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 the 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.

Key words: Regenerative Medicine, Stem Cells, Manufacturing, Automation, Characterisation.

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.

Regenerative medicines replace or regenerate human cells, tissues or organs to restore or establish normal function [1]. They have the potential to revolutionise methods of health care 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 $1billion; a large number of products are in clinical development having real long term potential for public health benefit. The market shift to commercial products based on stem cells is likely to mature in the next 5 to 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 aging 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, 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 continuous increase in demand for novel treatment strategies to treat tissue or organ damage, due to the rise in the 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, see [4] and [5]. Particularly important is the influence of industry specific regulation on the new product introduction process, focussed on ensuring safety and effectiveness of new products (for instance the requirements for clinical trials) and on the manufacturing process itself, with the requirement for GMP and cGMP, (current) Good Manufacturing Practice [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 industrialised 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 timescales 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 also require a transition in their manufacturing philosophy from biology carried out at the laboratory bench by experts to an engineered production method that frequently automates a process which initially shows many of the features of a craft [8].

As we have described above, only over very recent years has stem cell culture begun to be approached from a cost-effective manufacturing perspective – 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 Ramchandran Jaikumar in 1988, published posthumously by his colleague Robert Bohn [10], 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 more completely specify manufacturing procedures. This is exemplified by the statistical quality control and process design techniques pioneered by Shewart in the US (at AT&T) and Fisher in the UK. Deming applied these widely to great effect in both manufacturing and supply in the US during the second world war and with Japanese industry in their post war rebuilding [11], this approach ultimately leading to "six sigma" philosophies emphasising the control of variation and quality by design. 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 domains including the small-arms and the automotive industry, in electrical and electronic engineering domains including semi-conductors and complex assembly, and in the chemical engineering domains of the bio-pharmaceuticals 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 bio-manufacturing 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, emphasising 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 (C_p, essentially the "centred" ratio of specification to process output, and C_{pk}, the "un-centred" ratio, to ultimately parts per million (ppm) failure rates), with minimum waste and that have as their ultimate goal "quality by design" 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 of the process steps, where each process step has been designed to be capable. Such consistent production and the ability 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 characterise 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 [12]. 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 quality by design. 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 it did for the conventional manufacturing industries.

It is important to recognise 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), optimised (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 lab-based experimental disciplines to a nascent industry. Projected clinical demand indicates that within the next decade this industry will need to responsively and economically provide 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 due 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 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 that are well characterised 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

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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 characterised 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. In addition to the technical challenges outlined, manufacturing equipment, processes and facilities must be compliant with good manufacturing practice (GMP), the stringent regulatory framework controlling therapeutic product manufacture.

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. 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 standardised processes in many manufacturing industries. Automation also enables scale-out, replication of the unit process of production, 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 manufacture 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 bar code tracked adherent 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 exists) feasible. The commercial availability of the machine offers a significant advantage over prior non-commercialised or non-scalable bespoke robotic developments [13, 14].

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 human mesenchymal stem cells [15] and human embryonic stem cells [16] 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 products including neural stem cells [17] and smooth muscle progenitor cells. The cell types chosen were intentionally diverse. They covered examples of both autologous

and allogeneic 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, see figure 1.

Insert Figure 1

In parallel with the program 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 to automated production for an exemplar cell line manufacture process [18]. The process capability result is important for two main reasons. Firstly, it shows 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 failures rates are currently high, costly and add risk.

Insert Figure 2

(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 optimised automated manufacturing processes. A 'six sigma' type approach was used, figure 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 of process performance, a *data driven* process improvement intervention and, finally, process monitoring to demonstrate maintained control post improvement. 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 data for achieving an optimal process as some input factors cannot be optimised independently. Figure 5 outlines the DOE analysis of critical process input variables for the automated production of mesenchymal stem cells [19]. 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 optimising the culture of human embryonic stem cells (hESC) with a particular focus on controlling cost of goods [20].

