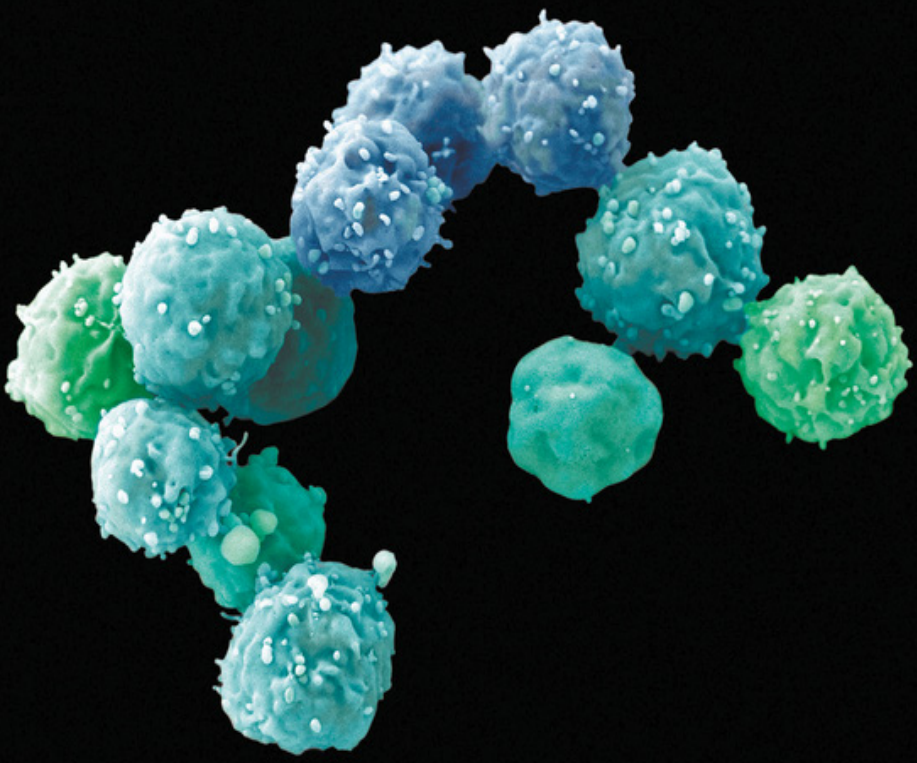

BIOPROCESSING FOR CELL-BASED THERAPIES

EDITED BY CHE J. CONNON

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Preface

Cell-based therapy is an exciting and rapidly developing field of medicine for the 21st century. However, this new paradigm brings new challenges in regulation and production, which requires cross-disciplinary approaches and greater collaboration between clinicians, academics and industrial scientists. This new alliance is reflected in the range of disciplines from which the distinguished contributing authors have been drawn to create this book, including Industry (EMD Millipore Corp., Athersys Inc., ReGenesys, Asymptote Ltd), Governmental (Process Innovation Centre – Biologics, Cell Therapy Catapult, UK Stem Cell Bank) and Academic Institutions (The Francis Crick Institute, and Newcastle, Birmingham, Loughborough, Aston, Edinburgh and Heriot-Watt universities). Together they represent the cutting edge, industrially focused frontline of bioprocessing for cell-based therapies.

The book is composed of eight chapters beginning with an introductory chapter that sets out the history of cell therapy leading up to the current and future challenges associated with the manufacture and distribution of this relatively new class of therapeutic modality. This includes a detailed discussion on the advantages and disadvantages of the one-one and one-many approaches in cell-based therapy. The book then moves into a series of chapters focusing on recent technical developments in bioprocessing for cell-based therapies. Chapter 2 discusses the use of stirred tank bioreactors for hMSC cultivation, specifically engineering requirements such as mixing phenomena, oxygen and heat transfer rates, metabolic demands of the cells and particular problems associated with adherent cells. Chapter 3 follows this theme by focusing on the important topic of cell characterization during scale-up of hMSCs. We are informed that

