capacitance trace of a perfused Hela cell culture evolving from batch (preset volume, increasing concentration) to fedbatch (increasing volume, preset cell concentration) growth conditions in shown in Figure 9 . The stable capacitance value can be seen when the culture is operated in a fedbatch mode with a preset cell concentration of 10^7 cells/ml. The peak observed during this step represents an increased cell concentration due to an insufficient fresh medium supply to maintain a stable capacitance.

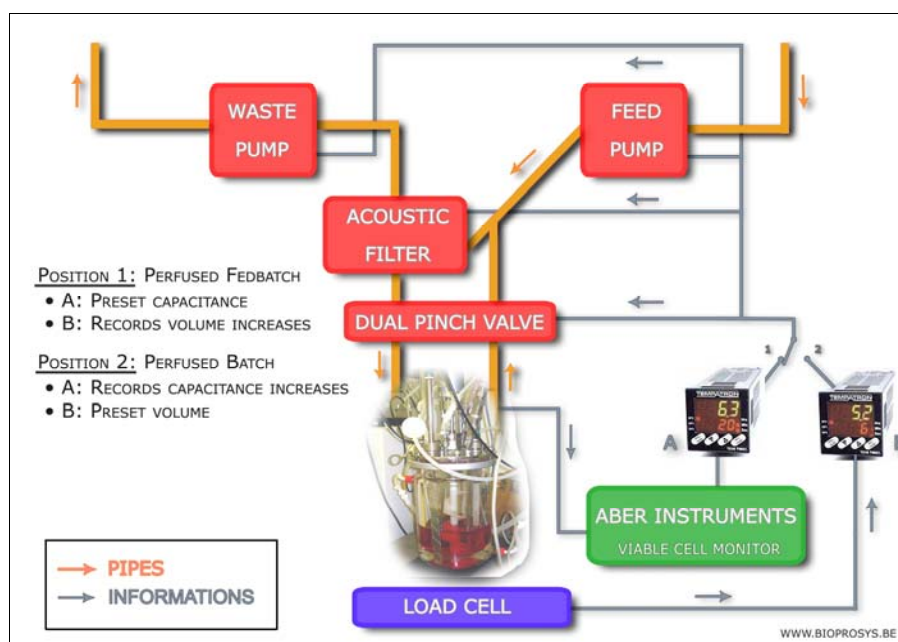


Figure 8 A robust automatic perfusion rate control system based on the RFI probe is now being used in cell culture manufacturing processes to control constant cell density.

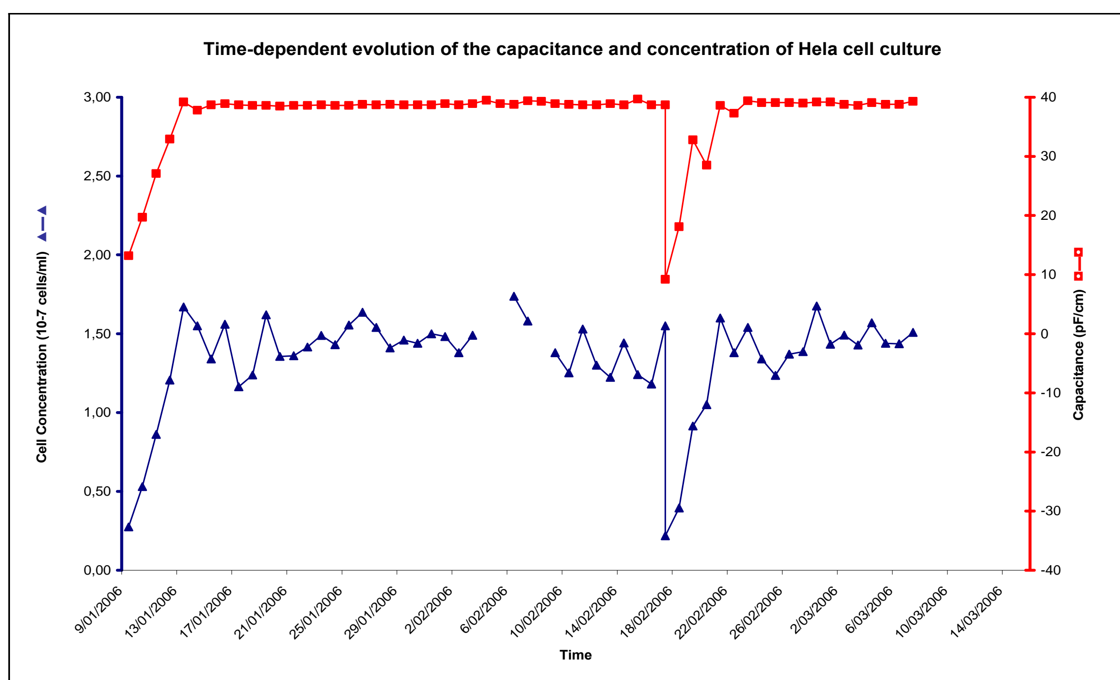


Figure 9 Using RFI to control a constant cell concentration in a bioreactor over a 2 month period

The RFI probe only measures the viable cell mass and is therefore ideal for this application and it has been applied for process control in sono-perfused cytostats, spin-filter perfused bioreactors and for maintaining steady-state, continuous culture of bioreactors with external loop filters (eg the Repligen ATF system) for monoclonal antibody and recombinant protein production.

In the second example of process control, data are taken from the FDA (Division of Biotechnology Review and Research-II OBP/OPQ/CDER). 5-L glass bioreactors (Sartorius Stedim) were run in batch, fed batch and perfusion modes. In the perfusion mode the bioreactor was equipped with an XCell ATF device (Repligen) and a RFI probe. A photograph of the experimental set up is shown in Figure 10.

