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Review

Advances and challenges in stem cell culture

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ABSTRACT

Stem cells (SCs) hold great promise for cell therapy, tissue engineering, and regenerative medicine as well as pharmaceutical and biotechnological applications. They have the capacity to self-renew and the ability to differentiate into specialized cell types depending upon their source of isolation. However, use of SCs for clinical applications requires a high quality and quantity of cells. This necessitates large-scale expansion of SCs followed by efficient and homogeneous differentiation into functional derivatives. Traditional methods for maintenance and expansion of cells rely on two-dimensional (2-D) culturing techniques using plastic culture plates and xenogenic media. These methods provide limited expansion and cells tend to lose clonal and differentiation capacity upon long-term passaging. Recently, new approaches for the expansion of SCs have emphasized three-dimensional (3-D) cell growth to mimic the *in vivo* environment. This review provides a comprehensive compendium of recent advancements in culturing SCs using 2-D and 3-D techniques involving spheroids, biomaterials, and bioreactors. In addition, potential challenges to achieve billion-fold expansion of cells are discussed.

Contents

Abbreviations: SCs, stem cells; 2-D, two-dimensional; 3-D, three-dimensional; ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; ECM, extracellular matrix; MEF, mouse embryonic fibroblast; LIF, leukemia inhibitory factor; MSCs, mesenchymal stem/stromal cells; HA, hyaluronic acid; PEG, polyethelene glycol; PLL, poly-L-lysine; PLA, poly-lactic acid; PGA, poly-glycolic acid; PCL, polycaprolactone; PGLA, poly-dl-lactic acid-co-glycolic acid; Dex-SH, thiol-functionalized dextran; PEG-4-Acr, PEG functionalized with four-arm acrylate; PDMS, polydimethylsiloxane.

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1. Introduction

Stem cells (SCs) have the capacity to self-renew and differentiate into specialized cells and are defined by their origin and degree of potency [1]. Pluripotent SCs are capable of unlimited self-renewal and differentiation into any of the over 200 types of cells in the body [2,3]. There are two sources of pluripotent SCs. First, embryonic stem cells (ESCs) are derived from the inner cell mass of a pre-implantation blastocyst [4] and pluripotency is controlled by an intrinsic regulatory network of core transcription factors, octamer-binding transcription factor 4 (OCT4), sex determining region Y-box 2 (SOX2), and Nanog homeobox (NANOG) [5]. Second, induced pluripotent stem cells (iPSCs) are derived by the ectopic or elevated expression of four transcription factors, OCT4, SOX2, Kruppel like factor 4 (KLF4), and MYC proto-oncogene (C-MYC) essential for induction of pluripotency in somatic cells [3].

Another type of SCs, mesenchymal stem/stromal cells (MSCs) are isolated from adult sources such as bone marrow and adipose tissues [6] or perinatal tissues, such as umbilical cord, cord blood, placenta and amniotic fluid [7,8]. MSCs are characterized by adherent growth to plastic culture plates, exhibiting clonal growth. They are positive for mesenchymal surface markers, CD90, CD73, CD29, and CD105 and negative for hematopoietic lineage markers, CD45, CD34, and HLA-DR [9]. Unlike pluripotent SCs, MSCs are multipotent and differentiate into only limited cell types such as osteogenic, chondrogenic, and adipogenic cells [10]. Also, their self-renewal and differentiation potential is dependent upon the source of isolation [11]. In addition, MSCs derived from adult tissues are also affected by aging and exposure to environmental stresses, which could alter genetic stability [8]. In comparison to adult MSCs, MSCs obtained from perinatal tissues exhibit higher growth and stemness potential [12].

As such, the study of SCs has contributed to the elucidation of basic biochemical and developmental processes [13–17]. They have also shown tremendous potential for *in vitro* disease models, tissue engineering, regenerative medicine, and cell therapy as well as pharmaceutical applications [18–21]. Furthermore, they can be used for drug discovery and development [22,23]. All these uses require large-scale production of high quality cells [24].

