Precision manufacturing for clinical-quality regenerative medicines

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Innovations in engineering applied to healthcare make a significant difference to people’s lives. Market growth is guaranteed by demographics. Regulation and requirements for good manufacturing practice—extreme levels of repeatability and reliability—demand high-precision process and measurement solutions. Emerging technologies using living biological materials add complexity. This paper presents some results of work demonstrating the precision automated manufacture of living materials, particularly the expansion of populations of human stem cells for therapeutic use as regenerative medicines. The paper also describes quality engineering techniques for precision process design and improvement, and identifies the requirements for manufacturing technology and measurement systems evolution for such therapies.

Keywords: regenerative medicine; stem cells; manufacturing; automation; characterization

1. Introduction

Regenerative medicine (RM) is widely seen as the next major innovation in healthcare. The ability to repair and replace damaged cells and tissue, using emerging technologies such as stem cells, offers the potential of lifetime cures for unmet medical needs, including conditions such as Alzheimer’s, heart failure, blindness and joint degeneration. The key to RM is that the product is the process. Creation of novel manufacturing technologies and skills gives an opportunity to secure a long-term industrial presence that captures the entire value system.

RMs replace or regenerate human cells, tissues or organs to restore or to establish normal function [1]. They have the potential to revolutionize methods of healthcare treatment and improve the quality of life for many. RM is now established as an important branch of medicine—the industry is starting to enjoy commercial success, with annual sales of over $1 billion; a large number of products are in clinical development, having real long-term potential for public

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health benefit. The market shift to commercial products based on stem cells is likely to mature in the next 5–10 years, with a series of therapies for cardiovascular conditions, cancer, arthritis and trauma in the pipeline. RM is an emerging industry with a unique opportunity to contribute to health and wealth. It is a high-value, science-based manufacturing industry whose products tackle the consequences of ageing and chronic disease. The industry, however, currently still faces a number of critical challenges, including problems of commercial viability and company growth, limited revenue and lack of investment. The key issue determining poor sales is the lack of clinical uptake of cell therapy products; this is mainly related to difficulties in establishing clinical utility and cost-effectiveness. Creating an appropriate evidence base is the key to addressing this deficit. Businesses therefore have a primary focus on successfully reaching ‘first in man’ clinical targets; this must be followed by the ‘one to many’ translation process to reach many patients, such that effective therapies can be produced at scale, and at a price society can afford. Although effective therapies that demonstrate positive health outcomes are being developed, the key barriers facing firms relate to important aspects of the translation process. These include establishing closer collaboration with clinical end-users, greater regulatory clarity, clearer reimbursement policies based on economic evidence of the cost benefit of product solutions in application in the market place, rapid post-approval adoption and the need to develop enabling technologies that lower manufacturing costs [2]. Recent regulatory decisions also demand more clarity in the criteria that define product performance.

(a) A glimpse of the clinical opportunity

There is a continual increase in demand for novel treatment strategies to treat tissue or organ damage, owing to the rise in mean life expectancy, coupled with a severe shortage of donor organs and the limitations of conventional treatment regimes. Allogeneic transplants, autografts, xenografts and medical devices all have their inherent shortcomings, replacing the diseased tissue or organ imperfectly, and with issues of availability in the case of transplants, as well as frequently requiring immunosuppressive treatment. Researchers globally regard stem cell therapies as a treatment option with the potential to alter the face of contemporary medicine, and ultimately give a new and effective dimension to medical therapeutics. Recent work in RM (see the review [3]) has provided the proof of principle for cell-based replacement for a number of structures ranging from skin, musculoskeletal and neuronal tissue to osteochondral grafts and complex organs such as the kidney, which may supplant more conventional therapeutics to revolutionize current medical practice.

2. Precision in a regulated market

Healthcare is one of the most attractive of the markets for high added value manufactured goods; it does, however, have characteristics that differentiate it from more conventional markets [4,5]. Particularly important is the influence of industry-specific regulation on the new product introduction process, focused on ensuring the safety and effectiveness of new products (for instance, the requirements for clinical trials) and on the manufacturing process itself, with the
requirement for (current) good manufacturing practice (GMP and cGMP) [6].

Overall, these regulations require tight definition and control of product and process from a very early stage; consequently, well-established, if conservative, manufacturing methods have been much preferred. Many product failures during development are attributable to the transition from laboratory prototype to industrialized product, leading to product safety problems and a lack of effectiveness, the main causes of failure in the clinic. The lack of integration of product and process design frequently leads to late-stage failures and extended product development project time scales because of significant requirements for rework. The business risks of operational quality failures in pharmaceutical and medical device technology supply cannot be underestimated, as they can include product recall costs, multi-million dollar fines, litigation, share price decline and compulsory plant closures.

Perhaps the most complex products to manufacture in this domain are those that represent the convergence of both medical device and pharmaceutical products. They add an extra layer of complexity to this already challenging process. RM/tissue-engineered products represent characteristic current and emerging generations of such products. Some of these products will also routinely include human living material and systems, adding biological complexity (variation and heterogeneity) to the relentless increase in the precision [7] demanded from manufacturing processes. These products are likely to require also a transition in their manufacturing philosophy, from biology carried out at the laboratory bench by experts, to an engineered production method that frequently automates or mechanizes a process that initially shows many of the features of a craft [8].

