DOI 10.1002/biot.201200226 Biotechnol. J. 2012, 7, 1522–1529

Perspective

How can measurement, monitoring, modeling and control advance cell culture in industrial biotechnology?

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This report highlights the potential of measurement, monitoring, modeling and control (M^3C) methodologies in animal and human cell culture technology. In particular, state-of-the-art of M^3C technologies and their industrial relevance of existing technology are addressed. It is a summary of an expert panel discussion between biotechnologists and biochemical engineers with both academic and industrial backgrounds. The latest ascents in M^3C are discussed from a cell culture perspective for industrial process development and production needs. The report concludes with a set of recommendations for targeting M^3C research toward the industrial interests. These include issues of importance for biotherapeutics production, miniaturization of measurement techniques and modeling methods.

Received 15 JUN 2012
Revised 10 JUL 2012
Accepted 31 JUL 2012

Keywords: Biopharmaceuticals · Gene therapy · Mammalian cells · Stem cells · Viral vectors

1 Purpose and background

Cell culture technology involves a wide range of cell types: insect, avian, rodent, primate, and human cells/cell lines, as well as embryonic, induced pluripotent, and adult stem cells. Their applications in industrial processes include products such as therapeutic proteins, viruses for vaccination and gene therapy purposes, and cells per se (Fig. 1).

Methods in measurement, monitoring, modeling, and control (M^3C) are critical for the production and characterization of biopharmaceuticals due to the complexity inherent in the host cells and molecules considered [1–4]. The success of and increasing use of animal cell culture technologies in industry make M^3C methodologies, especially for process design and optimization, an important challenge both in the academic and industrial communities.

This expert panel report intends to address issues relevant to all of these applications for users of cell culture

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Abbreviations: CQA, critical quality attribute; **M^3C**, measurement, monitoring, modeling and control; **MVDA**, multivariate data analysis; **PAT**, process analytical technology; **QbD**, quality by design

