TECH NEWS: STEM CELLS FOR MODELING AND CURING DISEASE

Freya Leask explores developments in cell culture and stem cell research that are revolutionizing how diseases are studied and treated

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Prior to 2015, options for patients with limbal stem cell deficiency (LSCD) were limited. Often caused by injury to the eye, LSCD causes patients to suffer pain, decreased vision and eventually blindness; success of long-term treatment depended on availability of healthy stem cells to replenish the corneal epithelium.

However, researchers working at the Centre for Regenerative Medicine (CMR) in the University of Modena (Italy) were working on another option.

A team led by Michele De Luca, Graziella Pellegrini and Andrea Chiesi – Director of R&D Portfolio Management of Chiesi Farmaceutici S.p.A. (Parma, Italy) and CEO of Holostem Terapie Avanzate, a University spin-off located in the CMR – developed a method where a tiny amount of patient tissue from an undamaged part of the limbus, the border of the cornea and the white of the eye, and a common site of injury, was used to produce a sheet of transplantable cornea.

Unlike previous treatments for LSCD, this technique avoided the need for invasive surgery to collect the tissue, reducing the risk to the rest of the eye. As a small amount of tissue is then cultured, it also removed the dependence on a healthy limbus.

Holoclar® was officially approved by the European Commission in February 2015, becoming, according to Chiesi, “the very first medicinal product based on stem cells to be approved and formally registered in the Western world.”

The approval provided not only inspiration to researchers and patients, but provided a blueprint for academic–industry

partnerships and a roadmap for other advanced therapies seeking commercialization and approval.

However, even with an example to follow, the road to approval for other stem cell-based therapies has proved challenging. In particular, navigating the scale up from research to clinical quantities and quality, and ensuring consistency, has remained a key obstacle. Ultimately, overcoming this hinges on a reliable manufacturing process and a quality source of raw materials.

A CULTURE OF CHARACTER

Part of the challenge in the manufacturing of a cell therapy lays in producing enough cells for an effective dose. A single dose of a stem cell therapy might contain 600 million cells; the fewer donor stem cells can be collected, the further the donation has to be expanded to meet that threshold.

“Scaling up can be challenging due to two reasons; first of all, due to spontaneous differentiation...small changes in their growth conditions and environment [can affect] characteristics of the undifferentiated stem cells... the ability to differentiate into a specific lineage ...or their multipotency”, explained Ankita Desai, Bioprocess Field Application Specialist, Eppendorf (Hamburg, Germany) [2].

“The second challenge is [that] supply of nutrients and oxygen to the cells at the center of the aggregate can be limited as the aggregate grows in size. This can also affect the characteristics and functionality of the

stem cells.”

In addition, the further the donor cells are expanded, the lower their efficiency in differentiating into effective cell types. “In particular (sic) for stem cells, rapid growth does not guarantee enhanced ▶

“Part of the challenge in the manufacturing of a cell therapy lays in producing enough cells for an effective dose.”

► *functionality. In fact, it may have the opposite effect,”* agreed Daniel J. Kota, Director of the Emory Personalized Immunotherapy Core at Emory University (GA, USA) [3].

To treat a clinically relevant population – for example, treating heart disease in 250,000 patients through cardiomyocyte replacement – might take 100 quadrillion (10^{16}) cells.

So how can manufacturers ensure the efficacy and number of their stem cells? The last 10 years have brought a number of advancements [4].

The first step towards reproducible manufacture came with the characterization of key components in the media required for cell culture, such as laminin and vitronectin, which are important for cellular adhesion.

Definition of these components meant that they could be produced artificially, limiting the biological variance in a culture system to the cells themselves and limiting the amount of process transfer required from the bench to the bedside. Development of fully “xeno-free” media continued but, due to cost, remained beyond the reach of larger scale production until 2011, when James Thomson and his lab at the University of Wisconsin-Madison (WI, USA) produced the first fully defined cell culture media, reducing the number of components from 70 to just eight.

Essential 8, consisting of insulin, selenium, transferrin, L-ascorbic acid, FGF2, and TGFβ (or NODAL) in DMEM/F12, with pH adjusted with NaHCO_3 , is now used around the world and commercially produced by multiple vendors.

However, the numbers of participants donating and receiving material involved in a clinical trial, and then a commercially available product, is several orders of magnitude greater than in basic research. Avoiding the multiple opportunities for human error and contamination (Figure 1) on this scale is a major headache for manufacturers. Could eliminating the former also reduce the latter?

CLOSING UP SHOP

Traditional manufacturing techniques for development and scale-up do not translate well to cell-based therapies.

“Conventional manufacturing processes for small molecules and biological therapies are well established... due to the inherent variability of cell therapies, cost-effectiveness remains a key challenge. High startup costs translate into significant barriers-to-entry for novel spinouts and startups, and for established biotech entities, attaining industrial-scale yields is challenging”, explained David Pettitt and David Brindley (University of Oxford, UK) [5].

A closed, automated system could avoid this, reducing manufacturing time through reduced operator handling or set-up and validation time. It also allows for continuous and real-time quality assessment, saving time and money that could be wasted on repeating a contaminated or ineffectual product.

“Handling your cell product in a closed system helps to reduce the risk of contamination and ensures consistent product quality,” agrees Thorsten Decker, a senior product manager for Miltenyi Biotec (Germany) [6]. *“In the discussion with your regulatory authorities, this may be helpful, for example, to agree on using a lower*

clean room class. Clean room costs are one of the major cost drivers for cell products.”