As we have described earlier, only over very recent years has stem cell culture begun to be approached from a cost-effective manufacturing perspective—the key to making the economic and business case for these potentially transformative new therapies. Critical aspects of the manufacturing approach are that the production system takes account of the number of products to be made and that machines are used as an alternative to people carrying out the process—both first identified by Charles Babbage in his discussion distinguishing between ‘making’ and ‘manufacturing’ in 1832 [9]. We have also learned through the work of the late Jaikumar [10], published posthumously by his colleague Robert Bohn [11], studying the evolution of manufacturing over 500 years, that epochal change in manufacturing is achieved via breakthroughs in process control—the ‘art to (manufacturing) science’ transition that increases our knowledge of the process and allows us to specify manufacturing procedures more completely. This is exemplified by the statistical quality control (QC) and process design techniques pioneered by Shewart in the USA (at AT&T) and Fisher in the UK. Deming applied these widely to great effect in both manufacturing and supply in the USA during the Second World War and with Japanese industry in their post-war rebuilding [12], this approach ultimately leading to ‘six sigma’ philosophies emphasizing the control of variation and quality by design (QbD).

The manufacturing community is also aware of the inevitable increase of process precision achieved [7]—roughly an order of magnitude every decade or so—driven by progressive and reinforcing increases in the precision of the manufacturing process and the measurement system applied. The manufacturing community has learnt and applied these trends and principles in mechanical engineering.

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domains, including the small-arms and automotive industries, in electrical and electronic engineering domains, including semiconductors and complex assembly, and in the chemical engineering domains of the biopharmaceuticals industry, including the manufacture of antibodies. Each of these principles is being applied and the trends being perceived as a new manufacturing community brings stem cell biomanufacturing from the laboratory of the expert biologist to the GMP facility of the manufacturer under the watchful eye of the regulator.

Process excellence and/or lean sigma approaches centre on the use of a portfolio of process-centred tools and techniques, emphasizing the use of data-driven and statistical thinking, including statistically designed experiments, that allow the creation of highly capable manufacturing processes with controlled and understood variation \( (\text{Cp}) \), essentially the ‘centred’ ratio of specification to process output, and \( \text{Cpk} \), the ‘un-centred’ ratio, to ultimately parts per million (ppm) failure rates, with minimum waste and that have as their ultimate goal ‘QbD’ or sometimes ‘robust design’. Rather than post-process inspection, the quality of the end-product is then assured by the quality of each individual process step and the robustness of the final quality to the variation in the process steps, where each process step has been designed to be capable. Such consistent production and the ability always to execute the same process in the same way is also at the core of regulated GMP manufacturing. Implementing later changes to the process is often problematic; the process must create the same product that successfully went through clinical trials and showed safety and efficacy. It is also usually required to make the ultimate product by the same processes that were used to make the large-scale (phase III) clinical trials batches, in part because it is very difficult with biological material to fully characterize the finished product and demonstrate equivalence with material made by another technique. Process control and more particularly early capable and scalable process design are consequently of great importance, particularly for products or processes that are novel [13]. It is this requirement for consistency together with increasing cost pressures within healthcare that are driving the increasing use of automation in these regulated industries.

These trends are echoed by the emphasis of the US regulator, the Food and Drug Administration (FDA), on process analytical technology, manufacturing science and QbD. The FDA argue that ‘a profound understanding of the interrelationship between product and process quality’ is required before real-time release, i.e. product release with no end-of-line testing, will be possible. To the FDA, ‘profound understanding’ means: all critical sources of variation are identified and explained; variability is managed by the process; and product quality attributes can be accurately and reliably predicted. Profound understanding may give ‘regulatory relief’ in submissions. To meet these challenges, companies are adopting ‘six sigma’ style programmes to develop a detailed understanding of their processes while improving their profit margins under increasing pressure on unit cost. The application and awareness of these techniques is happening somewhat later in these highly regulated industries than for the conventional manufacturing industries.

\[ \text{Cp} = \frac{\text{USL} - \text{LSL}}{6 \sigma} \]

\[ \text{Cpk} = \min \left( \frac{\text{USL} - X}{3 \sigma}, \frac{X - \text{LSL}}{3 \sigma} \right) \]

\( \sigma \) is the standard deviation of the process output distribution.

1\(^{\text{Cp}}\) = specification tolerance/process capability = \((\text{USL} - \text{LSL})/6\sigma\), where \( \sigma \) is the standard deviation of the process output distribution. For \( \text{Cpk} \) the lower of the two values of \((\text{USL} - X)/3\sigma \) or \((X - \text{LSL})/3\sigma \) is used where \( X \) is the process mean.

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It is important to recognize that such regulated manufacturing, in summary, requires:

— a consistent and well-documented approach to product and process design with effective change control and risk management;
— a detailed product definition and specification (with limits) traceable to clinical need;
— a capable (repeatable), optimized (frequently automated) process and a processing machine, with a process window for improvements;
— a capable measurement system, traceable to absolute standards; and
— statistical and first (physical) principle process models relating key input and output variables.

There is also a requirement for a more integrated approach to product and process design, particularly to the development, transfer and validation of manufacturing processes.

3. The manufacturing challenge

RM and tissue engineering are maturing through a translational phase from laboratory-based experimental disciplines to a nascent industry. Projected clinical demand indicates that, within the next decade, this industry will need to provide responsively and economically a diverse range of RM products, many of which will incorporate living cells, to a large market. This transformation is driving a need for novel, robust manufacturing systems for cell-based products that can meet the stringent regulatory requirements imposed on medical product manufacture.

The requirement to manufacture living products for RM applications poses significant new challenges. These challenges revolve around the complexity of the living product and its sensitivity to environmental conditions. A living cell is in a constant state of change in response to its environment, and therefore maintaining product quality requires precise process control. Cell products always incorporate some degree of heterogeneity owing to different micro-environments in the manufacturing process. Furthermore, the complexity of a living cell defies comprehensive definition; measurement of product quality is usually based on average population values of surrogate markers (i.e. critical gene or protein expression) that are at best indicative of critical product attributes (therapeutic efficacy, safety). The inherent pluripotency of stem cells—their ability ultimately to form a range of cell types—exacerbates the challenge.