In Figure 11, the viable cell densities for cultures run in the three different modes are compared between the Nova Bioprofile method (based on image analysis and Trypan blue) and the Aber values. The Aber VCD is based on converting the capacitance at 580 KHz to a viable cell density using a linear model. The capacitance probe data correlated well with the Nova Bioprofile VCD in both the fedbatch and perfusion modes. In the batch mode, the data were skewed to match the VCD during the death phase leading to a slight overestimation during the growth phase. The discrepancies that sometimes occur between the capacitance and trypan blue methods to derive the VCD during the cell death phase are now well understood and are explained in the literature (Braasch *et al*, 2013, Lee *et al,* 2014). In the perfusion mode

the capacitance probe was shown to immediately spot a sudden increase in live cell density after 12 days of culture caused by too much media being pumped out in error. This underlies an additional advantage of using a capacitance probe to measure the VCD so that errors or sudden failures can be picked up in real time, such that appropriate corrective action can be taken.

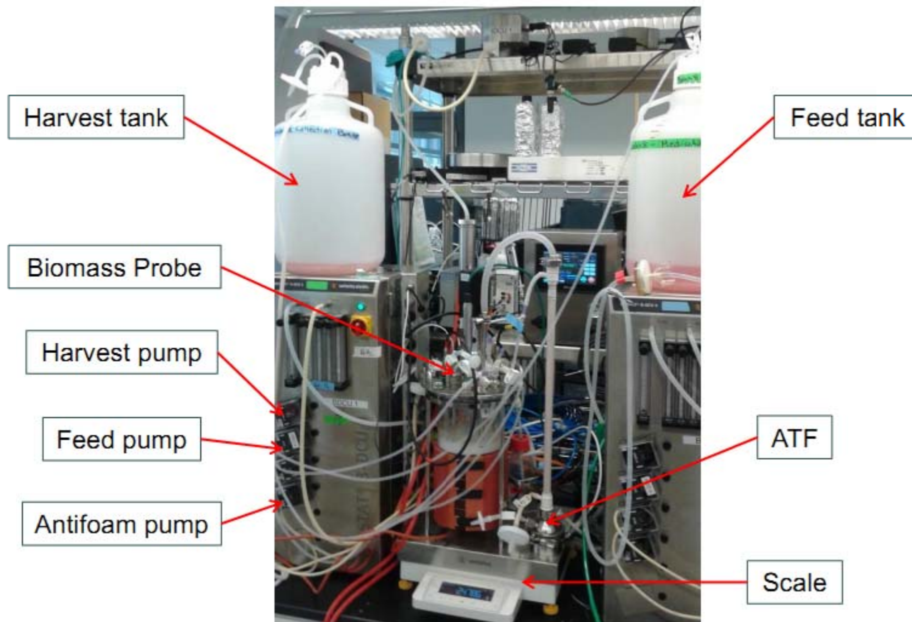


Figure 10. Perfusion set up at FDA using the XCell ATF and Aber RFI probe.

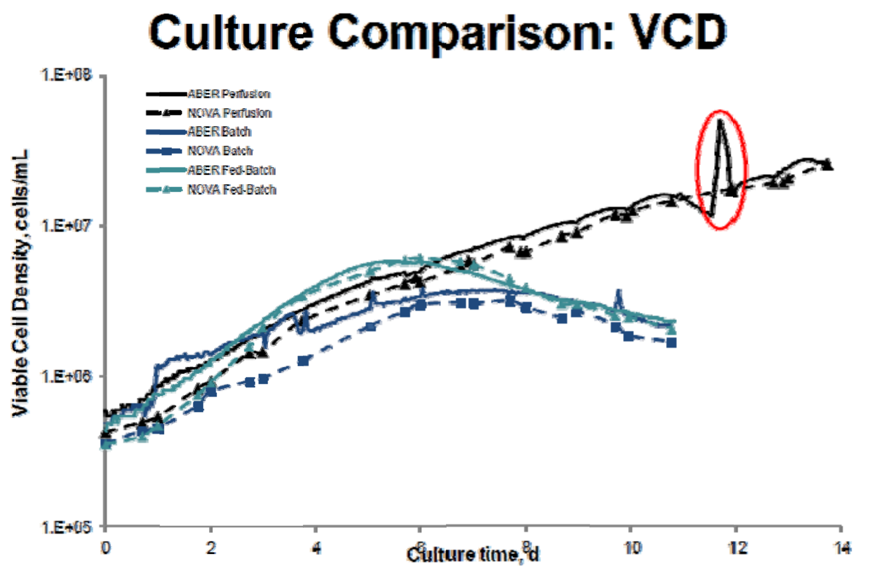


Figure 11. Viable cell density versus time for batch, fed batch and perfusion modes. The Aber values are based on converting the capacitance at 580 KHz to a viable cell density.

SCALE UP

Scaling is an important aspect with respect to any cell culture process, which involves transferring a bioprocess from one bioreactor size to another. Scaling includes scale-up and scale-down. Scale-up is required when a process is being developed for smaller, easy to handle volumes, with the final goal of commercial manufacturing at a much larger scale. Scale-down on the other hand is critical to troubleshoot large scale processes, for producing smaller quantities of product and to verify process condition changes.

When moving from a small scale system to commercial manufacturing, the critical quality attributes of the product being developed and manufactured should be identical. Therefore, it is imperative that the key parameters and performance during the scaling process are kept constant.

Here we show an example of cultivations in SSB RM 50 , RM 200, STR 50 , STR 200 and STR 1000 platforms (figures 12 and 13). Capacitance trends measured using the single use BioPAT Viamass were observed to be comparable across these platforms, thus indicating successful scale up. This demonstrates that not only can the RFI measurements be used across platforms, but can also be used to determine the success of the scaling strategy across different platforms.

This study was also performed successfully to compare the single use probe in RM and STR platforms vs the multiuse probe in the Cplus Sartorius bioreactors (data not shown).

