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PREVIEWS

Previews highlight research articles published in the current issue of STEM CELLS TRANSLATIONAL MEDICINE, putting the results in context for readers.

Stuart P. Atkinson

Centro de Investigación Príncipe Felipe, Valencia, Spain. Email: satkinson@cipf.es

The widespread application of stem cells and stem cell derivatives as part of clinically applicable reparative/regenerative strategies will require the safe and efficient generation of vast numbers of cells. Unfortunately, traditional two-dimensional adherent culture systems for the expansion and differentiation of stem cells suffer from limitations that include restricted surface areas, intensive labor costs, and challenging control, monitoring, and automation processes that hinder scale-up attempts.[1,2] Stem cell expansion under a mixed and controlled culture environment within stirred-tank suspension bioreactors represents an exciting alternative that allows scalability without negatively impacting quality attributes[3,4]; furthermore, bioreactor culture set-ups permit the comprehensive monitoring and control of parameters such as temperature, pH, and dissolved oxygen. While the bioreactor culture of stem cells remains an efficient process, recent research has sought to significantly improve cell expansion by developing novel subculturing techniques and delineating those mechanisms that support the maintenance of stem cell characteristics under hydrodynamic conditions. In the first of our Featured Articles published this month in *STEM CELLS Translational Medicine*, Chen et al[5] report on the development of a bead-to-bead cell transfer process for human mesenchymal stem cell (MSC) subculture in stirred-tank bioreactors that permits the expansion of cells while maintaining proliferation, viability, and a normal phenotype. In a Related Article recently published online at *STEM CELLS*, Nath et al described how the fluid shear stress generated in stirred-tank bioreactors supports the pluripotency of mouse embryonic stem cells (ESCs) through a pathway involving the translocation of β-catenin from adherens junction to the nucleus and the induced expression of pluripotency-associated genes.[6]

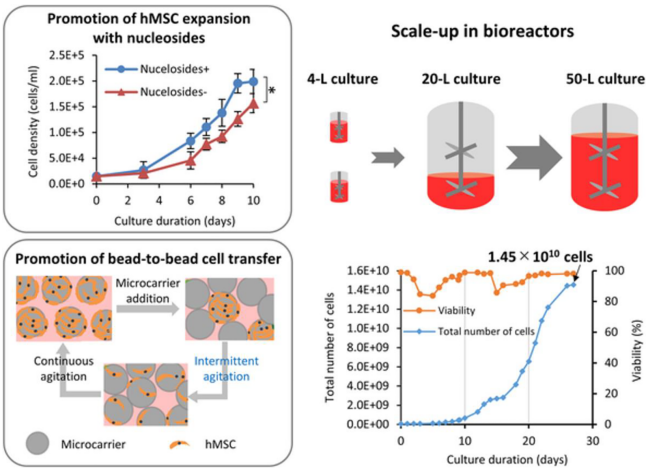
The ongoing development of therapies that rely on the large-scale derivation, expansion, and/or differentiation of a range of stem cell types will also require the implementation of quality control mechanisms to ensure both safety and efficacy post-administration/transplantation. While approaches to evaluate safety and functionality currently exist or are undergoing development at the small scale, their widespread use and/or adaptation to larger-scale cultures suffers from problems related to their high complexity, difficult standardization, and elevated cost. The analysis of cell morphology and dynamic behavior represents one of the most relevant alternate approaches, and this strategy has become particularly relevant with the introduction of live-cell imaging coupled with video bioinformatics analysis of time-lapse data, which permits the quantitative and noninvasive quality control monitoring of stem cell cultures.[7,8] Examples include a study by Wong et al,[9] who employed noninvasive imaging to predict the successful development of two-cell human embryos into blastocysts, and a study from Chan et al, who applied live cell imaging to identify those colonies most likely to give rise to bona fide induced pluripotent stem cells (iPSCs) during reprogramming.[10] In the second of our Featured Articles published this month in *STEM CELLS Translational Medicine*, Lin et al[11] present StemCellQC, a label-free video bioinformatics analysis tool for the in vitro evaluation of pluripotent stem cell morphology and dynamics, as an effective quality control methodology that can be adapted to large scale stem cell culture. In a Related Article published this month in *STEM CELLS*, Li et al[12] demonstrated the application of a patch-clamp technique to establish electrophysiological features as a reliable and sensitive quality control indicator for the therapeutically relevant cells within developing stem cell-derived retinal organoids.

