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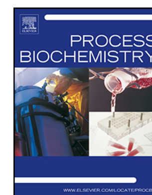

Review

Characterization of 3D pluripotent stem cell aggregates and the impact of their properties on bioprocessing

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ABSTRACT

Pluripotent stem cells (PSCs) have been traditionally expanded on a two-dimensional (2D) surface and require substrates coated with extracellular matrix (ECM) proteins. Recently, PSCs have been successfully expanded in suspension as undifferentiated PSC aggregates, which offer a means for large-scale production. Toward lineage-specific differentiation, PSCs can form aggregate-like structures known as embryoid bodies (EBs). The morphology and size of EBs have been shown to significantly affect the differentiation into specific lineages and three-dimensional (3D) tissue development, thus efforts have been devoted to form size-controlled EBs. The integration of both PSC expansion and differentiation in suspension promotes PSC-derived cell production in bioreactors. However, the cellular organization and differentiation potential of PSC aggregates, as well as the role of the cues provided by the reactors to regulate EB fate, have yet to be fully understood. Despite these challenges, integrated PSC aggregate-based culture provides a platform for a simple, scalable bioprocess for the potential application of PSCs in regenerative medicine, disease modeling, and drug discovery.

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1. Introduction

Pluripotent stem cells (PSCs) including embryonic stem cells (ESCs) and induced PSCs (iPSCs) have arisen as promising cell sources for regenerative medicine, disease modeling, and drug screening in recent years [1,2]. PSCs have shown encouraging data in treating heart failure, diabetes, stroke, and spinal cord injury [3]. To date, more than 10 clinical trials based on PSC technology have been approved worldwide [4]. In addition, PSC-derived cells have provided a platform for disease modeling and drug screening. For example, PSC-derivatives have been successfully used for evaluating cardiac and neural toxicity or developing mini-brains and other organoids [5,6]. For both therapeutic and drug screening purposes, a large quantity (>10^{10}) of PSC-derived cells is required. This amount of cells cannot be easily produced in two-dimensional (2D) culture vessels [3,7]. Thus, the potential of PSC technology relies on the scalable culture systems for expansion and lineage-specific differentiation.

During expansion stage, undifferentiated PSCs have been traditionally grown as an adherent monolayer, which requires a special medium and the coating of substrates with extracellular matrix (ECM) proteins [8]. Recently, new strategies to expand PSCs as three-dimensional (3D) aggregates as well as expanding PSCs on microcarriers have been investigated toward efficient cell production in stirred bioreactors [9,10]. The microcarrier cultures have been comprehensively reviewed somewhere else [11]. As discussed in this article, growing PSC aggregates eliminates the requirement for ECM substrates, thus providing a simple, scalable bioprocess to be integrated with PSC differentiation in bioreactors (Table 1).

During differentiation stage, PSCs are able to form embryoid bodies (EBs), the 3D differentiated aggregates mimicking embryonic development [12]. The spontaneously differentiated EBs contain the cells from three-germ layers. In the presence of inducing factors, EB formation can be altered to favor specific lineage differentiation. The structural properties of EBs, especially the morphology and the size distribution, have been found to affect the differentiation efficiency [13]. Recent advancements of using controlled methods, such as forced aggregation, micro-well arrays, micro-contact patterning, and agitation, have shown the capability to modulate EB morphology and size to minimize heterogeneity [14].

Various bioreactors have been investigated for scalable PSC expansion or EB differentiation toward large-scale production [15]. Bioreactors enable the spatial and temporal control of PSC biochemical environment and provide biomechanical cues to direct PSC aggregate fate decisions [16,17]. In addition, an efficient PSC-derived cell production requires the process integration of PSC expansion and differentiation in simple, robust, and scalable systems, which can be implemented in versatile bioreactors.

This review investigates recent progresses in the understanding of the dynamics of the complex structure and the biochemical environment of the PSC aggregates. This work attempts to understand how the spatial and temporal engineering of PSC aggregate-evolving microenvironment regulates stem cell fate decision and the possible translation into new bioprocess at large scale. The limitations of current bioreactors, the tools required for the on-process control and for the tight regulation of biochemical and biomechanical aggregate environment are discussed. Finally, this work highlights emerging strategies for process integration of PSC aggregate expansion and lineage-specific differentiation in bioreactors, for clinical applications.

2. Formation and biological properties of undifferentiated PSC aggregates

PSC aggregates promote cell–cell adhesion and endogenous ECM secretion compared to monolayers, which sustain the self-renewal of PSCs in the absence of ECM substrates [18,19]. While the culture systems of undifferentiated PSC aggregates have been well studied [20], the mechanism that promotes PSC aggregation is not well understood.

2.1. Role of cell adhesion molecules and endogenous ECM in PSC self-renewal

Undifferentiated PSCs express various cell adhesion molecules (CAMs), including connexins (Cxs) (e.g., Cx43, Cx45, Cx31, and Cx40), E-cadherin, and zonula occludens (ZO-1) [21,22]. Cx43 and E-cadherin have been demonstrated to play a key role in mouse iPSC reprogramming (e.g., as a downstream target of reprogramming factors) [7,23] and are required to regulate many PSC cellular processes such as maintaining PSC self-renewal and proliferation [24–26]. E-cadherin was shown to stabilize β-catenin and the leukemia inhibitory factor receptor (LIFR)/gp130 complexes, leading to the sustainment of the LIF/STAT3 signaling in mouse ESCs [27]. For human ESCs, E-cadherin was reported to maintain the self-renewal in association with fibroblast growth factor (FGF)-2 [28]. Cx43 promotes molecular diffusion between cells, increasing homogeneity of human PSC colonies [21]. Moreover, Cx43 modulates ESC proliferation through the regulation of epidermal growth factor (EGF) signaling [29]. Subsequently, the downregulation of ZO-1 promotes the activation of the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase/ERK kinase (MEK), STAT3, and transforming growth factor (TGF)-β/nodal signaling pathway, favoring ESC proliferation at the undifferentiated state [30]. Finally, the formation of PSC aggregates was shown to increase gap junctions and E-cadherin expression, leading to the increased expression of pluripotent markers (Oct-4, Tra-1-60, etc.) [18,19]. The enhanced expression of E-cadherin was also found to be associated with a decreased Wnt/β-catenin signaling, which is dependent on PSC aggregate size [18].

Undifferentiated PSCs express a set of integrins (i.e., $_{\alpha 5\beta 1}$, $_{\alpha 6\beta 1}$, $_{\alpha v\beta 3}$, and $_{\alpha v\beta 5}$), which interact with the endogenous ECM proteins [31]. ECM proteins differentially modulate PSC propagation and the expression of undifferentiated markers. For instance, collagen type IV or type I favors self-renewal, while fibronectin or laminin reduces Oct-4 expression for mouse ESCs [32]. In the case of human PSCs, endogenous laminin α5 promotes self-renewal in an autocrine and paracrine manner [33]. In addition, endogenous ECM proteins (e.g., laminin and nidogen) regulate initial human ESC aggregation [34]. Moreover, PSC aggregates secrete endogenous ECM proteins which are assembled into a complex network, serving as a reservoir of endogenous growth factors and providing unique cues to undifferentiated ESCs to sustain Oct-4 expression [35].

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