Insert Figure 3
Insert Figure 4
Insert Figure 5

(c) Achieving GMP standards of cell manufacture

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 laboratory 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 recognising 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 <3520 in a size range of 0.5 µm and larger. Furthermore, a quality control (QC) plan also needs to be put into place to ensure proper manufacturing oversight, as well as providing 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.

(d) The way forward

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 guidance document, potency assays must be specific, quantitative, meet pre-defined criteria, include appropriate standards and controls, be fully validated and measure both identity and strength of all active ingredients. Therefore, due 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 quality by design (QbD).

QbD focuses on building quality into the product through a thorough understanding of both the product and 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 carefully selected to maximise quality and minimise 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*. Since 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 stimuli. 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; organisation, 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 highlight the following issues that need to be addressed with respect to the manufacturing of RM products.

(a) Manufacturing and supply; Strategy and location;
Organisation, operations and people

The location of manufacturing and 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 centralised 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 (4ºC) 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 [21, 22].

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 is outsourced from a contract manufacturing organisation 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 recognised 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 to either secure manufacturing investments or to ensure 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 emphasise, 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 Good Manufacturing Practice [6] within a Quality System; and the ability to address COGs issues. These require the building of significant and often new capabilities within an organisation 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 [23, 24] 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 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 in 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 require 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 creating such process platforms and designing 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 happening perhaps once for an allogeneic therapy but requiring 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 above 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, see [25].

As has been indicated above, the process and more particularly the culture media used in the process can drive up COGS. Applying techniques for process optimisation and improving them to take account of the particular constraints of RM is particularly important to both 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 has many components, often whose presence is 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 straight forwardly validated. Key engineering considerations in unit process and machine design have to address aseptic processing, particularly the control of viable particulates, the ability to straight forwardly clean the device, 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 semi-conductor 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, characterisation, metrology and measurement systems analysis, the anticipated rate of change of precision

The core approach of this paper has been to present a quality engineering approach to the manufacturing problem for cell culture – this now needs to be applied to other process steps. Characterisation – the equivalent of metrology – is discussed at length below, with examples given in the discussion of the exemplar automated cell culture processes above. The

requirements for the level of process precision will inevitably increase – as they have in most other manufacturing fields. Regulatory concerns to more carefully define 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 [26].

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 due 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 quality control 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); 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 and limited technology leverage). 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 $\sim 10^7$ to 10^{17} cells/yr 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; 3D 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 human embryonic stem cells to grow in microcarrier or carrier-free suspension culture format [27, 28]. This is an adaptation to available technology from the existing bio-manufacturing 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 mode, if viable, and have excellent development of physicochemical monitoring and a heritage of regulated industrial use [29]. 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 these applications recent developments include multilayered culture systems aimed at increasing the efficiency and scalability of planar adherent manufacture. However, these have been only cautiously adopted due to insufficient scalability advantages and process control concerns relative to standard planar culture [30]. Further advances in 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 [31, 32]. 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 3D nature of these systems adds challenge in process control, scalability and operational procedures such as cell recovery [33].

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 standardised cell therapy manufacturing solutions.

6. Characterisation (of product and process)
– metrology of critical-to-quality characteristics

Characterisation is fundamental to the demonstration of adherence to good manufacturing practice (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 [34]. 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 characterisation 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 below and shown in figure 6.

Insert 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 [35]. These cells are hypothesised to act in several ways *in vivo*, including: repairing the function of the surrounding tissue by acting as replacement cells; by secreting various growth-promoting agents to encourage surrounding cells to act as repair agents; and, by mobilising existing niche stem cell populations to migrate to the affected area to repair the site of damage.