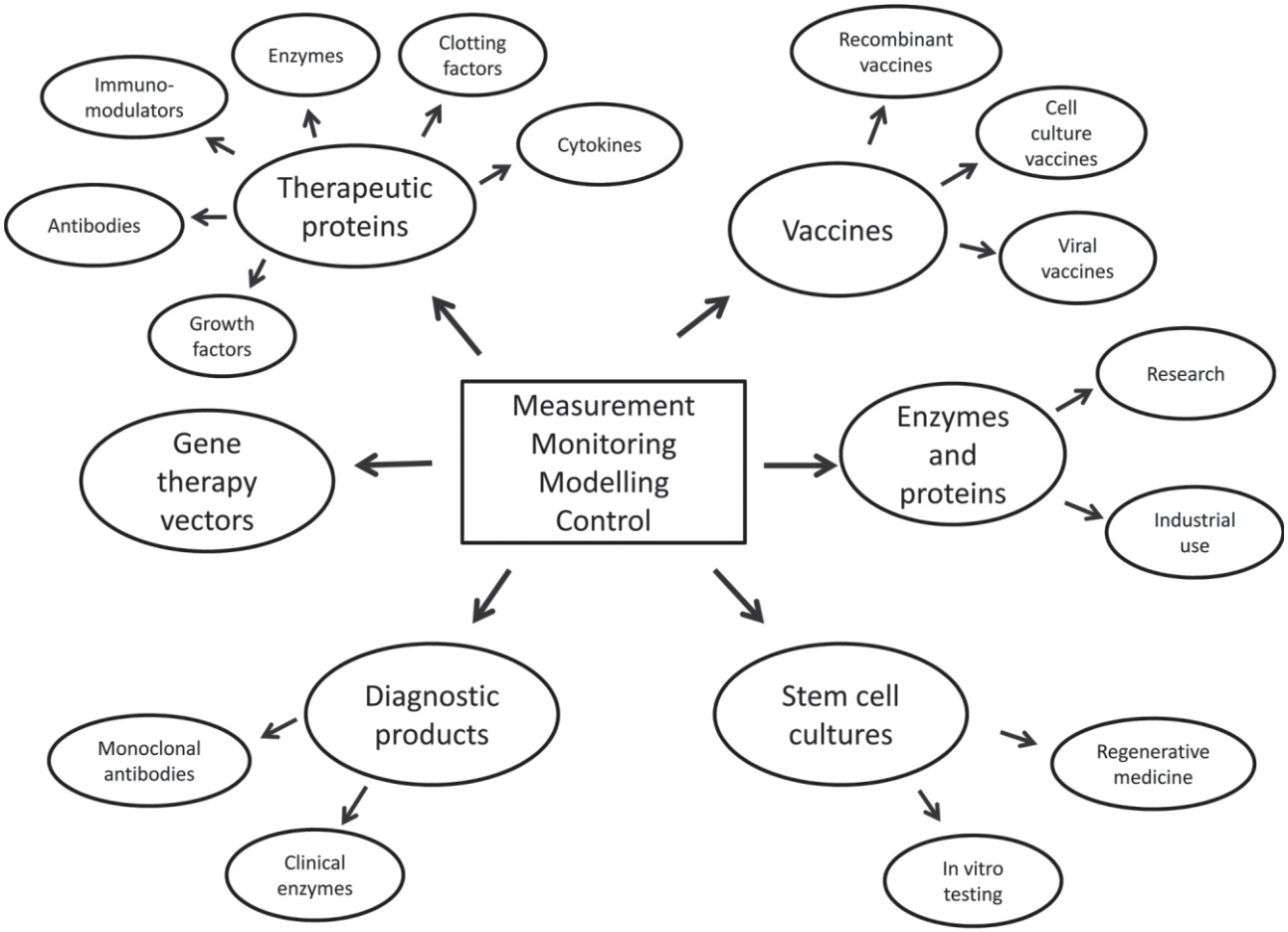

Figure 1. Main areas of cell culture applications where M^3C methods are required.

technology. It focuses on mapping how the academic and industrial competences in M^3C can jointly target the development of better solutions for purposes outlined in guidance documents [5–7] and, more broadly, by considering additional biotechnology manufacturing requirements.

2 The application of M^3C methods in the cell culture industry at present

M^3C methods applied in industry at present have largely been implemented to satisfy regulatory requirements but the sales success of many biopharmaceuticals has also driven industry to use M^3C methods to facilitate improvements in process development times, process understanding, and to drive down cost of goods.

The International Conference on Harmonization (ICH) quality Q1–Q11 guidelines [7] outline distinct recommendations for the pharmaceutical industry. This covers substantial parts of the measurement and monitoring methodology concerning quality attributes such as product identity, impurity levels, final product characteristics, cell banking and validation of the analytical procedures, sensors and equipment. It also clearly addresses the quality system and risk analysis aspects and, by that, adjoins to efforts in realizing process analytical technology (PAT) and quality by design (QbD) objectives [8, 9]. These guidelines can also be taken as guidance and advice for process analytical work in the non-pharmaceutical cell culture industry. The needs for good quality in combination with process economy may drive technical development along the same routes even if the regulatory authorities are not the driving force.

Table 1 lists areas in industrial cell culture technology where measurement, monitoring and, occasionally, modeling and control have significant impact. Examples of such activities are cell line engineering and development, plasmid transfection, stem cell differentiation and expansion, culture media optimization, bioprocess development and control, product recovery, quality control, and QbD.

Currently, mainly pH, DO, and CO_2 control loops are applied although more on-line (e.g., glucose and lactate