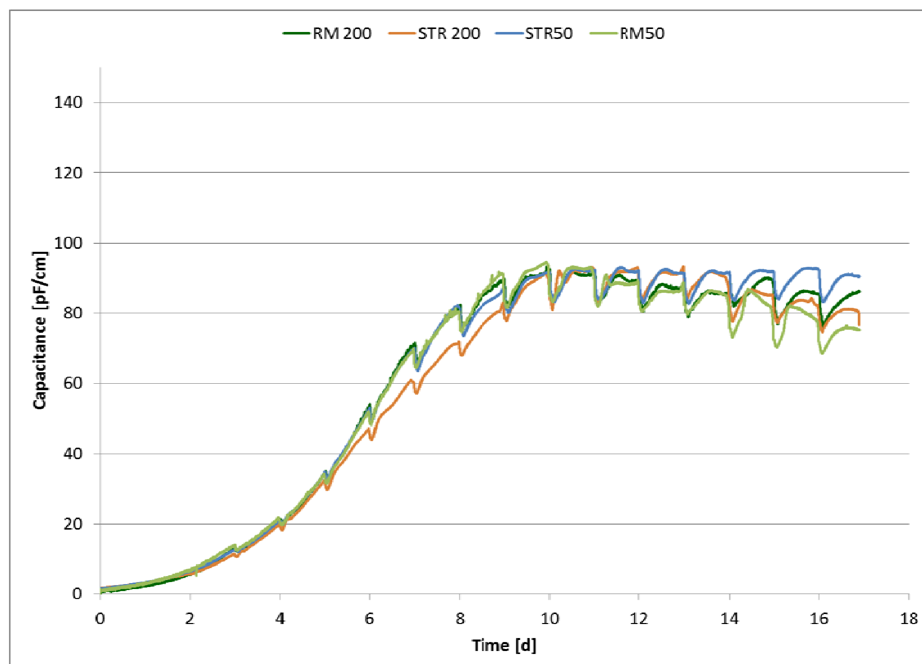


Figure 12 . Example of cultivations in SSB RM 50 , RM 200, STR 50 , STR 200 platforms.

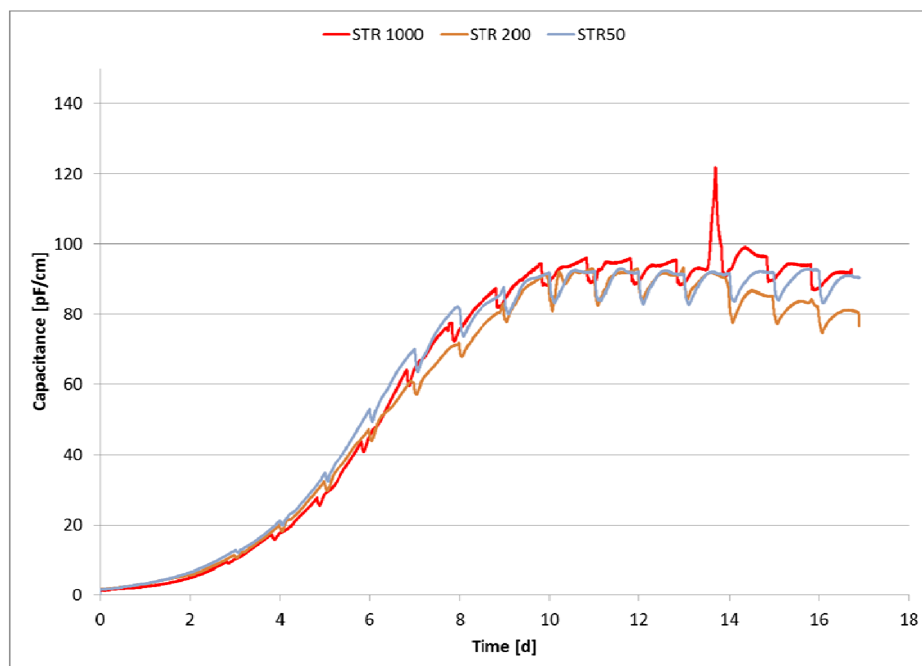


Figure 13 example of cultivations in STR 50 , STR 200 and STR 1000 platforms

INSECT CELL CULTURE

The insect cell baculovirus expression system has become one of the most widely used systems for routine production of recombinant proteins. After infection with the baculovirus, the cells undergo significant physiological changes resulting in cell enlargement, which is typically used as a parameter for successful infection.

Utilising RFI measurements to not only measure the concentration of insect cells, but to also determine successful viral infection has allowed for better understanding and control of these processes. Here is an example where capacitance measurement was used to determine the most appropriate point of the Sf-9 growth curve where the baculovirus could be added, for maximum recombinant protein production (figure xx). Excellent correlation was observed between the viable cell count and capacitance measurement before the addition of the virus. Moreover, after the addition of baculovirus (indicated by the vertical line) to Sf-9 insect cells, the monitor was used to track the progress of infection. Interestingly, even though the offline viable cell numbers are no longer increasing, the capacitance continues to increase rapidly after infection. It can be concluded that this represents an increase in the diameter of the Sf-9 cells and therefore, a successful baculoviral infection.