FEATURED ARTICLES

Novel Bioreactor Subculture Methodology Improves Large-Scale Culture of Mesenchymal Stem Cells

The large-scale manufacture of human MSCs in stirred-tank bioreactors for therapeutic purposes requires the optimization of subculture techniques, as current approaches for cell detachment and separation from microcarriers and reinoculation onto fresh microcarriers suffer from significant limitations.[13,14] The induced spontaneous migration of MSCs to fresh microcarriers that provide greater surface area for cell growth ("bead-to-bead" cell transfer) represents an interesting, but currently under-optimized, alternative. In their new *STEM CELLS Translational Medicine* article,[5] researchers from the laboratory of Katsuhiko Nakashima (Showa Denko Materials Co., Ltd, Yokohama-shi, Kanagawa, Japan) report on the development of an efficient bead-to-bead cell-transfer method for the culture of human MSCs in stirred-tank bioreactors and highlight a role for nucleoside supplementation.[15] Chen et al first established that nucleosides supported the proliferation and cell surface marker expression of human MSCs cultured in conventional and microcarrier-based suspension culture. Next, they demonstrated that intermittent agitation following the

addition of fresh microcarriers to cell-populated microcarriers induced the spontaneous migration of MSCs to the fresh microcarriers; furthermore, this strategy reduced microcarrier aggregation and improved overall yield. In small-scale spinner flasks, bead-to-bead cell transfer permitted serial MSC subculture for five passages; however, the authors also discovered that this strategy also functioned in larger scale cultures (from 4 up to 50 L) that employed single-use stirred-tank bioreactors. Overall, the application of nucleoside supplementation and this novel bead-to-bead cell transfer methodology supported a near 250-fold increase in cell number over approximately four weeks, with resultant MSCs displaying robust proliferation, trilineage differentiation, and potent immunomodulation and expressing known cell surface markers and maintaining a normal phenotype.



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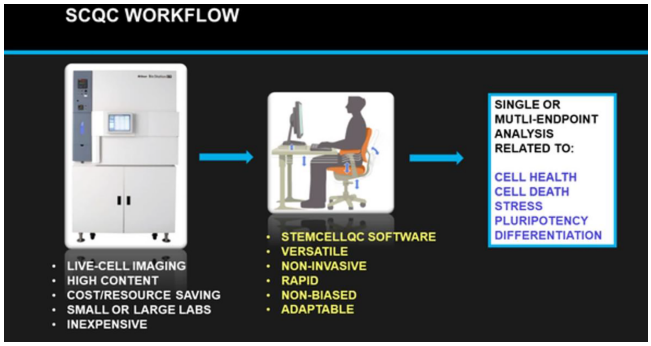
Supporting Pluripotent Stem Cell Culture Quality Control with Video Bioinformatics

A previous study from researchers led by Prue Talbot (University of California, Riverside, California) introduced "StemCellQC," a software package that employs phase-contrast images of ESCs/iPSCs to produce quantitative data on features related to colony morphology and dynamics.[16] In their recent *STEM CELLS Translational Medicine* article,[11] the team reported on the application of StemCellQC (or SCQC) to evaluate the impact of various substrates, culture media, cell types, and experimental treatments on the morphology and dynamic behavior of ESC/iPSC colonies from live-cell imaging data as part of a proposed quality control strategy. Lin et al highlighted the use of laminin-521 as a better growth matrix than other commercial alternatives by fostering better attachment and inducing lower levels of apoptosis. The analysis of various advanced growth media formulations revealed that media choice significantly affected ESC/iPSC morphology, growth rates, motility, and death rates; furthermore, the authors noted that different batches of the same growth media prompted different results. StemCellQC was also able to detect dynamic differences in undifferentiated and differentiating ESCs/iPSCs with regards to growth rate, general aspect, and motility. Finally, the authors reported that StemCellQC effectively detected cell line-specific alterations after exposing ESCs/iPSCs to an environmental chemical (in this case, cinnamaldehyde), a tactic often employed to evaluate potential alterations to early embryonic development.[17] Overall, the authors posit their StemCellQC methodology as a rapid, resource-saving, noninvasive tool for quality control in basic and translational research, toxicological/drug testing, and the development of stem cell-based therapies.



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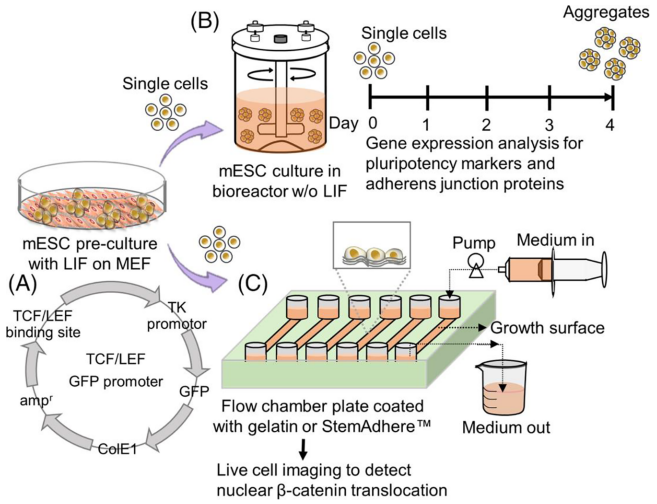
RELATED ARTICLES