Table 1. Typical cell culture activities requiring M^{3}C methodologies

Cell culture activity	Examples of M^{3}C methodology involved
Cell line engineering and development	Cell imaging
	Glycosylation profiling
	RT-PCR for mRNA expression analysis
	Microarrays for transcriptome analysis
Culture media optimization	HPLC, NMR, GC-MS, LC-MS
Bioprocess development and control	On-line monitoring of bioreactor state variables (temp., pO_2, pCO_2, pH, agitation, redox, conductivity)
	On-line spectroscopic techniques (NIR, fluorescence)
	Imaging, flow cytometry
	Microarrays for mRNA expression
	Glycoprotein profiling
	Control of state variables and feeding
	Mechanistic models of the bioreactor process
	MVDA and mechanistic models of the bioreactor process
Stem cell expansion and differentiation	Biomarker measurement, expression arrays
	Cell imaging, analysis of CQAs
Protein and vector recovery	Label-free protein quantification by absorption measurement
Protein production purification	HPLC, Spectrometric analysis, pH, conductivity
Product quality control	Immunosensors, HPLC

analyzer) or at-line (e.g., cell number estimation or on-line gas analysis) monitoring techniques are being available. Especially demanding measurement methods are found in microscopical analysis and omics methods.

During cell line development M^{3}C methods are largely restricted to product titer and cell growth analyses. Increasingly, methods to characterize some elements of product quality are being applied at this early stage, particularly methods amenable to high-throughput screening. Once a manufacturing cell line has been identified, production cell culture largely concentrate on pH, DO, and CO_2 control loops with at-line analyses of critical parameters such as cell number, viability, residual nutrient medium analysis (e.g., glucose, glutamine, lactate), and product titer if product is not the "cell" itself. In the relatively new area of stem cell expansion and manufacture the focus is to understand and characterize, as far as current methods allow any measurable impacts on cell phenotype, genotype, and any important epigenetic changes.

3 State of the art in M^{3}C research and its connection to industrial cell culture technology

M^{3}C research of relevance for cell culture technology does also take place at small and large companies involved in developing industrial measurement, instrumentation, and control systems for non-biopharmaceutical products. Indeed, the vast majority of M^{3}C users are in industrial sectors such as vehicle and aircraft manufacturing, the electronics, machining and food industry, mining, medical technology, etc. Commercial M^{3}C related products have significantly larger volumes in those areas than in biotechnology, making it likely that new valuable M^{3}C technologies may first be invented in a non-biotechnology environment as requirements and boundary conditions for research, such as state-of-the-art of technology and instrument development cost, are often the same.

Research and development of biotherapeutics is a complex and time-consuming process requiring significant effort and investment. Significant improvements in cell line and process development have been implemented by exploiting a range of high-throughput methodologies though exploitation of broader or novel M^{3}C technologies has been slow.

Nevertheless, the qualified research driven by industry's needs ongoing in biotechnology-related M^{3}C should be supported, recognized, and valued. Research should be separated into those M^{3}C methods with potential to impact on process development times, process robustness, process understanding, scale-up, and validation versus M^{3}C methods of monitoring (and controlling) the manufacturing process itself during routine operation.

Basic M^{3}C methods for measurement of bioprocess state variables, such as electrochemical electrodes, optical probes, spectroscopic methods, and biosensors are useful in cell culture processes and have been described in detail previously [1–3].

Examples of M^{3}C methods of particular interest for future application in cell culture processes which have recently undergone notable technological progress are compiled in Table 2 [10–31].

Specific developments include measurement methods based on cDNA microarrays, glycan profile analysis,

Table 2. Examples of recent research activities in M³C with high relevance for cell culture technology

M³C area	Research	References[a)]
Measure-	Microscopical imaging of cells for characterization purposes	[10, 11]
ment	Omics platforms for characterization	[12, 13]
	Flow cytometry analysis of vaccine production processes	[14]
	Biomarkers in stem cell expansion and differentiation	[15]
	Glycosylation profile analysis	[16]
	Metabolome analysis	[17, 18]
	Downstream processing characterization	[19]
On-line	Multisensor array technologies, e.g. electronic noses and tongues	[20, 21]
monitoring	Monitoring of main effluent gases (O_2, CO_2)	[22]
	Flow cytometry on-line	[23]
	On-line multi-wavelength fluorimetry	[24]
	NIR/MIR spectroscopy	[25]
Modeling	Soft sensor modeling (combined with on-line sensing)	[26]
	Hybrid models based on metabolic data	[27]
	Metabolic flux analysis modeling for process control	[28]
	Quality by design models for quality attributes	[29]
	Mechanistic modeling of chromatography	[30]
Control	Growth rate control	[22]
	Stem cell bioreactor control	[31]

a) References are either recent reviews or selected/representative applications.

and NMR or MS for metabolome analysis. Examples of methods useful for on-line/at-line monitoring of cell cultures include multi-way fluorescence spectroscopy, electronic noses for contaminations and cell concentration, and in situ near infrared and Raman spectroscopy probes for medium components and viability.