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(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 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 [36, 37]. Although suitable techniques with the necessary precision to assess these risks exist, many require long incubation times, for example 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 with their environment *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 realised 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 if immuno-suppressants will be needed [38]. Another concern regarding safety measurements is the current inability to remove all unwanted cells [39]. 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. Considering that current advanced cell sorters claim to only have a sensitivity limit of 98% [40], 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 accurate evaluation of cell source quality at a level that satisfies the stringent guidelines laid down by the regulators. DNA microarray technology can be used as a technique to assess relevant cellular pathways, such as senescence, as well as the recognised genetic changes that have been shown to occur with the extensive *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 insight into the genetic processes expressed in stem cells. Microarray data from tissue-specific reference files can be compared to 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 make a significant contribution to the biosafety issues of future cell-based therapies and RM products.

(b) Efficacy

Proper characterisation and understanding of cell function is the most important factor in determining whether a cell-based therapy will function effectively *in vivo*. However, as complete characterisation 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 *in vivo* situation. In some cases, *in vitro* assays can be used as surrogate measures [41]. Such measures can often provide more sensitive and useful data than *in vivo* trials in an animal model [42]. 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 end-points 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, due 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 focussed on once appropriate specific functional assays have been identified. With regards 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 [43] as well as more sophisticated measures of cell metabolic activity [44] - with both providing quantitative data. However, most of these viability percentages simply measure how many cells are "alive", not how many cells are actively metabolising 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 [45]. 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 [46, 47]. 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 [48]. 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 actual product failure.

There are many challenges associated with characterising regenerative therapies. From a regulatory perspective, these advanced treatments must not only be safe and effective for their designated indication, but must also be made by high quality manufacturing processes. Whilst a number of existing technologies are available to characterise 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 conclusion, there is a need for improved stakeholder involvement in product characterisation, to allow the

manufacture of the best possible regenerative therapies within the shortest timeframe 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 summarised 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 cost of goods supplied, critical to the uptake of these new therapeutics. With improving understanding of the evolutionary trajectory of processing platforms, and clearer perspectives on the measurement and characterisation techniques that will be applied in the coming decade, the next step is to take quality by design, 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 characterisation methods that are practical and demonstrable surrogates for measuring the way the product behaves *in vivo*; and because of the need to recognise 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.

Acknowledgements

References

1   Mason, C. & Dunnill, P. 2008 A brief definition of regenerative medicine. *Regenerative Medicine* **3**, 1-5.

2 Williams, D. J., Archer, J. R. & Dent, A. 2010 *Building a viable regenerative medicine industry, A guide for stakeholders*, Loughborough: Wolfson School of Mechanical and Manufacturing Engineering Loughborough University.

3 Singh, P. & Williams, D. J. 2008 Cell therapies: realizing the potential of this new dimension to medical therapeutics. *Journal of Tissue Engineering and Regenerative Medicine* **2**, 307-319.

4 Williams, D. J., Ratchev, S., Chandra, A. & Hirani, H. 2006 The Application of Assembly and Automation Technologies to Healthcare Products. *Annals of CIRP* **55/2**, 617-642.

5 Hourd, P. C. & Williams, D. J. 2006 Success in Healthcare Technology Businesses: Coordination of the Value Milestones of New Product Introduction, Financial Stakeholders and Business Growth. *Innovation: Management, Policy and Practice* **8**, 229-247.

6 MHRA 2007 *Rules and Guidance for Pharmaceutical Manufacturers and Distributors.* London: Crown Copyright.

7 Schellekens, P., Rosielle, N., Vermeulen, H., Vermeulen, M., Wetzel, S. & Pril, W. 1998 Design for Precision: Current Status and Trends. *Annals of CIRP* **47/2**, 557-586.

8 Archer, J. R. & Williams, D. J. 2005 Why tissue engineering needs process engineering. *Nature Bio-technology* **23**, 1353-1355.

9 Babbage, C. 1832 *On the economy of machinery and manufactures.* London: Charles Knight.

10 Bohn, R. & Jaikumar, R. 2005 *From Filing and Fitting to Flexible Manufacturing.* Hanover MA: now Publishers.

11 Gabor, A. 1990 *The man who discovered quality.* New York: Random House.

12 Pisano, G. P. 1997 *The Development Factory.* Boston, MA: Harvard Business School Press.

13 Joannides, A., Fiore-Hériché, C. & Westmore, K. 2006 Automated mechanical passaging: a novel and efficient method for human embryonic stem cell expansion. *Stem Cells* **24**, 230-235.