Delineating How Fluid Shear Stress in Bioreactor Cultures Supports Embryonic Stem Cell Pluripotency

Previous studies from the laboratories of Michael S. Kallos and Derrick E. Rancourt (University of Calgary, Calgary, Alberta, Canada) revealed that the fluid shear stress associated with the culture of mouse ESCs in stirred-tank suspension bioreactors permitted the maintenance of pluripotency in the absence of leukemia inhibitory factor (LIF),[18,19] a pleiotropic cytokine that supports mouse ESC expansion in the absence of embryonic fibroblasts feeders. In their most recent *STEM CELLS* article,[6] Nath et al reported on their investigations into how the hydrodynamic environment within stirred-tank bioreactors modulates mouse ESC behavior. Overall, mouse ESCs cultured in the absence of LIF exhibited a higher expression of pluripotency marker genes in bioreactor culture when compared to traditional two-dimensional adherent culture. Fascinatingly, the authors provided evidence for a mechanotransduction mechanism that supports pluripotency in bioreactor culture—a phenomenon they term "mechanopluripotency." The proposed mechanism involves the release of β-catenin from mouse ESC adherens junction in response to shear stress and the subsequent translocation of β-catenin to the nucleus to induce *c-Myc* expression, which then promotes the elevated expression of pluripotency marker genes such as *Oct4*, *Sox2*, and *Nanog* to support mouse ESC pluripotency. The authors believed

that a better understanding of mouse ESC responses to the complex hydrodynamic environment of stirred-tank bioreactors might permit the optimization of cell expansion protocols to safely and efficiently generate the large cell numbers required for the widespread application of stem cell therapies.



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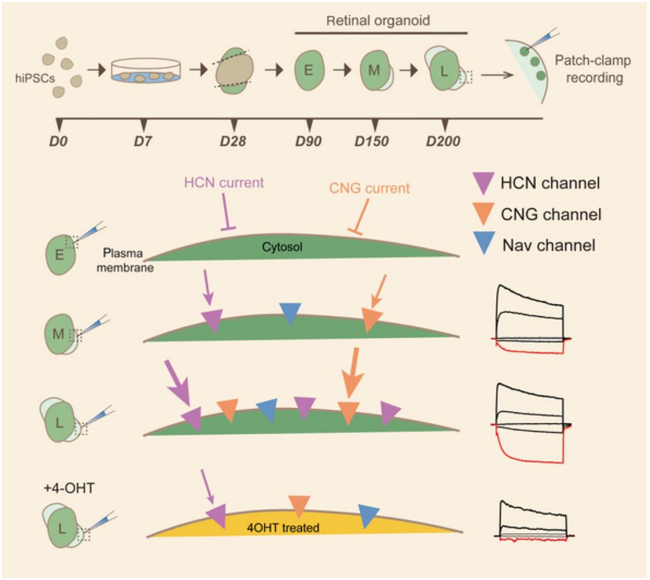
measures might function as an extra quality control step to ensure the safety and efficacy of retinal organoid-derived cells that may form part of cell replacement therapies for a range of vision-loss-associated conditions.



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New Quality Control Measure for Human iPSC-Derived Retinal Organoid-Derived Cells

Physiological approaches such as calcium imaging and multi-electrode array recordings function as quality control measures by assaying the functionality of the photoreceptors or ganglion cells that develop within maturing stem cell-derived retinal organoids.[20,21] In the hope of providing an accurate and sensitive physiological measure of retinal organoid maturation that may function as a reliable standard for quality control and interstudy comparisons, researchers led by Huan Zhao (Hefei University), Weiping Xu, and Tian Xue (University of Science and Technology of China, Hefei, Anhui, China) recently interrogated the electrophysiology of photoreceptor-like cells in human iPSC-derived retinal organoids. As reported in a recent *STEM CELLS* article,[12] Li et al evaluated patch-clamp recordings from photoreceptor-like cells in retinal organoids to determine electrophysiological properties that associate with physiological function. They discovered that specific ionic hallmarks (eg, hyperpolarization-activated cyclic nucleotide-gated [HCN] and cyclic nucleotide-gated currents [CNG]) matured progressively during differentiation and took on characteristics similar to macaque or human native photoreceptors after 200 days in culture. Furthermore, the authors established that the "inward-outward current ratio" accurately represented photoreceptor maturity, and they underscored the potential of this electrophysiological measure as an indicator of the pathological state. Overall, the authors hoped that these electrophysiological

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