Examples of modeling methods include mechanistic, hybrid, and metabolic flux analysis approaches. In this context, recent research efforts in the field of multivariate data analysis (MVDA) should also be highlighted and strengthened.

Measurement or monitoring methods for stem cell processes where the purpose is the control of cell expansion and differentiation have not yet been defined with few exceptions [15, 32], but progress with M³C methods for, e.g., toxicity testing might be relevant in this field. The same is valid for gene therapy production processes.

Several of the examples mentioned in Table 2 are the result of collaborations between academia and industry and often from publically funded projects. No doubt, M³C development work is also performed within companies. However, to our knowledge there are no fundamentally new undisclosed M³C methodologies within the industry R&D.

4 What are the most prominent needs for M³C in cell culture industry?

The needs to improve M³C in cell culture processes can be categorized in several ways.

Clearly, the requirements are both cell type and target product dependent. In the process operation, medium and cell line development phases, the list of needs can be very extensive. The panel identified certain needs that are more prominent (Table 3) and are commented on below.

For animal and mammalian cells, tools able to measure and monitor media components and their uptake rates are of particular interest. These include monitoring of starting materials as well as carbohydrates, amino acids (at least at-line when cell growth is not well characterized due to different process history or clones used), lipids, vitamins, and trace elements to evaluate whether limitations occur. Of significant value would be on-line or at-line monitoring of critical quality attributes (CQAs) to ensure that these are within the accepted control ranges; this is a particular challenge for lower titer processes.

Stem cell culture processes (from human embryonic stem cells, hESC, and induced pluripotent stem cells, iPSC), although more demanding, have similar M³C needs. M³C methods used to support cell manufacturing and cell-product release whether on-line or at line must deliver information quickly given the short "shelf" life of cell-products compared to protein or viral based products. However, a stem cell laboratory may benefit from the use of robotics and other automated procedures (entailing protocol standardization, decreased variability, and time effort) that may evoke other measurement needs such as developing and then applying to routine manufacture assays for assessing quality of cells, stem cell markers, differentiation status, and abnormal growth properties.

Single-use bioreactor technology is becoming increasingly popular for cell cultures and it provides an

Table 3. Industrial needs for M³C methodologies in cell culture technology

Need	Examples	Impact
Robust easy-to-use small sensors	Exploiting progress in nano- and microchip fabrication for cell culture parameters	Facilitating at-line process monitoring
Identify critical quality attributes	Specification of the QbD control space at the upstream stages of the process	Accomplishment of regulatory PAT initiative objectives
Basic sensors for bioreactors	Sensors for pH, pO_2 and CO_2 that cope with new construction materials, that manage with γ-ray sterilization	Strengthen new disposable or single-use designs
Disposable multichip-based sensors	Sensors tailored for a limited number of CQA	Easy-use, low need for experts, low cost for at-line monitoring
Systems biotechnology models that support cell and process engineering	Detailed mechanistic and metabolic flux models constrained by exometabolomics	Monitoring and control of processes under production conditions; Metabolic engineering of producer cell lines
Scaled-down models of the whole process sequence	Up- and downstream process development. Computational fluid dynamics	Trouble-shooting and improvement of existing commercial scale processes
Mapping glycoprotein pattern	Capillary electrophoresis and capillary gel electrophoresis with laser induced fluorescence	Quality control, optimization of titer of key product glycoforms, cell clone selection
Spectrometry methods using MVDA for defined applications	2D fluorimetry for cell growth and product quantity analysis	Online monitoring of culture performance
Speed-up expression array methods	Reduce/tailor the number of target genes per array	Decision-making
Methods for on-line gene vector monitoring	Monitoring adenovirus vector production	Optimization of vector production process
On-line glycoprotein monitoring in downstream processing	Monitoring of chromatographic column steps	Increased final product quality
Platform for collection of software programs	The same computer tool operates simultaneously Matlab™, DoE software, CAD-software	Would make exploitation and use of modeling methods and data doable in industrial environments and could even impact automatic control
Stem cell expansion biomarker (e.g., immunosensors)	Adapted to specific cell types	Increase quality of stem cell manufacturing