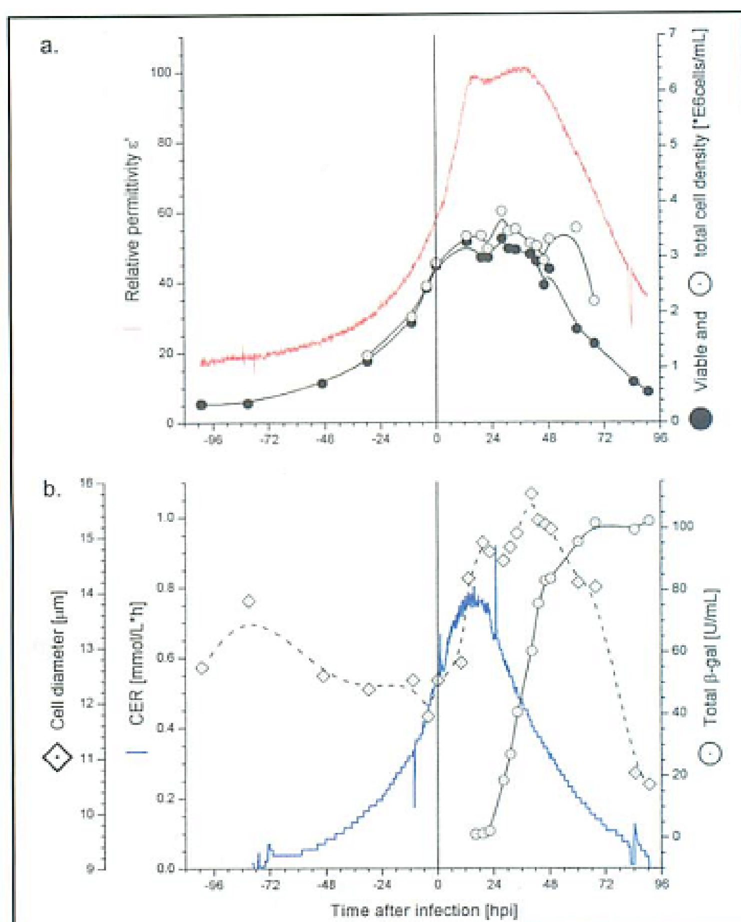


Figure 14 Using RFI to successfully monitor the infection of a SF9 cells.

Similar work was performed, but in a 50 L Sartorius rocking motion Cultibag system and the disposable capacitance patch probe. Yet again, the probe tracked viable cell density before the addition of a baculovirus for transient recombinant protein expression and successfully detected a valid infection by showing a rapid increase in signal caused by the increasing volumes of the infected cells. Interestingly, a noticeable noise increase in the capacitance profile was also observed right after infection, which was thought to be due to the changing electrical properties of the Sf-9 cell membranes.

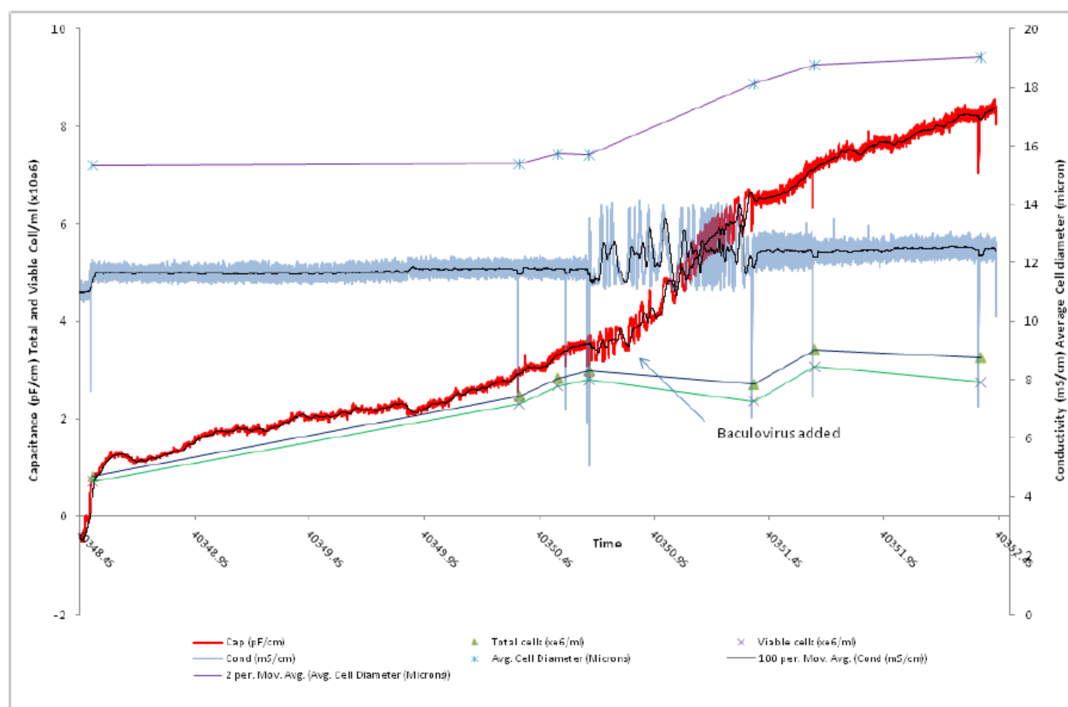


Figure 15 Capacitance and conductivity measurements in a 50L Sartorius Stedim Biotech (25 L working volume) RM Flexsafe® BIOSTAT® with BioPAT® ViaMass Sensor disc (Courtesy R. Tanner, GlaxoSmithKline, UK)

IMMOBILISED CELLS

Measuring the cell density of immobilized animal cells grown on carriers, within capsules or on inert packed beds can be very challenging. With microcarrier cultures, apart from the errors of sampling the heterogeneous suspension, the off-line method (usually based on a nuclear count) only provides a total cell count. For cell culture systems based on the packed bed system, it is very difficult to have access to a sample of the carriers in a sterile way and biomass can only be estimated by indirect methods such as oxygen uptake. In all cases, RFI provides a unique on-line method for estimating the live cell mass in real time.

IMMOBILISED CELLS - *MICROCARRIERS*

Figure 16 shows the application of RFI to monitor CHO cells grown on microcarriers during long term culture runs at Genzyme Inc (USA) for both the growth and harvesting phases. The process has been scaled up and the RFI probes are used in cGMP production.