to guarantee product consistency there needs to be uniformity in identity, maintenance of a unique product character and crucially demonstration of a consistent and therapeutically relevant product potency. Chapter 4 also focuses on hMSC culture, asking if we need to consider scale-up or scale-out. This chapter, then proceeds to highlight the technical difficulties involved in moving bioprocessing research from the lab-scale to large-scale, covering the challenges associated with choice of microcarriers and spinner flasks, then removing cells from chosen microcarriers. Chapter 5 continues by describing the importance of cell separation for cell-based therapies with an in-depth focus on the current methods for mammalian cell separation and their suitability for application in large-scale cell purification for clinical application. We then move on to Chapters 6 and 7, which discuss the importance of storage and transport of the cellular products. Chapter 6 focuses on cryopreservation, how to limit cell damage, large volume freezing, approaches to biobanking and regulatory requirements. Chapter 7 takes an interesting departure from cryopreservation, instead focusing on cold or hypothermic cell preservation, in which the logistics of short-term storage are described as are the use of hydrogels as a novel storage medium. The final chapter (Chapter 8) focuses on where the advancements in bioprocessing end up, such as in the clinical application of cell-based therapies. This chapter includes a case study describing the development of a new therapy against the background of a rapidly changing regulatory environment and the challenges this poses, before bringing the therapy successfully to the clinic.

1

Overview of the Cell Therapy Field

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1.1 The Context of Cell Therapies and Their Manufacturing Challenges

Cell therapies are not new. The first cell therapy was the transplant of bone marrow stem cells for patients with leukaemia in the late 1970s (Thomas et al., 1975). Over the next 20 years bone marrow stem cell transplants were adapted and adopted for bone marrow cancers and extended into other clinical indications, for example, inherited immunodeficiency. Bone marrow stem cell transplantation is now a routine clinical procedure for multiple indications.

The example of bone marrow stem cell therapies illustrates several of the characteristics that define cellular therapies more broadly. They were developed for less prevalent indications. They are allogeneic (see Box 1.1) one donor-one recipient therapies that were developed wholly by clinicians in a hospital context. They require interventional clinical procedures for administration of the therapy. They led to widespread clinician-led adoption by the clinical community through global clinician networks. They defined an approach to the **safety** of cell therapies based on risk and benefit to patients. They encouraged the development of cell processing expertise within hospitals and in many ways provided a basis of skills and expertise for clinicians to facilitate the development of other cell

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therapies (Foley and Whitaker, 2012). Though led by clinicians, the development and widespread adoption of bone marrow stem cell therapies was facilitated by companies who provided high-value goods and services to help manufacture and deliver the therapies in a hospital context. Around the turn of the millennium, two cell therapy products developed by companies were the first cell-based therapies to be approved by the US Food and Drug Administration (FDA). Apligraf and Dermagraft were competing skin-equivalent products designed to improve the healing of wounds and burns (Kemp, 2006).

These two therapies illustrate some of the characteristics of company-led approaches to cell therapies. They are allogeneic, one donor-many recipient cell therapy products that are manufactured at scale for prevalent indications. They do not require complex clinical procedures (Foley and Whitaker, 2012).

Apligraf and Dermagraft initially failed in the market (Lysaght and Hazlehurst, 2004). One key reason was cost of goods: the products were manufactured manually and had a short shelf life – two aspects

Box 1.1 Cell Therapy Definitions

Autologous

The patient's cells are the cells used in the therapy. No immune response is expected.

Allogeneic

The cell source is different to the patient receiving the cell therapy. There is a possibility of an immune response.

One to one

The cells used in the therapeutic dose are only of sufficient quantity to treat one patient; these treatments can be autologous or allogeneic.

One to many

The cells used are amplified to a scale able to treat many patients; these treatments can only be allogeneic.

of manufacture and distribution that are not well suited to prevalent indications. A contributing factor was cost relative to existing treatments, despite improved efficacy (see Box 1.2).

Box 1.2 Dermagraft and Apligraf – a roller coaster of investment, manufacturing costs and reimbursement

In the 1990s, Advanced Tissue Sciences invested around $300m to develop Dermagraft and Transcyte for the treatment of diabetic foot ulcers. In 2000, ATS formed a marketing partnership with Smith and Nephew, a global leader in wound care products. Dermagraft was approved by the FDA in 2001. In 2002, ATS filed for bankruptcy. In 2003, Smith and Nephew purchased ATS from bankruptcy and continued with manufacturing and sales. Smith and Nephew ceased production in 2005. In 2006, Advanced Biohealing purchased the Smith and Nephew manufacturing assets for an undisclosed amount (Jones, 2011), presumably at a value destroying discount, and in 2007 resumed manufacture, with a sales/reimbursement model that led to $147m sales in 2010. In 2011, Shire bought ABH for $750m (Smith, 2014). In 2013, Dermagraft assets were declared at $683m on Shire's balance sheet and 9 month losses for Dermagraft were $324m (Reporter, 2014).

