CELL & GENE THERAPY INSIGHTS

CELL & GENE THERAPY MANUFACTURING PATHWAY

SPOTLIGHT

FUTURE LEADER PERSPECTIVE

Key considerations in optimizing pluripotent stem cell bioprocessing

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In the same paper in which the first successful derivation of human pluripotent stem cells was reported, they were already being postulated as a potentially unlimited source of cell transplantation material [1]. In the following years, an entire research field has evolved to seek ways to control and harness the complexity of pluripotent stem cells, which has resulted in a slow but steady progression towards the clinic. Now it is the turn of bioprocess development to adapt existing tools and technologies, or develop totally novel approaches, to ensure the promise of these therapies can finally be delivered in a safe, efficacious and affordable manner.

PLURIPOTENT STEM CELLS' COMPLEX MoA MEANS COMPLEX PROCESSING

Cell therapies have long held promise to deliver transformative clinical benefits by administrating a product capable of complex mechanisms of action. However, to achieve such feats in a safe and efficacious manner requires an understanding of how to control the target phenotype. This issue is critical for pluripotent stem cells (PSCs) where the plasticity which enables them to transform into a huge range of potentially therapeutic cells types, also makes it very hard to measure and control the critical quality attributes (CQAs). Actually defining the CQAs is further complicated by the lack of clinical experience that is mirrored in regulatory uncertainty. There was disappointment in the field when Geron (California, USA), abandoned its trial in 2011 after significant investment. But now there is cause for cautious optimism. For instance Asterias (California, USA) picked up Geron's lead candidate for spinal cord injury and began enrolling a new trial in 2015. In addition Ocata Therapeutics (Massachusetts, USA), Healios KK/Riken (Tokyo, Japan), and Pete Coffey (UCL, London, UK) have all now implanted retinal pigment epithelium cells to treat blindness. In light of this it is pertinent that process development presses ahead even under an environment of many "unknown, unkowns" to ensure this initial momentum in the clinic is not derailed.

SAFETY CONCERNS OF PSC-DERIVED THERAPIES

The main safety concerns of PSCs are that they have a transient and often highly heterogonous phenotype during differentiation. This heterogeneity means the safety of a PSC therapy will be dependent on a process's proven ability to deliver only the intended cell type to patients, being free of potentially harmful contaminant cells. Cellular contaminants can be put into three broad categories:

1. Non-tumorigenic cells
2. Undifferentiated PSC
3. Proliferative cells

The presence of these cells, or masses they can form, could cause harm from either their metabolic activity and/or physical presence, particularly in anatomically sensitive areas such as the brain.

NON-TUMORIGENIC CELLS (CONTAMINATION FROM SPONTANEOUS DIFFERENTIATION)

During differentiation protocols the PSC population not only changes into the intended therapeutic cell but also differentiates into a multitude of other cell types. Differentiation yields can be very low, for example Klimanskaya *et al* reported <1% spontaneous differentiation of RPE from embryoid bodies after 6 weeks [2]. With optimization this same differentiation can be dramatically increased up to 80% through a complex regime of defined factors at specific timings [3]. However, this still leaves a substantial amount of contaminant "non RPE" cells (20%).

Such contaminants can cause issues with the regulators, for example Geron had their subsequently abandoned PSC clinical trial put on temporary hold when it was discovered that a certain unwanted cellular contaminant led to the formation of non-proliferative cysts [4]. This potential risk is termed "graft overgrowth" where limited benign *in vivo* proliferation of stem cells or their progeny could cause clinical harm [5]. Another example of this is where an autologous olfactory stem cell transplant into the spine at a Portuguese hospital had to be removed due to patient pain [6]. On removal the growth was determined to be non-cancerous containing nerve branches, bone and was secreting mucosal material. Therefore the manufacturing process needs to either have a purification stage to separate out just the desired population or be able to reproducibly deliver a defined heterogeneous population with pre-clinical assurances as to its safety [7].

TERATOMAS (PSC CONTAMINATION)

A test for the pluripotency of PSCs (ability to differentiate into the vast multitude of cell types) is to inject them into an immunodeficient mouse, where they form what is termed teratomas. These teratoma masses are then removed and interrogated for the three germ layers that all cells types are derivatives of, thus proving their pluripotency potential. However these PSC-derived teratoma-like masses do not meet the classic definition of teratomas which contain acquired genetic and epigenetic alterations [8]. Although benign this ectopic tissue either at

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the therapeutic site of implantation, or as a result of migration through the body's transport systems may cause harm.

When the FDA first started exploring the potential regulation of PSC therapies in 2008 a meeting with industrial stakeholders was held. Here the view was presented that undifferentiated cells in the final therapy were a safety concern and that the further down the pathway to specialization and terminal differentiation the lower the risk of "tumor" formation. Thus it is a key part any PSC-derived investigational new drug (IND) filing to have accounted for the issue of undifferentiated PSC contamination. In a manufacturing process this could mean tracking and ultimately confirming the absence of PSC markers in the final product.