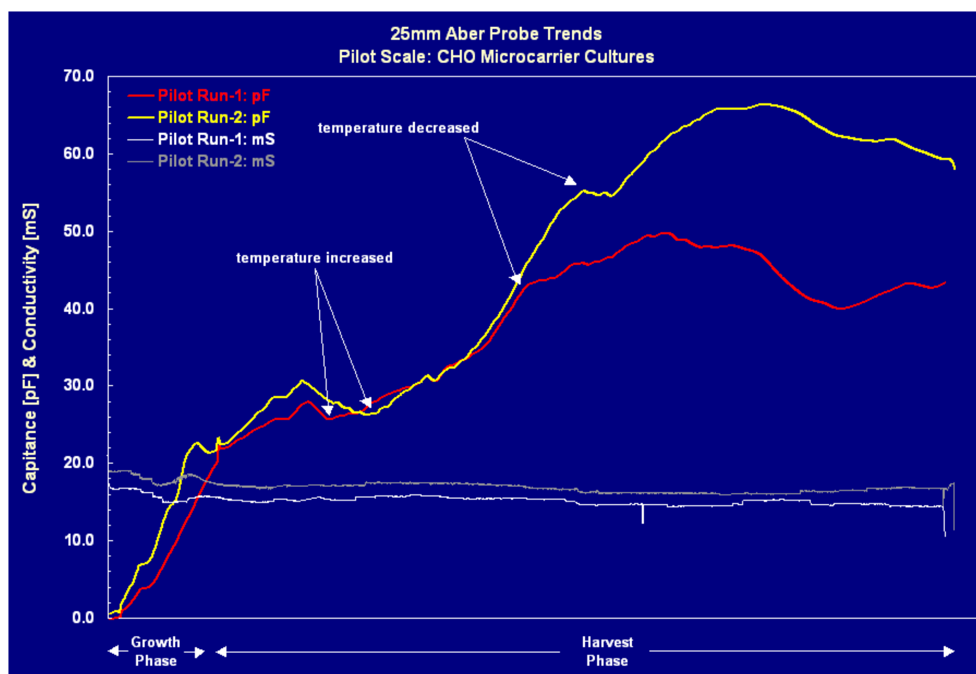


Figure 16 Using RFI probes at Genzyme (USA) to monitor CHO cells grown on micro-carriers

Figure 17 demonstrates the use of capacitance to monitor growth of Vero cells at Xenova (UK) on Cytodex 1 microcarriers with exchanges of media. Figure 18 presents data from a 20-day cultivation of baby hamster kidney (BHK) cells attached to macroporous microcarriers (courtesy of Novo, Denmark). Capacitance, conductivity, and the off-line cell counts are shown as a function of time. Samples were taken daily, and the numbers were estimated by crystal violet staining followed by manual counting of released nuclei in a hemocytometer. Vertical lines indicate the daily exchange of medium (the impeller is turned off to settle the cells). The off-line cell count on the macroporous carriers was unable to provide any meaningful data on the progression of the culture. By contrast, RFI provided valuable real-time information on the viable cell count. A small daily drop in capacitance signals indicates that essential nutrients are being depleted before the medium is exchanged.

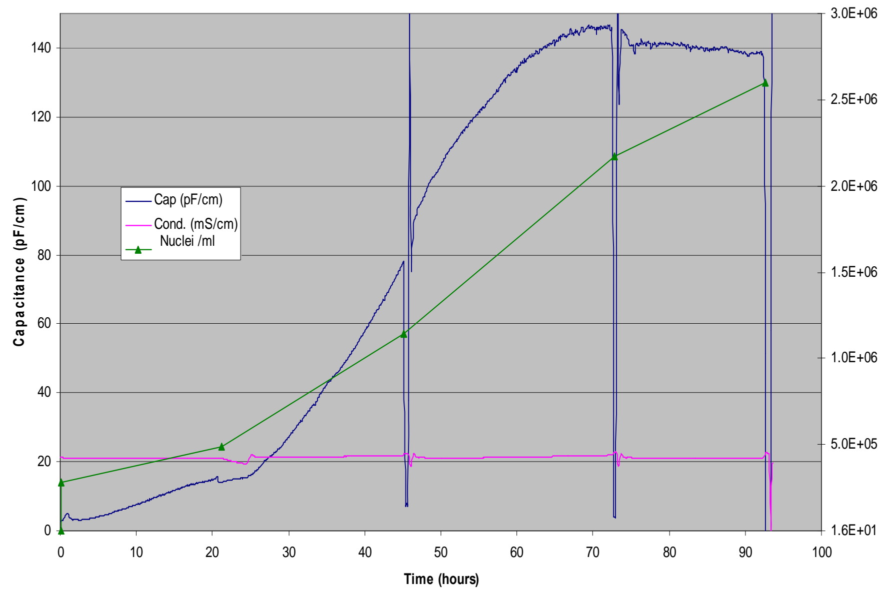


Figure 17 Vero cells grown on surface micro-carriers and monitored with a RFI probe.

IMMOBILISED CELLS - *MICROCAPSULES OR ALGINATE BEADS*

In this case study, CHO-DP 12 cells were grown in an encapsulated cell perfusion process (Cole *et al* 2015). Ca^{2+}-alginate-poly-L-lysine-alginate (APLLA) microcapsules had an initial cell density after inoculation of 0.3 × 10^6 vcells·mL^{-1} WV (viable cells per mL working volume) in a working volume of 1400 mL medium. The viable cell density was estimated using three different methods: offline cell counts using the microscope, continuous real-time RFI measurements and continuous real-time calorimetry measurements. The continuous capacitance measurements were computed into viable cell number by dividing the capacitance with the CHO-DP 12 specific capacitance, 1.44 × 10^{-6} pF·mL·cm^{-1}·$cell^{-1}$. The viable cell density, assessed by the three monitoring methods throughout the full culture time is displayed in Figure 18. Excellent correlation was observed between the cell density determined by RFI and microscopy, from the beginning of the culture.