opportunity for the production of cells for patient-specific therapies and toxicological studies. However, single-use technology introduces new demands. Polymeric construction materials may lead to new unexpected effects. The stability and sensitivity of traditional measurement and monitoring methods need to be evaluated in this new environment while single-use sensors capable of withstanding γ-radiation sterilization must be developed.

M³C activities in the subsequent downstream processing operations are often disregarded in favor of upstream operations.

When it comes to modeling, mechanistic and hybrid models should ideally be integrated with the upstream process operations. The use of mechanistic models integrating sequential purification steps has enabled trouble-shooting in the downstream process in real-time [33].

Non-mechanistic models or black box models may also be of great value. MVDA in different forms (PLS, ANN, hybrid structures) appears to be acceptable if properly verified.

PAT aims at gaining better process understanding. This understanding also includes factors that can be efficiently measured, monitored, and controlled. Relevant at-

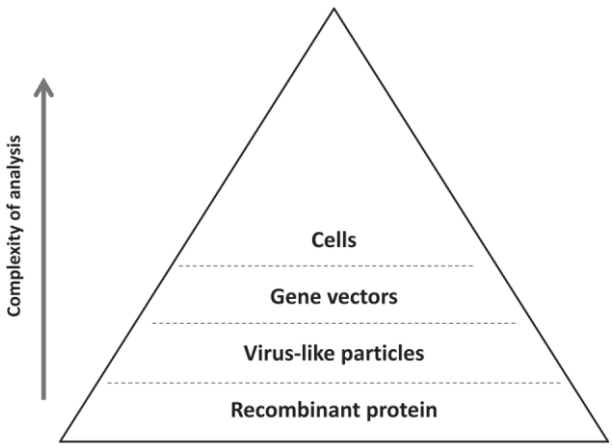

Figure 2. Need triangle illustrating the increasing demand for M³C when going from protein to cell production.

tributes, however, are often not sufficiently identified yet. Furthermore, some attributes can only be improved by cell line engineering while others, such as overflow metabolism, can be improved with on-line monitoring and control.

The lack of reliable PAT tools is largely due to the complexity of the biomolecular and cellular structures (Fig. 2). Identification of CQAs and product characterization should bear this in mind when searching for new M^3C applications.

5 What in modern S&T would best solve these industrial needs?

Today's state-of-the-art in M^3C has the potential to solve several of the abovementioned industrial needs. A key message is that there are opportunities for a more efficient utilization of existing technologies, and a transfer of new methodologies being established in academia to R&D and production in industry. But the M^3C research and methodologies must be targeted or driven by point of application, e.g., application to aid process definition and development versus process monitoring and control during routine (GMP) manufacture. If M^3C are not applied during first stages (i.e., R&D and clinical manufacturing) then it is very unlikely that M^3C get exploited for routine commercial manufacture. Also, the M^3C research community needs to understand how and why M^3C tools are applied in R&D and the criteria applied when they are being considered for application or exploitation in routine GMP manufacturing (clinical or commercial).

In general, priority should be given to methods that are simple, fast and easily integrated into the PAT concept for measurement of product quantity and quality.

Simplification of sensor technology in terms of smaller and cheaper mass produced sensors (e.g., by printing techniques and micro-electromechanical systems, MEMS) is a direction of development that is both technology-driven and pulled by the application demands in many industrial areas. Single-use integrated chip technology based on stabilized antibody/fragments is an example of this. Multi-sensor chips for medium components or other analytes in cell culture media would provide process engineers and QC analysts with convenient tools in a busy plant facility/QC laboratory environment. These devices must, however, be able to reliably measure, if not in real-time, at least in the required time-window to enable process changes.