Many of the solutions and technologies that have been developed for conventional biologics manufacture cannot be applied to cell therapies. Large-volume suspension bioreactors derived from a chemical or biochemical engineering tradition that are well characterized for cell line production are not readily adapted to the culture of adherent cell types such as those required for most cellular products. As the cells themselves, rather than an excreted culture by-product, are the product, conventional downstream purification also has limited use. The greater sensitivity of therapeutic cells and the difficulty of product measurement enforce greater reliance on process understanding and control to guarantee product safety and efficacy.
Achieving a controlled and characterized manufacturing process for cell-based therapies requires the development of new technologies, tools and techniques, as well as the transfer of manufacturing experience from diverse older industries. As indicated, in addition to the technical challenges outlined, manufacturing equipment, processes and facilities must be compliant with GMP, the stringent regulatory framework controlling the manufacture of therapeutic products.

Variation in the manufactured RM product can come from two sources: process input material and process conditions. If identical batches of input material are subject to identical processing conditions, they will produce identical product. Relative to conventional pharmaceutical or biologic production, both input material variation and process condition variation have been poorly controlled in cell therapy manufacture and can be a consequence of approach, particularly for autologous therapies. Although the complexities are specific to the RM industry, generic methods from other industries, in particular process automation and systematic process improvement methods, are instructive when approaching this challenge.

Process automation has been a key mechanism for achieving controlled and standardized processes in many manufacturing industries. Automation also enables scale-out, multiples of the individual units of the production process, with predictable process variation and therefore predictable process costs, in marked contrast to scale-up, an increasing of the physical volume, of manual laboratory operator processing. Systematic process improvement methods, such as the ‘six sigma’ approach, have been developed in electronics and automotive manufacturing in order to understand and control sources of process variation and thereby reduce the rate of defective products.

(a) Automated manufacture

The first step undertaken in the work reported here was therefore to remove manual operator processing from the manufacturing process of important therapeutic cell types and bring the processes under machine control. The CompacT SelecT robotic flask handling platform was developed as the current best candidate production technology to remove operator variation from the manufacturing process. The platform consists of a robotic arm in a clean processing environment adjacent to an incubator. The system can carry out most cell processing activities on barcode-tracked adherent

Figure 1. (Overleaf.) A selection of data from the automated production of key platform cell types with application in cell-based therapies. Automation is a major step in achieving scalability and reducing background variability in cell-based therapy production. Transfer from a manual (blue line) to an automated (red line with crosses) process requires demonstration of product quality equivalence using the best available measurement methods. Typically, this includes the growth rate of the cells. Other quality measures are more diverse. Here, we show a comparison of transcript levels to a master cell bank for 13 gene expression products (neural stem cells), karyotype and surface/intracellular pluripotency marker expression (embryonic stem cells), and typical surface marker expression and inducible alkaline phosphatase activity (mesenchymal stem cells). Morphology also varies between cell types and subjective visual assessment is considered important. (Online version in colour.)

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Figure 1. (Caption overleaf.)
Figure 2. The outcome of a process capability measurement and improvement exercise using quality engineering tools for an exemplar automated human cell production process. The process quality output assessed was cell yield. The process improvement team identified that the process mean of the product from the automation was lower than that from the manual but had lower variability (and therefore a better potential process capability if centred in the specification). A cause-and-effect analysis was used to identify potential reasons for the differences in performance. An improved, automated process was then validated to demonstrate a Cpk of 0.45. This is better than could possibly be achieved in the manual system, given the background process variation. (Online version in colour.)

cell culture flasks with relatively few deviations from conventional manual processing protocols that would be developed in the early stages of a product’s evolution. This similarity to manual flask processing increases confidence that cell product quality will not be affected and makes process automation of flask-based processes that are advanced in clinical development (where important historical process data exist) feasible. The commercial availability and regulated production heritage of the machine offer a significant advantage over prior non-commercialized or non-scalable bespoke robotic developments [14,15].

Key therapeutic cell types and associated commercial and academic partners (‘customers’ in a quality engineering sense) were selected to demonstrate the efficacy of automated cell production using the system. Successful automated production protocols were developed for the expansion of cell numbers for human mesenchymal stem cells [16] and human embryonic stem cells (hESCs) [17], as these represent the cell stock source for a significant proportion of cell therapies under development. Further work with collaborators also developed automated production protocols for niche proprietary cell lines and
Figure 3. Six sigma quality principles and statistical methodology applied to the quality improvement of automated, ultra-precision-engineered manufacturing processes for cell therapeutic products. A formalized six sigma quality improvement strategy, structured in five phases, define, measure, analyse, improve and control, is shown here to emphasize the objectives of each phase and to illustrate the type of activities, together with examples of the statistical tools (shaded boxes) that were applied to reduce variation and defect rates for an exemplar automated human cell production process. Six sigma is a business management strategy originally developed by Motorola, USA, in 1986. (Online version in colour.)
products, including neural stem cells [18] and smooth muscle progenitor cells. The cell types chosen were intentionally diverse. They covered examples of both autologous (self) and allogeneic (all) applications, different handling requirements and clinically acceptable production protocols. The automated production methods developed produced cells that met stringent quality specifications as identified by the customer and including cell proliferation, viability, genetic stability, biological markers and differentiation potency (figure 1).

In parallel with the programme to automate key therapeutic cell types, it was important to demonstrate the improved reproducibility (using the accepted manufacturing metric of process capability) achieved through moving from manual to automated production. Figure 2 shows the process capability of manual compared with automated production for an exemplar cell line manufacturing process [19]. The process capability result is important for two main reasons. Firstly, it shows that the automated process is in control, i.e. the variation is stable, and therefore enables the application of powerful statistical tools for process analysis. The manual equivalent usually shows increased variability due to variation between operators and operator-induced artefacts, thereby masking true process-related changes. Secondly, it also provides a higher probability of batch failure, allowing predictable production costs at scale when failure rates are currently high, costly and add risk.