GRAFT OVERGROWTH & KARYOTYPE DRIFT

During prolonged cell culture and adaptation to new methods PSCs can experience karyotypic drift (chromosomal changes) often resulting in decreased population doubling times which can lead to tumor formation *in vivo* due to uncontrolled proliferation [9–11]. However, this has not precluded the use of a karyotypically abnormal cell line being used in therapy for example Carpenter, Frey-Vasconcells, and Rao reported that the FDA approved the use of the human embryonal carcinoma cell line which has between 56 and 61 chromosomes for use in a clinical trial [12]. Yet their inclusion would still be undesirable as they present a significant safety concern. This makes karyotype testing to prove stability over a defined range of passages a likely prerequisite for the working cell bank used in manufacture.

Another potential complication arises from the use of induced pluripotent stem cells, where genetic engineering approaches can be used to revert cells into a plastic state. An inability to adequately control the reprogramming could lead to 'insertional mutagenesis' resulting in malignancies like those seen in past gene therapy trials [13]. However, the presence of genetic mutations after reprogramming does not necessary mean that cells are tumorigenic and therapy unsafe, as was reported by those familiar with the Riken iPSC trial – noting a lack of consensus and guidelines [14].

IMPLICATIONS WITH RESPECT TO PROCESSING TECHNOLOGIES & APPROACHES

Reducing contamination and enriching for the target phenotype as part of bioprocess development is severely hampered by a lack of rapid or non-destructive analytical assays available. This is a particular issue as the cultures are scaled up (or out) from simple flask culture into suspension cultures or multilayer plastic systems as the quickest analytical method of looking down a microscope is lost.

Therefore the use proxy measurements of media components, or metabolic by-products to track the process becomes vital as they often represent some of the quickest analytical tests to perform. This is exemplified in feasibility work which we conducted scaling

up PSC culture in a hollowfiber bioreactor whereby through empirical changes to the processing parameters in an attempt to maintain glucose and lactate levels within those seen in flask culture the bioreactor yield was doubled. But this still represented a 4-fold lower yield per cm^2 than standard flask culture [15]. This highlights the need to not just to be able to measure the system but have automated control systems – in this example it would be linking the perfusion system to a media analyser. For differentiation protocols taking many weeks the validation of proxy measures against the target phenotype will greatly improve process understanding allowing control. An example of this is work by Stachelscheid *et al* who tracked α-fetoprotein (AFP), β-human chorionic gonadotropin (ß-hCG) and activin in bioreactor cultures [16]. If suitable analytical tests were available to rapidly monitor PSC processes such as those described by Smith *et al* it would open the possibility of applying the principals of process analytical technologies (PAT) [17]. The use of PAT would enable the implementation of dynamic control to adapt to real time variation in the process to improve the consistency of the product quality [18]. Given the highly variable and transient nature of the PSC expansion and differentiation process the use of PAT could be key control of this complexity - reducing the chances of expensive batch failures where weeks worth of processing time might be lost.

The use of statistical tools such as Design of Experiments (DoE) have long been applied to biotechnology to understand complicated interactions between key variables and outcomes [19,20]. Such approaches typically involve an initial screening experiment whereby multiple factors are varied at maxima and minima in a rationalized way, meaning that not every permeation is run. This allows identification of which key factors impact the response whilst minimizing the experimental resources required. With the factors having the largest impact on the response identified these can then be focused in on to increases resolution. This increased resolution in the form of data points allows a model to be generated that predicts the interactions between both inputs and processing parameters and CQAs to be elucidated. Such statistical approaches have already shown to be effective in developing cell therapy processes, For example Ratcliffe *et al* and Hunt *et al* applied the approach to stem cell bioreactor operation, and explored key variables of PSC expansion in an automated culture platform [21,22]. We applied this statistical approach to the differentiation of PSCs into retinal pigment epithelium cells [23]. A mixture of variables where explored to cover factors that would impact both the process economics such as feeding regime and use of expensive growth factors. The model showed that decreasing the amount of media used had a positive impact on differentiation and it identified initial seeding density, nicotinamide concentration and time at which media times were made were the most important factors of the screen. The optimization experiment modelled their interaction with statistical certainty to predict an optimum process. However, the experimental effort to run

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large multifactorial experimenters over a 49 day differentiation protocol was considerable, and many of the responses measured were insignificant. This was most likely due to the experimental noise from a manual process being larger than the signal produced by the effect.

Therefore there is a real opportunity for new technological solutions to be developed for this next generation of cell therapy bioprocessing which will be applicable to both PSC and other classes of cell therapies such as CAR T-cells.

TRANSLATIONAL INSIGHT

If PSC therapies are to be both clinically and commercially successful it is critical that the processes can both optimize yield and control (or remove) the presence of unwanted cellular contaminants. To achieve this an information-lead approach is needed that will require a combination of quicker analytical tests that have been validated against anticipated predictors of efficacy in the clinic. Removal of manual handling and implementation of automated systems will be key to achieving the control needed and validate a process that can produce a safe and efficacious product.

FINANCIAL & COMPETING INTERESTS DISCLOSURE

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