For intracellular states, methods allowing the characterization of cellular metabolism with, for instance, HPLC, GC-MS, or ^{1}H NMR methods would be able to promote detailed metabolic models and new systems biotechnology findings.

Original efforts are necessary to meet several of the needs. Examples of such efforts include the use of high-throughput reversed-phase-like techniques for both upstream as well as downstream product analysis providing responses in seconds rather than minutes. Inventive solutions to NIR/MIR spectroscopic/fluorometric techniques for analysis of raw materials, product quality, and formulation could also be further exploited in cell culture technology, as they have been for other pharmaceutical production applications [34, 35]. The access to stable fluorescent markers, such as GFP for assay development could also be exploited further in combination with improved MVDA methods.

Innovative solutions should address analytical targets such as determination of viral titers or the ratio of full/empty viral particles, monitoring of population heterogeneity during long-term cultivation by single cell analysis, and computational fluid dynamics modeling of 3D complex multiphase flows, able to predict the ranges of shear stresses experienced by cells under specific production conditions. The data acquisition and analysis capacity has also to be adapted.

A very important issue is the education and training in using the M^3C methods. Several of the methods require substantial skills and experience such as data mining and handling of spectral information. New analytical methods must therefore be tailored for implementation at the user site.

6 Recommendations

(i) The PAT/QbD initiative of 2004 has evoked considerable activity worldwide in analytical research in both chemical- and bio-pharmaceutical development. For the latter, animal and human cell culture processes have an increasing share. It is therefore recommended that research efforts in analytical technology should address these needs.

(ii) Cell cultures have a number of unique properties that need to be analyzed with higher resolution if the analytical information is to be of value for automation and control. These include product glycosylation, critical intracellular variables, virus titers, and vector integrity as well as starting materials and medium components. Research on new M^3 methods should focus on these analytical needs.

(iii) Both data-based and mechanistic models are able to meet specific industrial needs in cell culture technology. Soft sensors based on on-line signals could use regression models or models based on advanced mechanistic modeling. Models that exploit metabolomic, transcriptomic, and other omic data should therefore be further developed and information obtained better integrated to facilitate interpretation.

(iv) Development of analytical technology should be enhanced significantly. General sensor technology and data processing methodology for industrial applications have undergone strong development mainly targeting areas other than biochemical and cell culture engineering. Thus, progress in e.g. nanotechnology, MEMS, microfluidics with soft lithography, screen printing, MVDA, bioinformatics, etc. could be adapted and integrated for cell culture applications.

(v) M^{3}C techniques should be developed toward easy and robust operation. Several research M^{3}C products have been too technically demanding and have failed to deliver solutions that can realistically function in a GMP-controlled process environment. Even with more relaxed regulatory requirements this boundary condition will remain. Multi-sensor approaches, such as microarray chips, disposable low-cost sensors, and a more extensive use of spectroscopic methods in combination with MVDA can contribute to achieving this aim.

(vi) New M^{3}C shall be compatible with the whole train of validation steps, materials, single-use equipment, sterilization, and γ-radiation. In cell culture technology, this is a more demanding task than in other biotechnology applications, because of lower titers and more sensitive cells for which analytical data tend to have higher variability and lesser accuracy.

(vii) Software for data management and analysis will most certainly continue to influence M^{3}C. Platform solutions, if possible via Internet access, where data and computation methods become interchangeable between powerful software (e.g., Matlab, Matematica, Simca, Modde) should be used within the M^{3}C applications in cell culture manufacturing and development.

(viii) A final recommendation is to encourage further M^{3}C research within consortia of partners including academia, tool vendors and the end-user (biotherapeutics industry), and, by that, improve the takeup of outputs by the biotherapeutics industry.

The authors would like to thank Dr. Bo Kara, Fujifilm Diosynth Biotechnologies, for valuable suggestions concerning use of M^{3}C in industrial animal cell cultures.

The authors declare no conflict of interest.

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