(b) Automated process improvement

It has also been important to demonstrate the value of systematic process improvement methods as a tool for achieving controlled and optimized automated manufacturing processes. A ‘six sigma’ type approach was used (figures 3 and 4) as the context for applying statistically designed experiments (DOE) in automated mesenchymal stem cell production. The six sigma method helps maintain a systematic approach to process improvement. It involves the definition of critical-to-quality process attributes, measurement of the process performance, analysis

Figure 4. (Overleaf.) A roadmap illustrating the application of a six sigma approach to evaluate and compare the behaviour of an existing manual process and a fully automated prototype process for the in vitro expansion of a selected anchorage-dependent cell line. Detailed process maps were generated for both processes to identify key sub-process steps. A cause-and-effect diagram and matrix were constructed to identify key process input variables. The measurement system for a selected key process output variable (KPOV = cell yield) was validated (gauge R&R). Plots of individual observations (I chart) and moving ranges (MR chart) for variables data were generated from manual and automated culture runs to test for special causes and verify statistical process stability. These data were used to construct short-term process capability (Cp, Cpk) histograms for the manual and automated processes to identify the process improvement opportunity and provide a scientific basis for determining the requirement for process adjustments. Informed by the differences between the manual and automated processes, categorized within critical sub-processes, the improvement team returned to the cause-and-effect matrix to identify root causes of the effects revealed by the capability analysis. An improvement strategy leveraging the identified key process input variables was targeted at increasing process output yield and reducing process output yield variation to create an automated, scalable process with a $C_p > 1$. (Online version in colour.)
initial evaluation of key inputs and outputs

cause-and-effect matrix

KPOV
- cell number
- cell viability
- cell function
- cell safety
- time and cost

seven key process steps

30 KPIV

process map

manual

automated

Figure 4. (Caption overleaf.)

baseline measurement and process capability analysis

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A selection of data from the application of factorial designed experiments to an automated, mesenchymal stem cell production process. This experiment was conducted as part of a systematic process improvement exercise. Initially, the critical-to-quality attributes were defined through consultation with end-users. The performance of the process for these key outputs was measured and the process analysed to identify key input parameters likely to impact on these attributes. The factors in the design, cell seeding density, foetal calf serum concentration, media volume and incubation time, were screened for the impact on the outputs. The data rank the input parameters by statistical significance of the effect on the process outputs (growth and a surface marker shown) but also show where critical interdependences exist. This is a necessary and efficient method to achieve process characterization and optimization. (Online version in colour.)

The power of DOE is to identify major process input effects on critical process outputs in the analysis phase with high experimental efficiency (i.e. results per experimental run). These multivariate experiments can also identify where input parameters are not independent (i.e. their individual effects are dependent on the levels of another parameter). This is critical for achieving an optimal process, as some input factors cannot be optimized independently. Figure 5 outlines the DOE analysis of critical process input variables for the automated production of mesenchymal stem cells [20]. Particularly important is the observed interaction between serum concentration and cell density. This shows the potential to reduce the use of undesirable production components, such as serum, with improved process understanding, and proves the futility of attempting process analysis...
using one-factor-at-a-time experimental approaches. Recent work has applied
response surface methods to optimizing the culture of hESCs, with a particular
focus on controlling the cost of goods [21].

\begin{enumerate}
\item \textit{Achieving good manufacturing practice standards of cell manufacture}
\end{enumerate}

As the automated process development work progressed, consideration was
given to the challenge of a production system that could meet the regulatory
requirements for clinical production. Regulatory authorities demand stringent
validation of equipment and facilities used in the production of therapeutic
products for human application. Therefore, the transition of facilities and
automation from non-GMP applications to GMP-validated equipment requires
careful consideration of the processing machine, as well as the surrounding
environment. Furthermore, the segregation of individual cell types, whether cell
lines or individual patient cells, is pivotal to maintaining the quality of cell- and
tissue-based products. If cross-contamination were to occur between individual
patient samples in an autologous therapy regime, there would be the potential
risk of adverse reactions due to the transfer of patient disease from one to
another, or even negative immune reactions due to the body recognizing cells
as non-self. However, in terms of manufacturing multiple autologous therapies
concurrently, the cost of complete segregation of patients’ cells in dedicated
incubators and biosafety cabinets may make the cost of otherwise valuable
therapies prohibitive.

From a regulatory perspective, full qualification of reagents and source
materials is required, along with appropriate manufacturing controls to ensure
consistency and product quality of each cell lot. Environmental monitoring is
of the utmost importance, with air quality, water quality, laboratory design,
cleaning, and personnel training and compliance all critical to product safety.
For example, FDA standards (Code of Federal Regulations, CFR 21) classify
a critical area, i.e. an area in which a product is exposed to environmental
conditions during manipulations, as requiring a per-cubic-metre particle count
of less than 3520 in a size range of 0.5 \( \mu \text{m} \) and larger. Furthermore, a QC plan
also needs to be put in place to ensure proper manufacturing oversight, as well
as to provide the following functions: examination of the various production
components; review and approval of production procedures, testing procedures
and acceptance criteria; assessment of each clinical batch based on a cumulative
review of completed production records and other relevant information; and
investigation and initiation of corrective actions if unexpected results or errors
occur during production. This QC plan then acts to prevent, detect and correct
any deficiencies that may produce poor-quality or unsafe products, such as the
transmission of adventitious infectious agents. Finally, it is important that the
QC plan establishes internal audits at planned intervals, and takes into account
relative risk factors, previous audit results and corrective actions, with the
completion of an annual audit of the complete operation.

\begin{enumerate}
\item \textit{The way forward}
\end{enumerate}

Once this has been addressed, the current biggest challenge for manufacturers
of cell- and tissue-based therapies is in the development of representative
potency assays to evaluate the final product. According to a recent FDA
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guidance document, potency assays must be specific, quantitative, meet predefined criteria, include appropriate standards and controls, be fully validated and measure both identity and strength of all active ingredients. Therefore, owing to the inherent heterogeneity in the cells themselves, these requirements can only be met if the product is fully defined and manufactured to the same consistent standards. This consistency will rely on strong quality systems controlling both the product and the manufacturing process itself. One way to combat this problem is through the concept of QbD.