Organogenesis was the first to receive FDA approval for a living, allogeneic, cell-based product (Apligraf). They were successful in securing a marketing agreement with Novartis in 1996 (Connolly, 2002a). However, the cost of producing Apligraf was too high and in 2002 Organogenesis filed for bankruptcy and terminated its marketing agreement with Novartis. A short-term deal with Novartis and company restructure (Connolly, 2002b) today means that Organogenesis develops, manufactures and markets its own products.

In early 2014, Organogenesis acquired Dermagraft from Shire, with a promise of a $300m payment based on future sales, but without accepting liability for the ongoing Department of Justice investigation into ABH sales and marketing practices (GenEngNews, 2014). Later that year, Medicare altered reimbursement rules (Carroll, 2013), suggesting that the $1,500 cost of Dermagraft would be reimbursed at a maximum of $840. Dermagraft is a very effective treatment for diabetic ulcers, but costs and reimbursement routes may prevent it reaching patients.

1.1.1 Regulation of Cell Therapies

Neither bone marrow stem cell transplantation nor the first two marketed cellular products were regulated as cell therapy products now are. A key question in the current regulatory landscape is whether cells are substantially manipulated before administration to the patient. Minimally manipulated cells, for example, using aseptic separation or enrichment, are governed by the same regulations that apply to any cell or tissue taken from a patient. Therapeutics that involve any more substantial manipulation including expanding cell numbers are now governed by different and more stringent rules akin to those used in the regulation of other medicinal products such as small molecule pharmaceuticals and biologics. These rules require that quality, safety and efficacy are demonstrated to the satisfaction of the regulators, both in order to undertake the clinical trials and for authorization as an approved medicinal product if the trials are successful. The rules include a requirement to show that the product has been manufactured according to Good Manufacturing Practice. So products similar to Apligraf and Dermagraft now require these new authorizations, while bone marrow stem cell transplantation with its minimal manipulation before administration does not and is overseen for Good Clinical Practice by organizations such as JACIE in Europe. In the US, the FDA oversees Good Clinical Practice for bone marrow transplantation, but this is separate from Biologics manufacture; in the US, the term "Biologics" encompasses cell therapies and the more traditional biopharmaceuticals (Oancea et al., 2012).

The uncertainties that preceded the introduction of the new regulations and the costs in time and money that were required for compliance with the new regulations led to a pause in the development of cell therapies, above all in the US and Europe. Nonetheless it is notable that both clinician- and company-led cell therapies have adapted to the regulatory change. As an example of the former, 12 of the 26 cell therapy manufacturing facilities in the UK are now accredited for Good Manufacturing Practice-compliant manufacture of cellular products (Foley et al., 2012). It should be acknowledged that the new regulations for cell therapies are very similar to those for biologics, and so are well understood by the pharmaceutical sector. They do however still pose a substantial manufacturing challenge, since it is the cells themselves, not a biotherapeutic product produced by cells, that are the medicinal product (the ATMP: Advanced Therapeutic Medicinal Product).

1.1.2 Manufacturing Challenges in Cell Therapy

The key raw material for cell therapy manufacture is a cell type obtained from a human source. A key distinction between cell therapies is whether the cell type or its differentiated or otherwise modified derivatives are destined for a single patient or for many patients. A second difference is whether the cells of origin are administered to the patient from whom they are taken. If they are, then they have the genetic identity of the patient and the therapy is autologous. If they are not, then the cells are genetically distinct from the patient recipient and the therapy is allogeneic. For the most part, allogeneic therapies are one to many, while autologous therapies are one to one, though there are examples of one to one allogeneic therapies in which a single patient is treated with cells from a single genetically distinct donor (see Box 1.1).

The manufacture of one to many cell therapies closely resembles the manufacture of biopharmaceuticals (Figure 1.1). Cells from the donor are grown, separated and characterized to make a master cell bank (Box 1.3). Working cell banks can be derived from the master cell bank and used to manufacture patient doses, as for biopharmaceuticals. However, there are important and challenging differences (see Figure 1.1).

We have already briefly mentioned one difference. It is the cells themselves, not their products that are the therapy; as is often remarked, in biopharmaceutical manufacture, one throws away the cells, while in cell therapy manufacture, one throws away the medium. A second crucial difference is that biopharmaceutical manufacture relies on a few standard cell types in standard media; cell therapy manufacture is bespoke to each therapy and does not have the benefit of well-developed platform technologies, for the time being at least.