Traditionally, SCs are propagated as a monolayer in two-dimensional (2-D) plastic culture plates and often require undefined or xenogenic materials including but not limited to attachment substrates, cytokines and growth factors, as well as serum. Use of xenogenic or animal derived media can potentially transmit pathogens and limit reproducibility between cultures due to lot-to-lot fluctuation of the material used [25]. Monolayer culture necessitates routine passaging to maintain self-renewal and potency of cells, which is highly inefficient for large-scale expansion of cells. In addition, 2-D attachment alters cell shape and geometry [26], leading to cell flattening and changes in the internal cytoskeleton and nuclear shape [27], which in turn modifies gene and protein expression [28,29]. Furthermore, studies have shown that the composition and organization of the extracellular matrix (ECM) can also send biochemical and mechanical signals for cell differentiation [25,30,31].

2-D culture techniques and applications have been practiced for the majority of primary and established cell lines and standardized for analytical assays ranging from microscopy and counting cells to the study of disease processes and drug testing [32]. Notably, 2-D culture has been used for directed differentiation of SCs into many specialized cells, including chondrocytes, osteocytes, adipocytes, cardiomyocytes, smooth muscle cells and hepatocytes [33,34]. However, 2-D culture often results in a lack of functional derivatives [35]. Studies have also shown that 2-D monolayer culture fails to accurately reproduce animal physiology [36], and proves insufficient to validate drug discovery [37].

Overall, 2-D culture conditions lack the intricacy necessary to mimic the SC niche, dynamic and specialized three-dimensional (3-D) microenvironments, which are responsible for the regulation of SC fate *in vivo*. Native 3-D niches allow for complex spatial interactions between cells, ECM components, and gradients of nutrients, oxygen, and waste [38]. The effect of the 3-D microenvironment can be easily demonstrated in pluripotent SCs, which retain their pluripotency when injected into the inner cell mass of another embryo, but spontaneously differentiate into all three germ layers when injected elsewhere in animals due to external cues [39]. Furthermore, supportive niches for multipotent SCs, have been identified in the gonads, intestinal crypts, hair follicles, and bone marrow *in vivo,* whereby anchored cells release signals that govern continued self-renewal or lineage-specific differentiation [40]. Transplantation of multipotent SCs outside the niche results in differentiation into various cell lineages dependent upon the cues in the surrounding *in vivo* microenvironment [41].

To address the various problems facing 2-D culture of SCs, 3-D culture methods have been developed to control cell fate by incorporating physiologically relevant and biomimetic microenvironments by mimicking the ECM composition and stiffness of the SC niche *in vitro* [27,38,42,43]. However, 3-D culture for SC maintenance and expansion still remains a challenge [44], primarily, due to need to optimize culture conditions for different types of cells and analysis. Therefore, is it necessary to elucidate the mechanisms important for SC maintenance, including biomaterial signaling and mechanical forces that would aid in the uniform and reproducible expansion of SCs without loss of genetic stability or differentiation potential.

This review is focused on the recent progress in 3-D culture for expansion of SCs, both in quality and quantity, acceptable for clinical, pharmaceutical and biotechnological applications. In addition, we will report some important recent advancements in 2-D culture, since these methods have historically led to significant developments in SC biology and early studies have been reviewed previously [22,45]. Overall, this review assesses the latest advances in proliferation and differentiation of pluripotent and multipotent SCs.

2. Two-dimensional culture techniques

2.1. Maintenance and differentiation of pluripotent SCs

2-D culture studies have focused on the maintenance of stemness, a self-renewing state characteristic of undifferentiated SCs, by extrinsic factors such as mechanical forces between both cells and their 3-D microenvironment, which are transduced to biological cues that can control cell shape and function [25,46–49].

Usually, pluripotent SCs are grown on plastic culture dishes coated with ECM components (such as gelatin, Matrigel, or collagen) or a mouse embryonic fibroblast (MEF) feeder layer to aid in