QbD focuses on building quality into the product through a thorough understanding of both the product and the process, combined with a clear knowledge of manufacturing risks along with appropriate mitigation strategies. This system can aid manufacturers by reducing time to approvals, but can also build significant cost into the manufacturing process through the volume of testing required throughout production. Therefore, the choice of quality system needs to be selected carefully to maximize quality and minimize cost. Once this quality system is in place, potency assays can be developed as in vitro surrogate assays for the eventual efficacy of the therapy in vivo. Because the manufacturing process is now controlled, any variations in results will be due to the potency of the cells themselves, whether it be their potential to form colonies, or their ability to secrete proteins in response to a specific stimulus. In addition to this, there needs to be a strong focus on real-time monitoring of product manufacture and non-destructive, and preferably non-invasive, testing methods that will avoid cell wastage through onerous quality testing regimes. By improving these techniques, the cost of cell- and tissue-based therapies should be reduced, as fewer cells will be needed to provide an effective treatment, as well as to provide the evidence to satisfy the regulator that the treatment will be safe and effective.

4. Manufacturing (systems) issues that remain to be addressed

As has been implied above, manufacturing approaches have now been applied to therapeutic stem cell culture; however, there is still much to do. When systematically considering manufacturing issues, it is helpful to structure the perspective using a hierarchical model of manufacturing. Key areas to be examined should include: manufacturing and supply; strategy and location; organization, operations and people; design and operation; the production system; individual machines and processes; the unit process; quality engineering, metrology and measurement systems analysis, including the anticipated rate of change of precision. Those within the field are currently attempting to identify and create platform technologies and approaches for each level of this hierarchy.

Discussions with industrial collaborators have highlighted the following issues that need to be addressed with respect to the manufacturing of RM products.

(a) Manufacturing and supply; strategy and location; organization, operations and people

The location of manufacturing and the structure of the supply chain are strongly dependent on the product and its method of preservation. Allogeneic products can be made remotely from the patient, much as a conventional pharmaceutical is; however, autologous products, dedicated to a single patient,
are usually processed near to the patient, perhaps within a hospital location or at a centralized location serving a number of clinical sites. The supply process has to fit with the processes available at the ultimate destination in the hospital. Critically, this depends on whether the product is shipped at room temperature, shipped at a cold chain temperature (4°C) or cryopreserved—the latter requiring temporary storage in freezers and subsequent cell resuscitation before reaching a patient. Cell therapies are relatively fragile living materials and require careful management of transport and the supply chain—achieving a consistent and long as possible shelf life is critical to this, as is the design of transport packaging and preservation systems [22,23].

Many companies begin to make their first experimental products within their own or academic laboratories. The key manufacturing strategy decision for any company considering launching an RM product is the ‘make or buy’ decision of whether manufacturing of the product at higher production volumes is outsourced from a contract manufacturing organization at some time during the new product introduction process. The decision made is strongly dependent on the culture and experience of the company in the regulated manufacturing of these living products—such experience is rare, for example, even in large pharmaceutical companies. The decision is also influenced by the high capital cost of the necessary facilities and the regulatory burden associated with their licensing and operation. Critically, unlike a conventional small-molecule pharmaceutical, for RM products, manufacturing defines a significant proportion of cost of goods supplied (COGS); this in turn defines profitability and commercial viability. Control of manufacturing is therefore considered very important by some RM companies; this importance is reinforced by the recognition that ‘the product is the process’ and that business must have absolute confidence that its product is made consistently. It should also be recognized that, even if the process is automated, high added value manufacturing businesses provide significant numbers of jobs in their location. This in turn can lead to regional interventions either to secure manufacturing investments or to ensure that expansion of facilities takes place on an existing site. The financial implications of this can significantly modify location and ‘make or buy’ decisions.

As our discussion had begun to emphasize, the manufacturing of cell therapies is complex, requiring: the handling of living human materials where the product is the living material; the ability to process these materials in a way that satisfies a regulator, including GMP [6] within a quality system; and the ability to address COGS issues. These require the building of significant and often new capabilities within an organization and its people.

(b) Design and operation of facilities; the production system

That the ‘product is the process’ means that there are very significant couplings [24,25] between the design of the product and the design of the process and production system. A critical variable still to be established for RM products is the allowable variation in the critical parameters required for product performance—key components of the specification of the product defining what has to be achieved by manufacturing. In industries with traditional manufacturing processes, we have, over long experience, developed a good understanding of the variation that can be achieved by the process. We have not yet measured this for
the processes required to make RM products—we cannot therefore guide those specifying the products on the variation that manufacturing can achieve and in consequence determine realistic targets for allowable variation.

Whether the product is designed to be used in an allogeneic or an autologous application has a particularly important effect on the design of the manufacturing facility. In the first case, the process is able to build on conventional engineering approaches as exemplified by the factory, or on chemical engineering approaches as exemplified by the process plant. In the second case, however, any manufacturing approach has to be effective for a ‘batch of one’. Furthermore, cell-based products usually require the pre-preparation of cell banks. Depending on the scale required, cell banks may need to be started from a number of cell sources, with each cell source being likely to have slightly different expansion and other characteristics. This highlights that there is actually a continuum of approaches spanning autologous and allogeneic and that the production system must suit the particular position on the continuum of the product that is being processed.