One to one cell therapy manufacture uses many of the underlying processes and principles of biopharmaceutical manufacture, but is markedly different in scale and separation technologies. Each dose is manufactured for a single patient from a single donor and multiple doses must be manufactured in parallel (Box 1.4).

1.2 The Cell Therapy Landscape

The REMEDiE project has identified around 700 companies that are working in regenerative medicine products or services worldwide, with the large majority located in the US or Europe. More than 90%

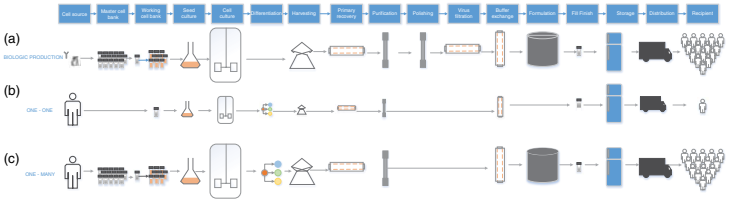

Figure 1.1 Process flow diagram of the sequence of unit operations used in biopharmaceutical manufacture and their relative scales: (a) general process used for the manufacture of monoclonal antibodies; (b) manufacturing for a one on one therapy; and (c) manufacturing process for a one to many cell therapy process.

Box 1.3 How many cells? The scale of manufacture

Mason and Dunhill (2009) and Simaria (2014) have provided some estimates of the numbers of cells that may need to be manufactured. Individual cell doses range from 10^5–10^8. A one to one therapy such as cartilage repair needs a dose of around 10^7 cells and can scale to around 10^4 patients. Treatment for heart failure (one to many) may require a 10^9 dose in 10^7 patients, that is, the manufacture of 10^{16} cells. If cells weigh around 10^{-8} kg, this is around 100,000 metric tonnes: a manufacturing challenge.

Box 1.4 Major manufacturing challenges

Bespoke laboratory based manufacturing processes for early clinical trials may not scale economically to satisfy demand related to disease prevalence. Thus there is a need for early thinking in process development:

- developing robust, replicable processes that can be scaled up or out
- moving early lab-based processes to GMP-compliant processes, materials and equipment; and
- incorporating supply chain and clinical delivery in process development.

Funding process development is a challenge given current public and private funding frameworks.

of these companies are SMEs. Regenerative medicine is defined as including cell therapies, but not exclusively, so it is likely that the number of companies working specifically on cell therapies is somewhat smaller.

1.2.1 Licensed Cell Therapy Products

There are currently 11 cell therapies licensed by the FDA (2014). Of these, five are cord blood derived haematopoietic progenitor cells (HPCs), three are based on fibroblasts or keratinocytes or both, two are chondrocyte-derived and one is a modified dendritic cell. In Europe, only two cell therapies have so far been approved by the EMA and both are chondrocyte-derived (Tozer, 2011). The HPCs are

allogeneic one to one therapies for bone marrow disorders delivered by clinicians; of the three skin cell therapies, two are allogeneic and all are marketed by companies; the chondrocyte therapies are autologous and also marketed by companies. Licensed therapies in Europe and the US are described in Figure 1.2.

1.2.2 Companies, Clinicians, Products and Procedures

In analyzing the cell therapy landscape we have found it useful to distinguish between a cell therapy that can be readily administered to a patient and another that requires a more complex clinical intervention to deliver it to the site of choice. The former we have called a product because it is closest to an off-the-shelf drug or biopharmaceutical. We have classified the latter therapies as requiring a procedure (Foley and Whitaker, 2012). From clinical trial data, the tendency is seen to be for procedure-based therapies to be set up and delivered by clinicians, while product-focused cell therapies tend to be developed by companies. Mapping of the existing licensed therapies shows that 10 involve a clinical procedure and 3 a product. Companies have developed all the licensed products, while the procedures have been developed equally by clinicians and by companies (Figure 1.2).

1.2.3 Cell Therapy Clinical Trials

In an exhaustive analysis of worldwide clinical trials databases, around 1,000 cell therapy trials were found that were not investigating established cell therapies for an established indication (Li et al., 2014). Of these 1,000 trials, just over 400 studied mesenchymal stem cells and an equal number used haematopoietic stem cells. The number of trials based on other stem cell types was 208, including only 6 that used embryonic stem cells, the remainder being somatic stem cells. Around 600 trials used autologous cells and 300 allogeneic cells as a therapeutic agent; around 100 trials involved stimulating endogenous cells with a non-cellular therapeutic.