14 Terstegge, S., Laufenberg, I. & Pochert J. 2007 Automated maintenance of embryonic stem cell cultures. *Biotechnology and Bioengineering* **96**, 195-201.

15 Thomas, R. J., Chandra, A., Liu, Y., Hourd, P. C., Conway, P. P. & Williams D. J. 2007 Manufacture of a human mesenchymal stem cell population using an automated cell culture platform. *Cytotechnology* **55**, 31-39.

16 Thomas, R. J., Anderson, D., Chandra, A., Smith, N. M., Young, L. E., Williams, D. J. & Denning C. 2009 Automated, scalable culture of human embryonic stem cells in feeder-free conditions. *Biotechnology and Bioengineering* **102**, 1636-1644.

17 Thomas, R. J., Hope, A. D., Hourd, P. C., Baradez, M., Miljan, E. A., Sinden, J. D. & Williams, D. J. 2009 Automated, serum-free production of CTX0E03: a therapeutic clinical grade human neural stem cell line. *Biotechnology letters* **31**, 1167-72.

18 Liu, Y., Hourd, P. C., Chandra, A. & Williams, D. J. 2009 Human cell culture process capability: a comparison of manual and automated production. *Journal of Tissue Engineering and Regenerative Medicine* **41**, 45-54.

19 Thomas, R. J., Hourd P. C. & Williams D. J. 2008 Application of process quality engineering techniques to improve the understanding of the *in vitro* processing of stem cells for therapeutic use. *Journal of Biotechnology* **136**, 148-155.

20 Ratcliffe, E., Hourd, P. C., Guijarro-Leach, J., Rayment, E. A., Williams, D. J. & Thomas, R. J. 2011 *Application of Response Surface Methodology to scalable, automated expansion of human embryonic stem cells for prediction of a high cell quality, economic production strategy.* Loughborough: RGC Working Paper/103.0/10.

21   Nikolaev, N. I., Liu, Y., & Williams, D. J. 2010 Effects of Dynamic Mechanical Forces on Potential Cell Therapy Products in Cold Transportation. In, WCB 2010, IFMBE Proceedings **31** (eds Lim, C. T. and Goh, J.C.H.) pp 1137-1140. Berlin: Springer.

22   Whiteside, T. L., Griffin, D. L., Stanson, J., Gooding, W., McKenna, D., Sumstad, D., Kadidlo, D., Gee, A., Durett, A., Linbald, R., Wood, D., & Styers, D. 2011 Shipping of therapeutic somatic cell products, Cytotherapy **13**, 201–213.

23   Suh, N. P. 1990 *The Principles of Design.* New York: Oxford University Press.

24   Suh, N. P. 2010 *Axiomatic Design, Advances and Applications.* New York: Oxford University Press.

25   Niang, M. W. & Williams, D. J. 2011 Invited review: 3D Culture and Bioreactors for Cellular Therapies. *Cytotherapy* at press.

26   Ratcliffe, E., Thomas, R. J. & Williams, D. J. 2011 Invited Review: Current Understanding and challenges in bioprocessing of stem cell-based therapies for regenerative medicine. *British Medical Bulletin* at review.

27   Steiner, D., Khaner, H., Cohen, M., Even-Ram, S., Gil, Y., Itsykson, P., Turetsky, T., Idelson, M., Aizenman, E., Ram, R., Berman-Zaken, Y. & Reubinoff, B. 2010 Derivation, propagation and controlled differentiation of human embryonic stem cells in suspension. *Nature Biotechnology* **28**, 361–364.

28   Oh, S. K. W., Chen, A. K., Mok, Y., Chen, X., Lim, U., Chin, A,. Choo, A. B. H. & Reuveny S. 2009 Long-term microcarrier suspension cultures of human embryonic stem cells. *Stem Cell Research* **2**, 219-230.