Each process within an RM manufacturing facility will need to be carried out within a controlled environment. Consequently, the facility will have a high capital cost, the capital (and operating) cost for the facility usually being determined by its footprint. Production machines have correspondingly high costs because of the requirements for, for instance, aseptic processing within the machine’s own environment and high levels of cleanliness, because many RM products cannot be terminally sterilized or irradiated subsequent to processing. Process automation is increasingly being applied in the production of RM products because the automation, by eliminating the variation due to operators, brings the process under control. Process control techniques are also being explored and supplied for similar reasons and to reduce variation. Many solutions are as yet bespoke, with only a few standard processing platforms emerging for key process steps—bespoke solutions are expensive. A key technological challenge is to create such process platforms and to design production systems capable of changing with innovations in the process platform.

\[(c)\] Individual machines and processes; the unit process

There are a number of unit processes for the production of cell therapies. Production begins with the isolation of the cell sources from a biopsy—this happens perhaps once for an allogeneic therapy but needs to be carried out for each patient receiving an autologous product. Isolation is necessarily variable depending on source. Cells for therapeutic use are frequently enriched—increasing the proportion of cells with therapeutic value—by sorting or centrifugation, for example. ‘Minimal manipulation’ approaches may be required to comply with the regulatory pathway. Many cell therapy approaches require cell number expansion in culture to generate sufficient cells for therapeutic use—this has been discussed earlier in some depth—and may require a subsequent differentiation step to convert stem cells to the cell of therapeutic interest. Two-dimensional adherent culture ‘on plastic’ or a cell suspension equivalent may not be sufficient to achieve a particular cell type, and three-dimensional structure systems may be required that allow mechanical as well as biological inter-cellular signalling [26].

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As has been indicated earlier, the process and more particularly the culture media used in the process can drive up COGS. Applying techniques for process optimization and improving them to take account of the particular constraints of RM is particularly important both to define practically achievable process variation and to improve processes. A key application area is the design of media formulation. This is a particular target because of its impact on COGS. Media have many components, whose presence is often based on historical precedent, including many that are either naturally derived or their recombinant equivalents. There is a requirement to establish which of the many components are really necessary to achieve a successful culture to specification.

In addition to being able to achieve the biological requirements of each process step, process and production system design also has to be engineered so that it can satisfy the constraints of GMP, in particular that it can be straightforwardly validated. Key engineering considerations in unit process and machine design have to address aseptic processing, particularly the control of viable particulates, the ability to clean the device straightforwardly, and the management of contamination and cross-contamination between batches. The challenges here are exemplified by the standards required for human stem cell processing for therapeutic use—one colony-forming unit (CFU) per cubic metre. While the non-viable particulates allowed are higher, the viable particulate requirement reflects the levels of environmental control conventionally required for semiconductor processing. Many of these issues are addressed by the design of processing consumables—‘plastic ware’—again, however, these add very significantly to COGS.

(d) Quality engineering; characterization, metrology and measurement systems analysis; anticipated rate of change of precision

The core approach of this paper has been to illustrate a quality engineering approach to the manufacturing problem for cell culture—this now needs to be applied to other process steps. Characterization—the equivalent of metrology—is discussed at length later, with examples given in the discussion of the exemplar automated cell culture processes mentioned earlier. The requirements for the level of process precision will inevitably increase—as they have in most other manufacturing fields. Regulatory concerns to define more carefully the products in use are likely to drive this in the short term. In essence, the regulator takes the view that if you can better measure a relevant parameter, then you must. Together with this increase in level of precision, the number of product and process variables for which precision is required will also inevitably increase [27].

5. Alternative process platforms

The evolution of manufacturing systems for therapeutic cell-based products is being driven by investment directed at process scalability, reproducibility and manufacturing cost reduction. The methods to achieve these goals are not yet established owing to limited process understanding and conflicting incentives. Manufacturing technology developers are searching for a process specification generic enough to satisfy an adequately large market to support a commercially viable platform; this is difficult, given the diversity, and sensitivity
to process, of the products. Conversely, product developers are inherently cautious regarding manufacturing process modifications and therefore search for a bespoke manufacturing capability fitting their development specification; they lack the sophisticated QC measures to robustly assess the consequences of process change or the economic models to guide manufacturing technology decisions. These drivers can explain the three major directions of development for the manufacture of cell-based therapies: the technology push to implement existing platforms from the biopharmaceutical or cell product industry (low risk for machine makers who lack a clear specification); the scale-out of existing methods (low risk for therapy developers but limited in scale and control); and the development of niche bioreactors within cell product development companies (bespoke environments but with high development costs, limited technology leverage and little regulatory heritage). These approaches all continue to evolve and can each cite examples of success, but rarely without compromise. In most cases, therefore, the process arising is rarely optimal.

A serious constraint on developing manufacturing solutions is both the diversity and poor definition of manufacturing requirements. Although the products are classified by their cellular component, they are extremely divergent in other characteristics relevant to the manufacturing process. It is misleading to think of development of a single manufacturing technology for RM. For example, the range of production scale requirements is likely to span at least approximately $10^7$–$10^{17}$ cells yr$^{-1}$ for differing therapeutic indications. In the former case, adherent scale-out of current methods may be economically possible and controllable. In the latter case, large high-density systems will be required with associated high-technology monitoring and control. Cost build-up in manufacture is likely to vary between products, potentially changing the emphasis for systems between criteria such as media efficiency and processing footprint. The monitoring and feedback control requirements are likely to vary depending on regulation, control of input materials and critical-to-quality cell characteristics. Cell niche design (or the process design space) in the manufacturing technology will vary depending on the environmental sensitivity of the cell type and the acceptable clinical specification. Almost all of these factors are currently speculative and largely empirical. In addition to these considerations, autologous therapies and allogeneic therapies will require differences in batch size, product separation and other manufacturing parameters. These issues apply to simple cell suspension therapies; three-dimensional tissue-engineered products will add further complexity.

