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Review

Progress and challenges in large-scale expansion of human pluripotent stem cells

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ABSTRACT

The constant supply of high cell numbers generated by defined, robust, and economically viable culture processes is indispensable for the envisioned application of human pluripotent stem cells (hPSCs) and their progenies for drug discovery and regenerative medicine. To achieve required cell numbers and to reduce process-related risks such as cell transformation, relative short batch-like production processes at industry- and clinically-relevant scale(s) must be developed and optimized. Here, we will review recent progress in the large-scale expansion of hPSCs with particular focus on suspension culture, which represents a universal strategy for controlled mass cell production. Another focus of the paper relates to bioreactor-based approaches, including technical aspects of bioreactor technologies and operation modes. Lastly, we will discuss current challenges of hPSC process engineering for enabling the transition from early stage process development to fully optimized hPSC production scale operation, a mandatory step for hPSCs' industrial and clinical translation.

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1. Introduction

1.1. The need for large-scale production of human pluripotent stem cells and their progenies

Human pluripotent stem cells (hPSCs), including human embryonic (hESCs) and induced pluripotent (hiPSCs) stem cells, represent a unique cell source for the, in principle, unlimited production of functional human cell types *in vitro*. In this regard, hPSCs hold great promise for revolutionizing drug discovery, drug safety assays, *in vitro* disease modeling, and ultimately cell-based therapies (Fig. 1) [1,2].

The evolutionary conservation of mammalian genomes has resulted in numerous drugs that were discovered by assays employing ubiquitous cell lines and further validated in typical rodent models to lack efficiency or cause detrimental side effects after clinical translation [3,4]. Systematic research indeed revealed limitations of animal models regarding their predictability of drug function and toxicity in man. Underlying reasons include substantial species-specific differences in (i) cell and tissue physiology (such as liver metabolism, beating rate of the heart, *etc.*), (ii) inflammatory response, (iii) structure and specificity of the immunological system, and (iv) others [5]. This underscores the necessity for using human cells, ideally tissue-specific cells, for drug discovery, validation, and safety pharmacology [1,6,7].

Moreover, for many sporadic and rare diseases caused by genetic mutations, such as cystic fibrosis [8], hereditary pulmonary alveolar proteinosis (hPAP) [9], or Huntington's disease [10], novel drug candidates should ideally be screened and validated in human cells carrying the respective mutation(s). In contrast to immortalized cell lines, which are typically used for high throughput screening (HTS) drug discovery assays, this deems straightforward by using disease-specific *in vitro* models based on patient-derived hiPSC lines differentiated into functional cell types relevant to the respective disorder [8].

Beyond drug discovery and disease modeling *in vitro*, first patients have recently received hPSC progenies aiming for novel approaches in regenerative medicine. For treating age-related macular degeneration in the eye, both hESC- [11] and hiPSC-derived [12] retinal pigment epithelial cells were readily applied. The implantation of hESC-derived insulin-producing cells in patients with type 1 diabetes was announced by the company ViaCyte, and early hESC progenies were readily transplanted to the left ventricle of a first heart failure patient *via* a tissue engineering approach [13,14].

At present, functional hPSC progenies are mainly generated by protocols in laboratory scale and quality. However, the envisioned routine application of these cells will require appropriate large-scale production processes, ultimately by standardized and economically viable procedures and technologies.

Rough estimations suggest that for replacing disease-induced loss of hepatocytes, pancreatic β-cells, or cardiomyocytes, approximately $1–10 \times 10^9$ functional cells per patient will be required. Even higher needs were calculated for the visionary production of "in vitro blood", since approximately 2.5×10^{12} red blood cells are required per patient in transfusion medicine [15].

It is worth noting that equivalent cell numbers are readily required ahead of treating patients; for example, for pre-clinical studies in large animals such as pigs or non-human primates, which represent more physiologically and functionally relevant models of human diseases such as heart failure compared to rodents [16–18].

The need for developing well-defined large-scale hPSC expansion and differentiation processes is not dictated by cell number requirements alone. Another impelling necessity is to comply with the currently evolving regulatory framework for hPSC-derived therapeutics, including the application of relevant "current good manufacturing practice" (cGMP) guidelines [2,19,20].

Taken together, many of the envisioned clinical and industrial applications of hPSCs will depend on the constant, controlled production of billions of cells. In principle, bioprocesses for the production of recombinant proteins by common mammalian cell lines, which have been established in >1000 L scale, may serve as a blueprint [2,16,18,21]. In this scenario, the established bioreactor systems provide effective technologies to replace laborious and poorly controlled research-type processes.

By combining process automation, monitoring, and control with scalability, bioreactor systems are applied to reduce operator-dependent variability, paving the way for more robust and cost-effective hPSC production [22–24].

However, due to their intrinsic potential, hPSCs may switch from pluripotency toward (uncontrolled) differentiation not desired during the cell expansion phase. Moreover, subsequent differentiation into desired lineage(s) is a highly complex process altered by a multitude of overlapping parameters, which also includes effects of the proceeding expansion strategy. Therefore, hPSC processing is substantially more challenging than long-standing strategies for the cultivation of transformed and relative unpretentious cell lines typically used in industry and thus requires a high degree of innovation in process development and control [2,16,18,21,25].

2. Culture platforms for hPSC expansion

2.1. 2D culture systems and process scale-out

Undifferentiated hPSCs are conventionally maintained and expanded at two-dimensional (2D) conditions with cells adhering to the matrix-covered surface of culture plates or flasks. The cumbersome co-culture of hPSC colonies grown on mitotically inactivated fibroblasts ("feeder cells") – as initially described for their routine maintenance [26,27] – has been largely replaced by semi- or fully-defined matrices such as Matrigel [26], recombinant proteins (such as laminins [28]), or synthetic polymers [29].

To generate larger cell amounts, scale-out of the 2D approach has been suggested simply by multiplying culture dishes or by using multi-layered flasks marketed as "Cell Factories" or "Cell Stacks" [30]. Thus, the term "scale-out" refers to keeping a manufacturing lot size constant but multiplying the number of parallel unit operations (see Fig. 2A) [31]. However, although some degree of process automation for 2D culture has been published [32,33], the approach remains relatively cost-, space-, and labor-intensive. The method also restricts the online monitoring and control of key process parameters including vital cell counts, dissolved oxygen (DO), pH, and glucose and growth factor concentrations. It should be mentioned though that a culture system for large-scale 2D processing of hPSCs based on multilayered plates was recently introduced, which allows pH and DO monitoring and feedback-based control [34].

However, 2D cultivation typically relies on static culture conditions known to induce the formation of undesired gradients, including media components, metabolic waste products, paracrine factors, and gases. Together, despite its advantageous simplicity, 2D cultivation raises a number of issues that limit the strategies' utility for the systematic development of hPSC mass production [24,25,35].

2.2. 3D culture systems and process scale-up

The field is recently reaching consensus that three-dimensional (3D) culture (synonymously termed suspension culture) is a potent approach to achieve the extensive hPS cell number requirements