29   Becker, T., Hitzmann, B., Muffler, K., Pörtner, R., Reardon, K. F. & Stahl, F., Ulber, R. 2007 Future aspects of bioprocess monitoring. *Advances in Biochemical Engineering Biotechnology* **105**, 249-93.

30   Auniņs, J. G., Bader, B., Caola, A., Griffiths, J., Katz, M., Licari, P., Ram, K., Ranucci, C. S. & Zhou, W. 2003 Fluid mechanics, cell distribution, and environment in CellCube bioreactors. *Biotechnology Progress* **19**, 2-8.

31   Narkilahti, S., Rajala, K. & Pihlajamäki, H. 2007 Monitoring and analysis of dynamic growth of human embryonic stem cells: comparison of automated instrumentation and conventional culturing methods. *Biomedical Engineering Online* **6**, 11.

32   Downes A., Mouras R. & Elfick A. 2010 Optical spectroscopy for non-invasive monitoring of stem cell differentiation. *Journal of Biomed Biomedicine and biotechnology*, http://dx.doi.org/10.1155/2010/101864.

33   Wang, D., Liu, W., Han, B. & Xu, R. 2005 The bioreactor: a powerful tool for large-scale culture of animal cells. *Current Pharmaceutical Biotechnology* **6**, 397-403.

34   Rayment, E. A. & Williams, D. J. 2010 Concise Review: Mind the Gap: Challenges in Characterizing and Quantifying Cell- and Tissue-Based Therapies for Clinical Translation. *Stem Cells* **28**, 996-1004.

35   Preti, R. A. 2005 Bringing safe and effective cell therapies to the bedside. *Nature Biotechnology* **23**, 801-804.

36   Lepperdinger, G., Brunauer, R., Jamnig, A., Laschober, G. & Kassem, M. 2008 Controversial issue: Is it safe to employ mesenchymal stem cells in cell-based therapies? *Experimental Gerontology* **43**, 1018-1023.

37   Mannello, F. & Tonti, G. A. 2007 Concise Review: No Breakthroughs for Human Mesenchymal and Embryonic Stem Cell Culture: Conditioned Medium, Feeder Layer, or Feeder-Free; Medium with Foetal Calf Serum, Human Serum, or Enriched Plasma; Serum-Free, Serum Replacement Nonconditioned Medium, or Ad Hoc Formula? All That Glitters Is Not Gold! *Stem Cells* **25**, 1603-1609.

38 Li, L., Baroja, M. L., Majumdar, A., Chadwick, K., Rouleau, A., Gallacher, L., Ferber, I., Lebkowski, J., Martin, T., Madrenas, J. & Bhatia, M. 2004 Human Embryonic Stem Cells Possess Immune-Privileged Properties. *Stem Cells* **22**, 448-456.

39 Halme, D. G. & Kessler, D. A. 2006 FDA Regulation of Stem-Cell-Based Therapies. *New England Journal of Medicine* **355**, 1730-1735.

40 Bd Biosciences. 2008. BD FACSAria Cell-Sorting System [Online]. Available:http://www.bdbiosciences.com/immunocytometry_systems/products/display_ product.php?keyID=53 [Accessed 21 July, 2009].

41 Collins, N. H. 2009 Product Review, Release, and Administration. In: GEE, A. (ed.) *Cell Therapy: cGMP Facilities and Manufacturing*. New York: Springer.

42 Klausner, M., Ayehunie, S., Breyfogle, B. A., Wertz, P. W., Bacca, L. & Kubilus, J. 2007 Organotypic human oral tissue models for toxicological studies. *Toxicology in Vitro* **21**, 938-949.

43 Liu, Y., Hourd, P. C. & Williams, D. J. 2007 Measurement System Capability: A Comparison of Manual and Automated Cell Counting Systems, *Tissue Engineering* **13**, 1722.