Despite these difficulties, there are significant continuing advances within the three areas of development. The most significant recent progress is the adaptation of hESCs to grow in microcarrier or carrier-free suspension culture format [28,29]. This is an adaptation to available technology from the existing biomanufacturing industry and, for the first time, provides a possible route to produce the cell numbers required for high-dose, high-patient-number therapies. Such systems are generally considered the most economic and easily controllable production method, if viable, and have excellent development of physico-chemical monitoring and a heritage of regulated industrial use [30]. However, they require a significant change from development processes, predominantly designed on planar polystyrene growth surfaces. Many other therapeutic cell types or specific differentiation pathways are unlikely to tolerate this adaptation without losing clinically critical features. For such applications, recent developments include
multi-layered culture systems aimed at increasing the efficiency and scalability of planar adherent manufacture. However, these have been only cautiously adopted owing to insufficient scalability advantages and process control concerns relative to standard planar culture [31]. The automation of standard planar culture methods has shown promise, and improved control, but is afflicted by high entry costs and limited scalability. These planar culture system developments have necessitated parallel development of new non-invasive analysis methods, such as image analysis or spectroscopy [32,33]. Finally, systems have been designed for high-density support of adherent cells, often by virtue of diffuse mass exchange via perfused fibres or suspended particles. However, the complexity and three-dimensional nature of these systems adds challenge in process control, scalability and operational procedures such as cell recovery [34].

Manufacturing for cell-based therapies has, as yet, an unclear future direction and the design of future manufacturing technology will only become more rational as our understanding of the constraints improves. It is likely to involve multiple strands of technology development, a key criterion for which will be reducing the cost of both developing and producing high-quality product. For a significant period, the industry will rely on point solutions for common process areas and adopting technology from related industries. However, as the understanding of manufacturing requirements increases, we anticipate the emergence of increasingly standardized cell therapy manufacturing solutions.

6. Characterization (of product and process): metrology of critical-to-quality characteristics

Characterization is fundamental to the demonstration of adherence to GMP and underpins efforts to obtain product regulatory approval. Specifically, there is a need to demonstrate safety, efficacy and purity of manufacture of regenerative therapies [35]. Safety is of prime concern to ensure therapies do not have a deleterious effect on the patient. Efficacy generally refers to the ability of a product to cause a functional response in the patient, and is related to the potency of the therapy. Purity of manufacture assessments can be used to determine the quality and capability of manufacturing processes. Suitable characterization strategies will depend on the type of product being assessed. Appropriate tools and techniques for the assessment of devices, cell-based therapies and combination products are discussed later and shown in figure 6.

Cell-based products involve the use of either a single cell type or a combination of cell types to provide a positive therapeutic response once implanted in the patient [36]. These cells are hypothesized to act in several ways in vivo, including: repairing the function of the surrounding tissue by acting as replacement cells; secreting various growth-promoting agents to encourage the surrounding cells to act as repair agents; and mobilizing existing niche stem cell populations to migrate to the affected area to repair the site of damage.

(a) Safety

The sterility of cell-based products also needs to be assured. For this purpose, existing quantification techniques for measuring safety parameters such as bacterial and fungal load, virus contamination and endotoxin levels appear to be
<table>
<thead>
<tr>
<th>technique</th>
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<th>efficacy</th>
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Figure 6. The range of techniques that can be applied to analysing regenerative medicine products and how these techniques contribute to the measurement of safety, efficacy and purity of manufacture for medical device-based products, cell-based products and combination products that have both device- and cell-based product characteristics. The additional complexity associated with taking the cell-based approach is highlighted. Also it is important to recognize that a cell-based product may require a purpose-designed delivery device and consequently be regulated as a combination product. PCR, polymerase chain reaction; qPCR, quantitative PCR; GTL, gas to liquid; ELISA, enzyme-linked immunosorbent assay; FRAP, fluorescence recovery after photobleaching; NMR, nuclear magnetic resonance.

Sensitive enough to prevent adverse patient events. Contamination from culture media needs to be tested for and eliminated. In addition, cells are often passaged for extended amplification times, which can lead to cellular senescence, as well as genetic and epigenetic changes [37,38]. Although suitable techniques with the
necessary precision to assess these risks exist, many require long incubation times (e.g. 14 days for bacterial testing), and thus new rapid test methods are required. There are currently a number of methods available to monitor the interactions that occur between cells and their environment \textit{in vitro}, including those based on the assessment of immunochemical and biomolecular markers. However, whereas each has its own merits, no one provides for the non-invasive, rapid, specific and non-destructive analysis of living cells. Of the techniques that might provide some of these attributes, Raman spectroscopy has shown promise, as evidenced by its increasing utility in the life sciences sector in recent years. One of the drivers for this transformation has been the evolution of the instrumentation to the stage where the technique’s potential can be realized in complex solutions.

In the case of allogeneic therapies, where cells are derived from an unrelated donor, the autoimmune response needs to be assessed, particularly to determine whether immuno-suppressants will be needed \cite{39}. Another concern regarding safety measurements is the current inability to remove all unwanted cells \cite{40}. For this to occur, current cell detection methods will need to be sensitive enough to detect as few as tens of cells in a large cell suspension of many million cells. Considering that current advanced cell sorters claim to have a sensitivity limit of only 98 per cent \cite{41}, there needs to be significant advances in this technology to ensure patient safety. However, the multiple purification steps required to ensure a sufficient standard of quality may add significant and unsustainable costs to the product, in terms of reagents, work hours and also initial cell numbers required to give enough purified product.