These figures are broadly comparable to an analysis undertaken in 2011 (Foley and Whitaker, 2012) that used a sampling algorithm using data only from www.clinicaltrials.gov, which might be expected to show a bias towards US and European cell therapy trials. Using the same methodology, we have now analyzed the trials database again; however, this time including additional trials that were registered up

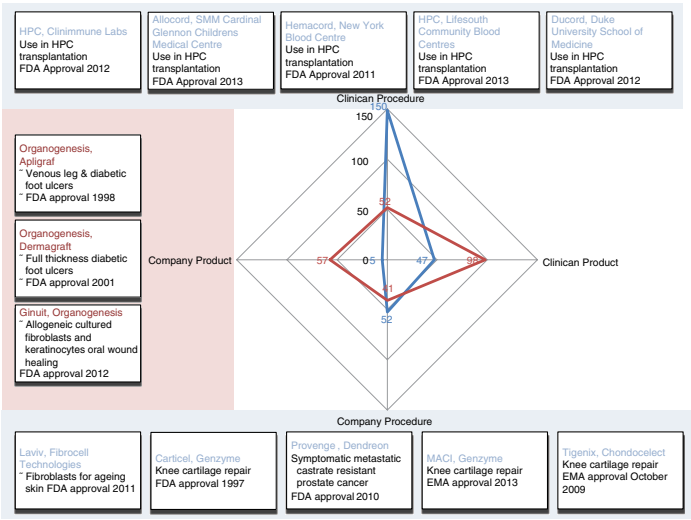

Figure 1.2 Heuristic classification of a selection of cell therapies to the three dimensions. Therapies shown in red are one to many, and therapies in blue are one to one. Numbers are our estimate of all stem cell trials in the clinical trials database that met our criteria. Therapies in boxes have received a market authorization. Abbreviations: EMA, European Medicines Agency; FDA, US Food and Drug Administration; HPC, human progenitor cells.

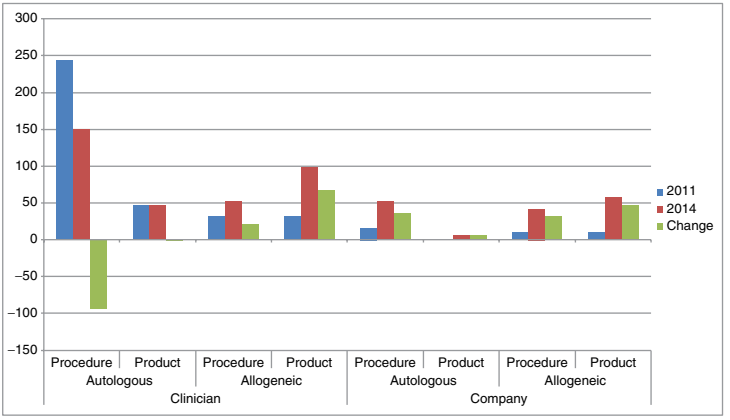

Figure 1.3 A comparison between 2011 and 2014 of the change in cell therapy clinical trials using www.clinicaltrials.gov and the search term "stem cells". Hematopoietic stem cell clinical trials and those terminated before completion were excluded. The trials were analyzed using three dimensions, clinician-company, autologous-allogeneic and procedure-product.

to March 2014 and excluding those that were terminated before completion (Figure 1.3).

There have been some substantial changes in the categories of cell therapeutics and in the relative involvement of companies and clinicians in the three years from March 2011. The number of trials using easily administered products has more than doubled; the number of trials involving procedures remains unchanged, thus products are now represented in around 40% of trials, up from around 17% in 2011. The number of companies sponsoring cell therapy trials has increased four-fold: these company-led trials now account for 30% of trials in the database, up from 10% three years ago. The number of trials of allogeneic therapies has also risen substantially, tripling in the last three years; now half the trials involve allogeneic therapies, up from 20%. Strikingly, the number of trials of clinician-led autologous procedures has fallen by 40% and there have been marked increases in trials of allogeneic therapies sponsored by both clinicians and companies (Figure 1.3).

The analyses of cell therapy clinical trials presented here and previously (Foley and Whitaker, 2012; Li et al, 2014) exclude cellular therapies for cancer. Cellular cancer therapies broadly involve the modification