44 Berridge, M. V. & Tan, A. S. 1993 Characterization of the Cellular Reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT): Subcellular Localization, Substrate Dependence, and Involvement of Mitochondrial Electron Transport in MTT Reduction. *Archives of Biochemistry and Biophysics* **303**, 474-482.

45 Tura, O., Barclay, G. R., Roddie, H., Davies, J. & Turner, M. L. 2007 Absence of a relationship between immunophenotypic and colony enumeration analysis of endothelial progenitor cells in clinical haematopoietic cell source. *Journal of Translational Medicine* **5**, 1-10.

46 Dobra, K., Andäng, M., Syrokou, A., Karamanos, N. K. & Hjerpe, A. 2000 Differentiation of Mesothelioma Cells Is Influenced by the Expression of Proteoglycans. *Experimental Cell Research* **258**, 12-22.

47 Walen, K. H. 2005 Budded karyoplasts from multinucleated fibroblast cells contain centrosomes and change their morphology to mitotic cells. *Cell Biology International* **29**, 1057-1065.

48 Brinkmann, M., Lütkemeyer, D., Gudermann, F. & Lehmann, J. 2002 New technologies for automated cell counting based on optical image analysis `The Cellscreen'. *Cytotechnology* **38**, 119-127.

Figure 1. This figure shows 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 to an automated 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 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.

Figure 2. This figure shows 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). 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.

Figure 3. Six sigma quality principles and statistical methodology have been 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 the following 5 phases: define, measure, analyse, improve and control (DMAIC) is shown here to emphasise 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.

Figure 4. This roadmap illustrates 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 was constructed to identify key process input variables (KPIV). The measurement system for a selected key process output variable (KPOV = Cell Yield) was validated (Gage 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. This data was 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 Cp>1.

Figure 5. This figure shows 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 impact on the outputs. The data ranks the input parameters by statistical significance of effect on the process outputs (growth and a surface marker shown) but also shows where critical interdependencies exist. This is a necessary and efficient method to achieve process characterisation and optimisation.

Figure 6. This figure shows 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 figure highlights the additional complexity associated with taking the cell based approach. Also it is important to recognise that a cell based product may require a purpose designed delivery device and consequently be regulated as a combination product. (Acronym definitions: PCR - polymerase chain reaction and q – quantitative PCR, GTL – gas to liquid, ELISA - Enzyme-linked immunosorbent assay, FRAP - Fluorescence recovery after photobleaching).
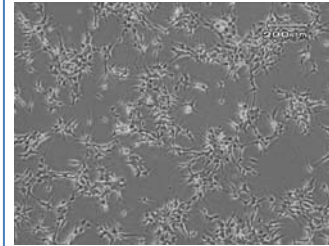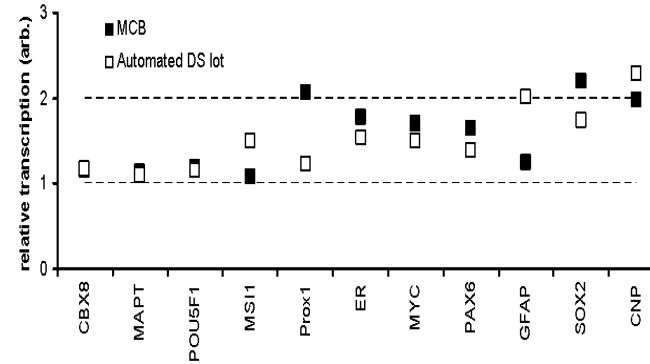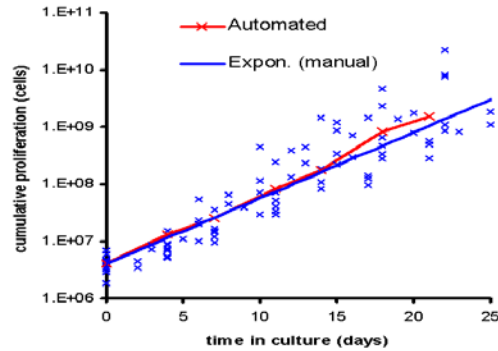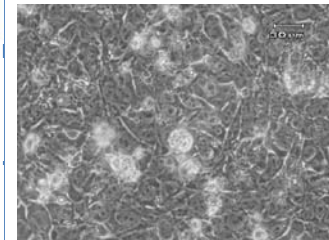
Quality assay Cell type	Growth rate	Surface proteins/gene expression /stability	Morphology
Neural Stem Cells			
Embryonic Stem Cells			
Mesenchymal Stem Cells			