Cells cultured using closed and automated systems have been shown to exhibit population doubling over double that of cells cultured in traditional flasks [7]. Specific closed systems, such as the CliniMACS Prodigy, have been shown to produce T cells encoding chimeric antigen receptors (CAR) for CD19 (CAR19), a widely targeted antigen in lymphoma, comparable with existing procedures [8].

With all these developments in manufacturing, you’d think we’d

be closer than ever to stem cell therapies for all. However, for developing therapies, *“going from a Phase I clinical trial to an advanced phase clinical trial has proved to be insurmountable – a valley of death”,* according to Bruce Levine, Deputy Director, Technology Innovation and Assessment, Center for Cellular Immunotherapies, University of Pennsylvania (PA, USA) [9].

Could the unique properties of stem cells be applied in another way to overcome this?

SMALL ORGANS, BIG IMPACT

In a lab in The Netherlands, a tiny model of the human mammary system lies in a petri dish. ►

“Cells cultured using closed and automated systems have been shown to exhibit population doubling over double that of cells cultured in traditional flasks”

Figure 1. Factors that can affect levels of contamination in a stem cell-based product. Reproduced with permission from *Regenerative Medicine* [1].

► Although this miniature organ isn't attached to the rest of a body, it's demonstrating signs of cancer, allowing a number of experimental treatments to be tested on it. This seems like science fiction, but it's a scenario happening in labs around the world.

Mini organs, or organoids, can be created from stem cells by taking advantage of their predilection for differentiation. *"Organoid cultures have virtually unlimited expansion, and they are untransformed, genetically and phenotypically stable and retain biological and functional properties of the original tissue,"* says Farzin Pourfarzad, Group leader, Hubrecht Organoid Technology (The Netherlands). *"The cultures can be tailored and scaled up as necessary for high-throughput screening and therefore provide a unique and robust in vitro model for drug development, diagnostics, and patient stratification."*

The most common diseases being studied are shown in Figure 2, which illustrates the results of a 2018 survey conducted by RegMedNet [10], an online community of scientists interested in regenerative medicine and stem cell research.

"Identification of LGR5 crypt stem cells was the key discovery by Hans Clevers that led to gastrointestinal tract organoid establishment. Rapid turnover and regeneration, simple and highly polarized epithelium, presence of all cell types made gastrointestinal tract organoid one of the most commonly used organoids," Pourfarzad agrees. *"Currently lung, breast, pancreas organoids are also of interest for cancer patient stratification and immunomodulation modeling."*

Subsequently, these diseased organoids can be used for high-throughput testing of drug candidates, and linking multiple organoids to create 'humanoids', which could eliminate the need for animal testing. Pourfarzad is convinced the benefits of developing these models and process transfer away from traditional models outweigh the initial cost and complexity.

"Preclinical assessment of newly developed medicinal compounds is of utmost importance to predict their safe and efficacious use in humans. Organoids preclinical drug assessment projects...avoid costly traditional animal experiments and provide quick indication of drug safety and efficacy allowing decision making at early stages of drug development."

THE ONLY PLACE FOR A CURE

Stem cells hold a huge amount of potential to treat disease, particularly when there is a defect in just one kind of cell or in a rare disease where the patient population and the disease are well understood.

For sickle cell disease, the future is bright. Gene-edited stem cell therapies have shown promise in encouraging normal blood cell growth. For example, researchers from the Boston Children's Hospital (MA, USA) are trialing a treatment using lentiviral gene editing to modify the *BCL11A* gene, which represses fetal hemoglobin

Figure 2. Excerpt from "Organoids: technology and challenges around the world" infographic illustrating the most common applications, indications and tissue types being studied using organoids. Reproduced with permission from RegMedNet.

production, in stem cells collected from the patient's bone marrow. This cell therapy is then infused back to the patient, suppressing production of sickled cells and protecting patients against the progression of their disease.

Stem cell-based therapies are also being investigated for neurodegenerative diseases, such as Parkinson's disease (PD). Jun Takahashi at the Center for iPS Cell Research and Application at Kyoto University (Japan) is leading the first-in-human trial assessing the safety of dopaminergic progenitor cells derived from induced pluripotent stem cells (iPSCs) as a treatment for PD, which is an amenable target for stem cell therapy.

"The pathology [of PD] is relatively simple: a single type of neuron (dopaminergic neuron) in a small area is degenerated [and] there is evidence that fetal cell transplantation can improve the motor symptoms of the PD patients," he explained [11].

In the study, neural cells will be differentiated from iPSCs stored in a cell bank, the iPS Cell Stock for Regenerative Medicine (Kyoto, Japan), which have been HLA matched to the patient. The benefits of using banked cells from a donor (allogeneic cells) as opposed to cells collected from the patient (autologous cells) are cost and availability.

"Autologous therapies use a complicated 'service-based' business [whereas] allogeneic therapies will utilize a much simpler 'off-the-shelf' business model, where batches of doses will be produced at ►

► a manufacturing facility and then shipped to hospitals for use as and when required,” explained Nafees Malik, Asklepios Consulting (London, UK) [12]. Allogeneic therapies will be cheaper and available quicker, allowing more patients access to these kinds of treatments.

Whatever the source of the stem cells, patients and researchers alike are hopeful that stem cell-based treatments could revolutionize medicine.

“For me”, says Adrienne Bell-Cors Shapiro, Founder of Axis Advocacy (CA, USA), an advocacy group for people with sickle cell disease [13], “stem cell science is the only place where I can really look [for my daughter] to be cured.”

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