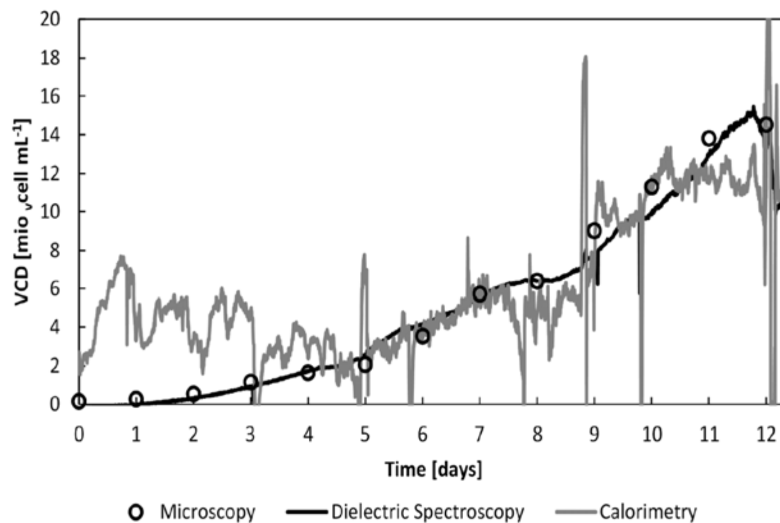
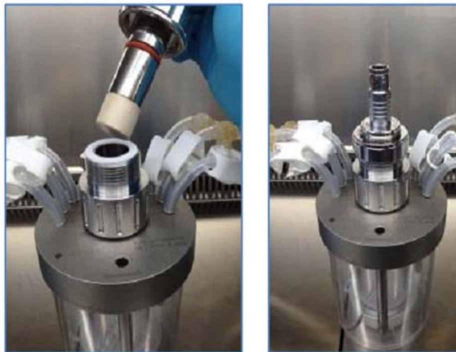


Figure 18 Using RFI to monitor cells grown in alginate beads. RFI data is compared with microscopy and calorimeter data.

IMMOBILISED CELLS - *PACKED BEDS*

The iCELLis range from Pall have fully-integrated, high-cell density with a fixed-bed system. Central to the iCELLis bioreactor technology is the use of a compact fixed-bed, filled with proprietary macrocarriers. This matrix is made of medical grade polyester (see Figure xx). However, monitoring and control of a cell culture is complicated as it is difficult to have access to samples of the carriers in a sterile way. The RFI technology is well suited to monitoring the packed bed and this is done by using a sterilizable multi-use RFI probe for the iCELLLis Nano and a patch style single RFI probe on the production scale iCELLis 500.

The example presented here was made with a 2.67 m^2 bioreactor iCELLis Nano (4 cm bed height) to produce adenovirus type 5 in HEK293 cells (protocol described in Lesch *et al* 2015, one of the process development runs). A cell density of 7500 cells/cm^2 was inoculated, cells were cultured for 5 days, and the adenovirus production was assessed during the culture days 6–7. The cell growth was measured by nuclei count from the top carriers and the biomass signal (pF/cm). The biomass signals measured in pF were comparable to the cell count up until infection (Fig. 19) although there is a slight difference in Biomass probe signal on the last day. This is most probably due to fact that cells are dying at that stage due to the infection, and the difference in the definition of cell death for the different technologies becomes apparent.



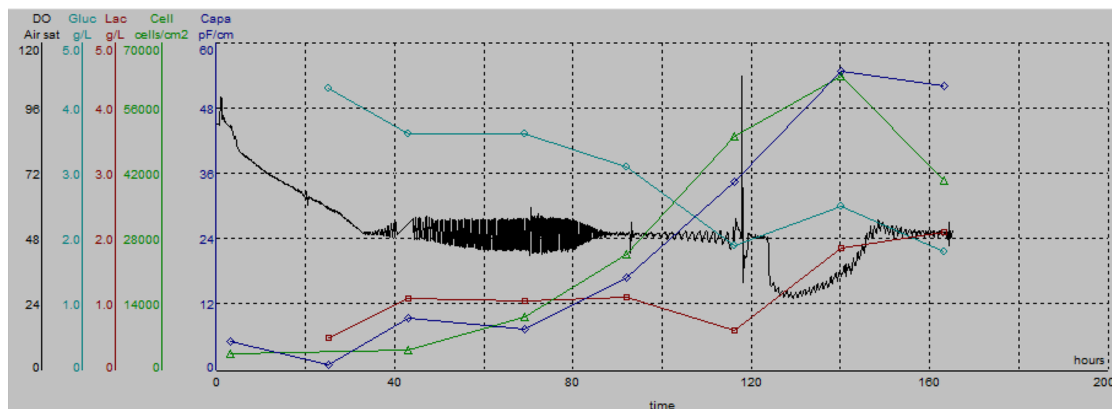


Figure 19 . Comparison of cell counts (based on nuclei staining) versus biocapacitance for HE293 cells grown in the Pall i-cellis Nano system(Data courtesy of Hanna Lesch, Finvector)

Thus, the biomass probe can be a tool to monitor cell growth in the iCELLis 500, where no carrier samples can be taken. In this case the biomass signal represents the cell concentration on the top carriers and the assumption has to be made that the cells are evenly distributed.

Figure 20 shows some earlier work where RFI had been used at the industrial scale to monitor the live cell concentration of CHO cells in a packed bed reactor with Fibra-Cel carriers in a 40 Litre Celligen Plus bioreactor (NBS) through the initial growth phase (up to 4 x 10^7 cells/ml of packed bed) followed by a stationary production phase with a high specific protein production rate (Ducommun *at al* 2002). The same authors showed that this packed bed at the 5 Litre scale had a homogenous distribution of the cells and concluded that the RFI measurement was representative of the complete packed bed. (Ducommun *at al* 2002).