Figure 1

Figure 2

| Define | Measure | Analyse | Improve | Control |

Objectives

Define project goal, scope, process and customers (internal & external)	Measure status of current process operation & quantify the problem	Analyse sources and potential root causes of process variability and/or defects	Determine solution alternatives that address root causes	Maintain improvement by monitoring the process

Activities

Define customer & business requirements	Develop detailed process map. Identify key process steps	Identify value/non-value added process steps	Create process model; Y=f(x), to relate KPIV with KPOV	Validate & Implement monitoring & control systems
Identify and quantify the improvement opportunity	Identify key process output variables (KPOV). Define specification limits	Develop hypotheses: causes of variability (common or special cause) and/or defects	Confirm which KPIV give the most leverage on the outputs	Implement Statistical Process Control
Establish performance standards for CTQs	Define relationship between KPOV and CTQs	Identify critical KPIV for potential leverage (vital X's)	Define Control Limits for KPIV	Measure short term process capability (KPOVs) (KPIV)
Define customer critical-to-quality characteristics (CTQs) to be improved (defects)	Identify key process input variables (KPIV)	Analyse & identify the KPIV that have greatest impact on KPOV; develop Y=f(x) relationship	Determine optimum levels for KPIV to achieve desired KPOV and CTQ	Verify the business benefits (increased profitability/savings)
Develop top level process map	Validate measurement systems for KPOV		Validate measurement systems for KPIV	
Create the Project Charter.	Measure baseline stability & capability (KPOVs)		Implement (adjust the process), confirm & verify improvement	

Toolkit

Project Charter	Detailed Process Maps	Cause &Effect (C&E) Fishbone matrix	Design of Experiments (DOE)	Process Capability Measurement (CpK)
Process flow diagram (SIPOC)	Data Collection Plan	Pareto Charts	Risk Analysis (FMEA, FMECA, FTA, PHA, HACCP, HAZOP)	Control Plans
Kano Analysis	Run Charts	Graphical Data Analysis	Measurement System Analysis (Gage R&R)	Statistical Process Control Charts
Voice of the Customer (VOC)	Process Capability Measurement (CpK)	Risk Analysis (FMEA, FMECA, FTA, PHA, HACCP, HAZOP)	Robust Design	Time Series Charts
Voice of the Business (VOB)	Measurement System Analysis (Gage R&R)	Root Cause Analysis	Response Surface Methodology (RSM)	
Quality Function Deployment (QFD)	Cause &Effect (C&E) Fishbone diagrams	Hypothesis Testing		
CTQ tree	Pareto Charts	Variance, Regression & Correlation Analysis		
Historical Data Plots		Design of Experiments (DOE)		

