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Culture methods and environmental cues of human pluripotent stem cells

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Culture methods

Feeder based culture

Standard human pluripotent stem cells (hPSCs) cultures have supportive cells such as inactivated mouse embryonic fibroblast (MEF) feeder cells that aid cell growth, secrete several important growth factors into the medium, which help maintain pluripotency and prevent differentiation [1]. Feeder based cultures are suitable for routine maintenance of hPSCs colonies, and MEFs are the most frequently used feeder cells, particularly because they support the robust growth of all types of embryonic stem cells as colonies [2]. However, since MEFs have complex and undefined heterogeneity, a variety of human cell types such as human fibroblasts, tubal, foreskin, and bone marrow-derived stromal cells can be used as feeder cells instead of MEF [2]. Feeder based cultures are suitable for routine hPSCs maintenance, genetic engineering and single cell cloning, but are not used for clinical applications.

Feeder-free culture

Feeder-free culture, such as KSR supplemented with Activin A and FGF-2, has been developed which has been shown to support self-renewal in the undifferentiated state of human embryonic stem cells [1].

Non-colony type monolayer (NCM) expansion

Single cell-based non-colony type monolayer (NCM) cultures have been developed since colony culture or suspension culture is not suitable due to disadvantages such as heterogeneity and suspension environment for drug screening or single cell analysis [3]. 2D monolayer cultures of pluripotent stem cells require sufficient cell numbers and cell density. HPSCs can be propagated by adhering monolayer to a weakly minus charged plastic container. Extracellular matrix proteins and proteoglycans are first secreted from the cells to form a matrix, which is attached to plastic containers such as negatively charged polystyrene. Cells are attached to the matrix through specific cell surface receptors on the cell surface. Thus, using existing culture vessels with better surfaces for attachment results in faster cell attachment [2].

Dissociated cells (single cells) at high density (ie, ~ 1.4×10^5 / cm^2) were cultured on Matrigel coated polystyrene plates using Rho kinase (ROCK) inhibitor or JAK inhibitor 1, resulting in high single cell plating efficiency for early 24 h. In addition, hPSCs can be cultured on NCM at the surface of human recombinant laminin-512 coated polystyrene without the use of a ROCK inhibitor, and generally hPSCs under these growth conditions have genetic stability and pluripotency [3].

The advantages of this culture are the feeder-free, adjustable growth rate, the production of homogeneous hPSCs, rapid cell growth, rapid (2-4 days) cell recovery after cryopreservation (vs. 1 to 3 weeks of colony culture). In particular, hPSCs grown with NCM are highly effective in teratoma formation and can be converted into colony-type culture if grown as clumps [2].

Batch culture

Batch incubation is a partially closed system in which most of the required material is loaded into the bioreactor vessel and only the substances necessary for gas exchange and pH adjustment are added or removed [2].

Perfusion culture

The perfusion culture is a culture method in which a new culture medium is continuously supplied by perfusion and the consumed medium is removed to thereby form a constant culture environment and enable cell culture for a much longer period of time [4].

Suspension Cultures

To expect the clinical effects of hPSCs, ranging from 10^7–10^{10} or beyond are essential. Suspension cultures of bioreactors enable large scale production of hPSCs for this purpose, and hPSCs grown by suspension culture remain pluripotent and chromosomally stable. Among many types of bioreactors, the spinner vessel and stirred-tank bioreactor provide a glass container with a working volume of 50 mL to 200 L and a homogeneous growth environment and can precisely control culture conditions by real-time monitoring of a temperature, oxygen level, acidity, growth factor consumptions, and metabolite concentrations [5]. A major disadvantage of suspension culture is cell damage associated with hydrodynamic shear forces [5]. Another disadvantage is that growth rates vary according to different suspension culture conditions. As the size and irregularity of cell aggregates increases, apoptotic cell loss, cell differentiation and heterogeneity may occur. In addition, the long-term effect of ROCK inhibition on hPSCs is unclear.

Environmental cues

In addition to the aforementioned culture media and extracellular matrix, there are physical and physiological environments that promote

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hPSCs growth such as temperature, humidity, osmosity, acidity, stiffness of growth surface, and cell density. Traditional hESC cultures proceed at normal oxygen levels whereas early in vivo mammalian embryos develop in hypoxic conditions. Low oxygen concentration (2 - 3%) at the physiological level reduces spontaneous differentiation of hESCs, improves hESC clone recovery by up to 6-fold and reduces chromosomal aberrations without affecting the expression of pluripotent markers [6]. Pluripotent stem cells have traditionally been grown on MEF on Matrigel as colonies and differentiate as embryoid body (EB) aggregates. The intercellular connections through intracellular signaling, cell density, ligand-receptor interaction, etc., have a great influence on the microenvironment of hPSCs. With the discovery of single cell death and survival mechanisms, various forms of culture can be selected, such as colonies, single-cell, single cell-based non-colony type monolayer, and suspended aggregates [1].

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