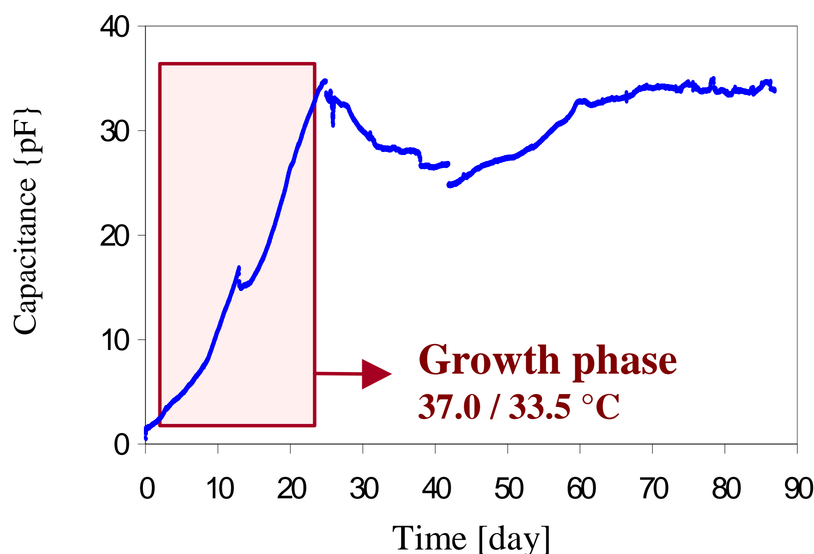


Figure 20. Example using RFI to monitor a 40L packed bed bioreactor at the industrial scale.

3D CELLULAR CONSTRUCTS

In any three dimensional (3D) biofabrication process, assessing critical biological quality attributes of 3D constructs such as viable cell number, cell distribution and metabolic activity is critical to determine the suitability and success of the process. One major limitation in current state-of-the-art is the lack of appropriate methods to monitor these quality attributes in situ in a non-destructive, label-free manner. In the following study (Narayanan *et al*, 2017), the feasibility of using dielectric impedance spectroscopy to address this gap was investigate. The experimental setup can be seen in Fig 21.

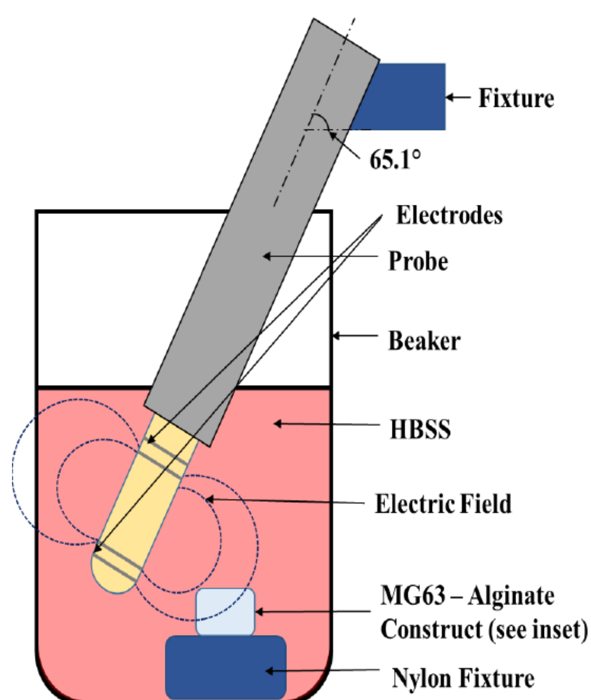


Figure 21: Experimental setup to measure 3D cellular constructs with the Aber annular probe.

The relative permittivity of 3D alginate constructs with four different concentrations of encapsulated MG63 cells (1 – 6.5 million cells/mL) was first measured, and was found to be statistically significantly different ($p < 0.05$). Within the tested range, the relationship between cell concentration and relative permittivity was noted to be linear with an R^2 of 0.986 (Fig 22).

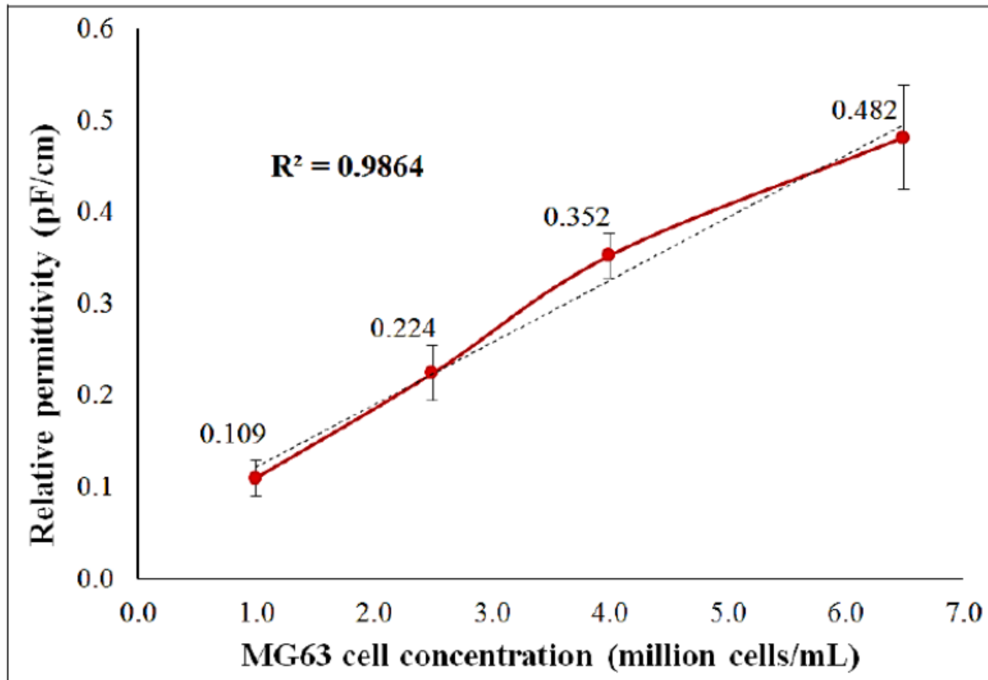


Figure 22: Average permittivity of MG-63-alginate hydrogel constructs measured across different cell concentrations. The average permittivity is given in data labels and the standard deviation is represented by the error bars.