Figure 3

Manual   **Automated**

Process Map

71 KPIV

Cause & Effect Matrix
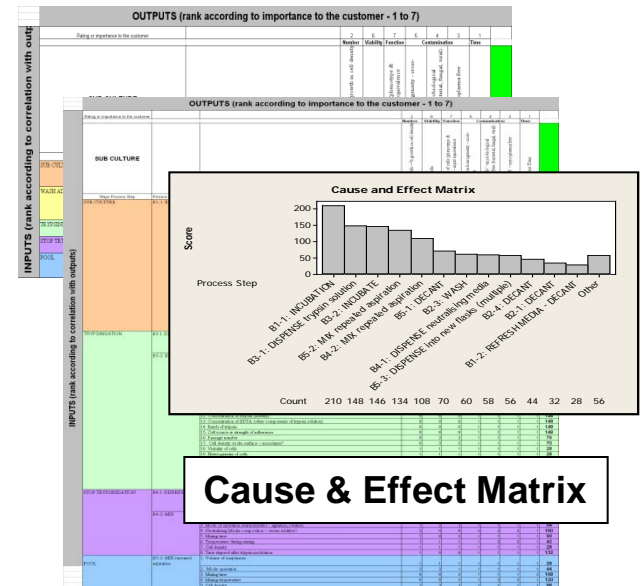
Initial Evaluation of Key Inputs & Outputs
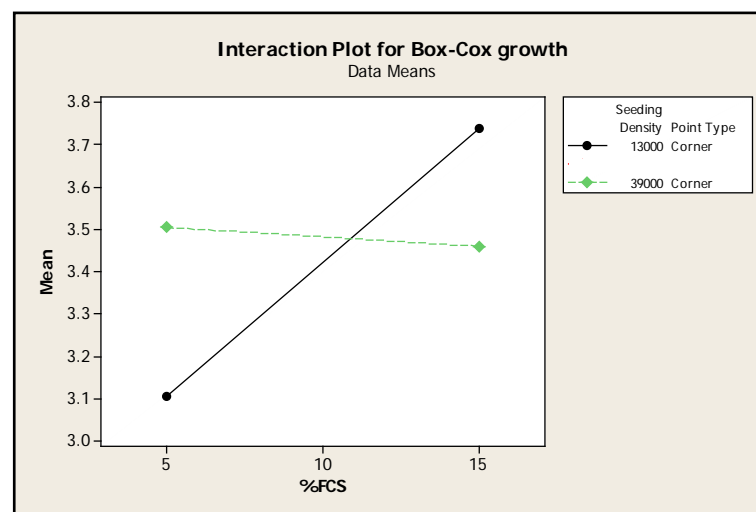
Baseline Measurement & Process Capability Analysis

- **6 Key Process Steps**
- **30 KPIV**

KPOV
- **Cell number**
- **Cell viability**
- **Cell function**
- **Cell safety**
- **Time & cost**

1

<u>Figure 4</u>

Figure 5

Technique	Analysis Capability	Device	Cell-based	Combination	Safety	Efficacy	Purity of Manufacture
Broth & agar	Bacterial growth	✓	✓	✓	✓		
PCR assay	Bacterial and viral presence	✓	✓	✓	✓		
Blood pathology testing	Donor contamination of biologics		✓	✓	✓		
***In vitro* culture**	Cytotoxicity, endotoxin	✓	✓	✓	✓		
GTL banding of metaphase spreads	Karyotyping		✓	✓	✓		
Fluorescence *in situ* hybridisation	Karyotyping		✓	✓	✓		
Flow cytometry	Cell number, viability and phenotyping		✓	✓			✓
Animal models	Toxicology, immune response, cell effectiveness	✓	✓	✓	✓	✓	✓
ELISA	Cell effectiveness		✓	✓		✓	
Trypan blue assay	Cell number and viability		✓	✓			✓
Metabolic assay	Cell viability		✓	✓			✓
Optical microscopy (including fluorescence)	Morphology, structure and function	✓	✓	✓		✓	✓
Histology	Morphology, structure and composition	✓	✓	✓	✓	✓	✓
Diffusion cell	Diffusion of nutrients and waste	✓		✓		✓	
FRAP	Diffusion of nutrients and waste	✓		✓		✓	
NMR techniques	Spectroscopy: composition; Imaging: structure and diffusion	✓		✓		✓	✓
Compression testing	Mechanical properties	✓		✓	✓	✓	✓
Optical spectroscopy (Including Raman)	Composition, Cell Viablity	✓	✓	✓		✓	✓
DNA Microarray Technology - qPCR	Gene expression, SNP analysis		✓	✓	✓	✓	✓

Figure 6