The role of these cell-based products, and, in particular, the evaluation of human mesenchymal stem cells for cell-based therapies in tissue injury and degenerative diseases, requires rapid and accurate evaluation of cell source quality at a level that satisfies the stringent guidelines laid down by regulators. DNA microarray technology can be used as a technique to assess relevant cellular pathways, such as senescence, as well as the recognized genetic changes that have been shown to occur with the extensive \textit{ex vivo} expansion that is a prerequisite to obtain the cell numbers that are necessary for human cell-based therapy protocols.

Understanding the genes that dictate the special properties of stem cells has implications for both stem cell biology and RM. Microarray analysis measures the global expression of genes and can thereby provide an insight into the genetic processes expressed in stem cells. Microarray data from tissue-specific reference files can be compared with microarray data of stem cells, making it possible to identify similarities in particular geno/phenotypes while also revealing other novel signatures. Hence, microarray analysis can be used to better understand stem cell differentiation and to make a significant contribution to the biosafety issues of future cell-based therapies and RM products.

\textit{(b) Efficacy}

Proper characterization and understanding of cell function is the most important factor in determining whether a cell-based therapy will function effectively \textit{in vivo}. However, as complete characterization of some cell processes is still unknown, it is very difficult to accurately predict every consequence of a particular cell once placed within a patient. Efficacy tests should always be cell-specific, and ideally test the function of the cell that will be required in an
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in vivo situation. In some cases, in vitro assays can be used as surrogate measures [42]. Such measures can often provide more sensitive and useful data than in vivo trials in an animal model [43]. Clinical endpoints have to be defined at an early stage to allow for proper evaluation of cell-based therapies in patient trials. For cell-based treatments, the endpoints will have to be patient-specific, relative to the age of the patient, as well as being related to when the disease was diagnosed, to account for how any existing complications will affect the treatment outcome [3]. However, owing to the complexity of several clinical applications, optimal efficacy measurements may evolve over time due to improved clinical information being available to inform the decision. Quantifying efficacy measurements is another challenge for product developers, but can only be focused upon once appropriate specific functional assays have been identified. With regard to in vitro testing, the sensitivity of measurements will always depend on the detection system that is being used. Therefore, the design of the functional assay, and identifying its key output requirements, is likely to be more challenging than the sensitivity of the detection system itself.

(c) Purity of manufacture

Many scientists consider cell viability as the primary factor for determining the cellular effect of these advanced therapies once implanted in the body. This can be measured using various simple assays [44] as well as more sophisticated measures of cell metabolic activity [45]—with both providing quantitative data. However, most of these viability percentages simply measure how many cells are ‘alive’, not how many cells are actively metabolizing and playing a productive role in their environment. In terms of these advanced therapies, identifying the cell phenotype, function and mode of action will be critical for specific clinical applications. Biomarkers may be important in distinguishing different cell phenotypes, but they do not always provide a correlation to cell function [46]. Therefore, in terms of cell-based therapies, how the cells act in the body might be more important than their immunophenotype in vitro. Cellular morphology can also be used to analyse cell populations, using various microscopy techniques to determine if cells appear true to their phenotype [47,48]. In all these cases, though, the safety and efficacy of the product is being determined essentially by implication rather than by understanding of its mode of action.

To meet product specifications, cell number and cell viability measurements must be accurate so that specific product dose can be determined. However, the accuracy of cell counts can also be variable, from both manual and automated systems [49]. Therefore, while automated cell counting systems have several advantages over manual counting, improvements still need to be made. Of note, the final product acceptance range should be carefully considered, as the limitations of the instrument must be taken into account. This may include the tolerancing of specifications, as well as factoring in measurement system errors that may contribute to misleading data. An extremely narrow range might cause products to be rejected due to these inaccuracies rather than to actual product failure.

There are many challenges associated with characterizing regenerative therapies. From a regulatory perspective, these advanced treatments not only must be safe and effective for their designated indication, but also must be
made by high-quality manufacturing processes. While a number of existing technologies are available to characterize regenerative therapies, many are time-consuming, expensive and disruptive. In general, there is a need to identify suitable surrogate in vitro tools, define clinical endpoints early on and develop product specifications that can be met by current manufacturing processes. In particular, characterization methods should allow the establishment of limits on product specification and be able to resolve whether process changes have impacted the specification [50]. In conclusion, there is a need for improved stakeholder involvement in product characterization, to allow the manufacture of the best possible regenerative therapies within the shortest time frame and at an economic cost.

7. Conclusions

This paper has described the challenges associated with the precision manufacturing of living stem cell therapies. In particular, it has summarized the results of work that has taken an automation and improvement approach to removing the process variation inherent in manual operations for cell number expansion and the subsequent improvement of the biological process to a higher precision by the application of process quality engineering techniques in this new domain. This approach has allowed the measurement of the current achievable variation in the underlying processes and assisted in the reduction of COGS, critical to the uptake of these new therapeutics. With improved understanding of the evolutionary trajectory of processing platforms, and clearer perspectives on the measurement and characterization techniques that will be applied in the coming decade, the next step is to take QbD, design-led, approaches earlier in the new product introduction process. Such approaches are challenging in this domain because of: the requirement to understand and work around the natural variation in the living, plastic, product; the need to apply biological characterization methods that are practicable and demonstrable surrogates for measuring the way the product behaves in vivo; and the need to recognize and accommodate the regulatory constraints on manufacturing and on clinical trials. With more experience in manufacturing, there will be increasing understanding of the variation that can be achieved from the manufacturing process and consequently the tolerance ranges it can deliver. The application of such therapies will give increased understanding of the variation allowable in the product while ensuring therapeutic effect. Matching what is required with what can be achieved and the inevitable increases in precision demanded with time will identify the requirements for new, more precise processing platforms. Increased understanding of the underlying biological processes and their interaction with approaches to manufacturing technology will be needed to create the step change required for the next generation of scalable precision production systems capable of more than replicating and incrementally improving the performance of the human operator.

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