Furthermore, the β- dispersion parameters for MG63-encapsulated in alginate (6.5 million cells/mL) was characterised (Figure 23).

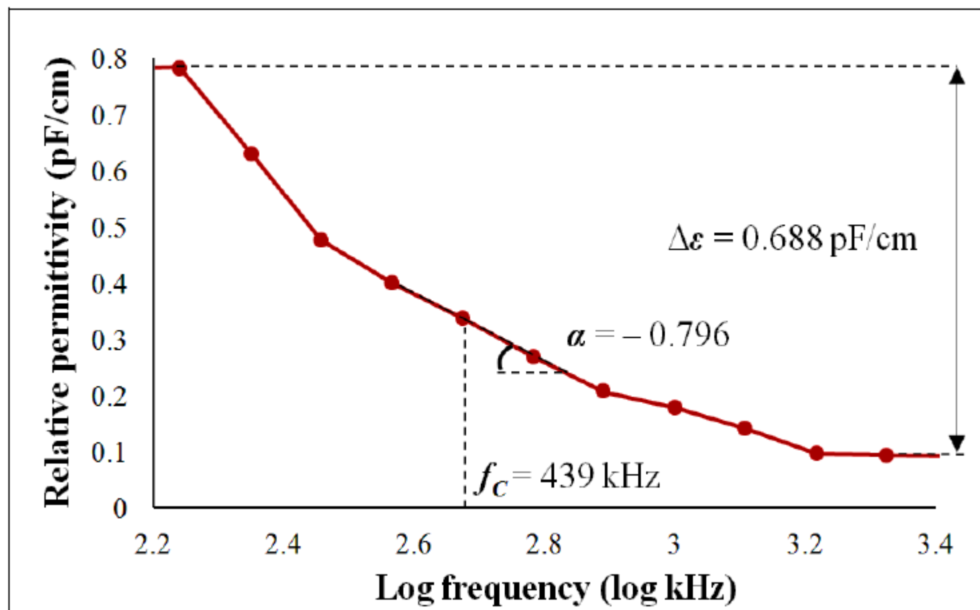


Figure 23: The characteristic β-dispersion curve obtained from six MG63-alginate hydrogel constructs with a concentration of 6.5 million cells/ml.

In addition, the characteristic β-dispersion curves were used to distinguish between different cell types (MG63 and stem cells) as shown in Fig 23 (Narayanan *et al* 2017). As seen from Fig 23 and 24, the frequency scan parameters, α and f_c are noticeably different for these different cell types.

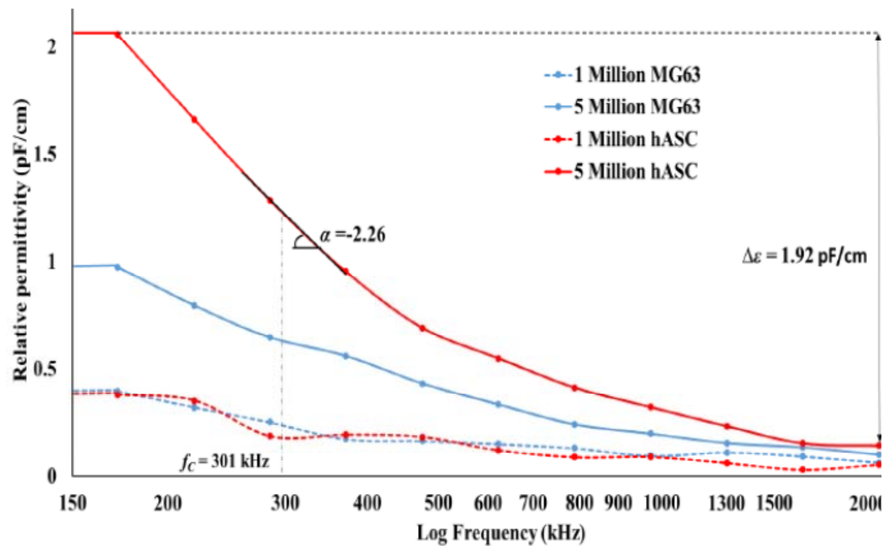


Figure 24: The characteristic β-dispersion curve obtained from MG63 and hASC constructs at different concentrations.

These results demonstrate that dielectric impedance spectroscopy can be used to monitor critical quality attributes of cell-encapsulated 3D constructs. Owing to the measurement efficiency and non-destructive mode of testing, this method has tremendous potential as an in-process quality control tool for 3D biofabrication processes and the long-term monitoring of cell-encapsulated 3D constructs.

CONCLUSIONS

- Aber RFI measurements provide a real time and online estimate of the total live biovolume of a cell culture process.

- RFI measurements do not require the need to sample, hence mitigating the risk of bioreactor contamination, and reducing the time and effort associated with offline measurements.

- With Aber's offering, the RFI measurements can be used across various platforms - from small glass vessels, to large stainless steel reactors and single use platforms.

- RFI measurements can be used to determine the success of a scale up strategy, by providing biomass fingerprints for each step.

- RFI measurements have been used to not only monitor, but also control cell culture processes - controlling cell concentration in perfusion processes, and an approach to a flexible nutrient feed strategy in production environments has been demonstrated.

- It has also been shown to successfully monitor Sf-9 insect cell culture, determine the appropriate time of baculovirus addition and identification of successful infection.

- In immobilised cell culture platforms, such as packed beds, microcarriers and alginate beads, where offline cell concentration measurement is challenging at best, RFI measurement have been shown to be successful in tracking the culture in real time.

- RFI measurements are thought to have tremendous potential as an in-process quality control tool for 3D biofabrication processes and the long-term monitoring of cell-encapsulated 3D constructs.

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For 30 years we at Aber™ have pioneered the development and use of dielectric instrumentation for the measurement of cell membrane capacitance and media conductivity. For 27 of those years we supplied the Biotech industry with three generations of biomass monitors. Now with the recent introduction of the FUTURA pico system, ABER technology is available for even smaller bioreactors.

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