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Research review paper

Application of human mesenchymal and pluripotent stem cell microcarrier cultures in cellular therapy: Achievements and future direction

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ABSTRACT

Mesenchymal stem cells (MSCs) have recently made significant progress with multiple clinical trials targeting modulation of immune responses, regeneration of bone, cartilage, myocardia, and diseases like Metachromatic leukodystrophy and Hurler syndrome. On the other hand, the use of human embryonic and induced pluripotent stem cells (hPSCs) in clinical trials is rather limited mainly due to safety issues. Only two clinical trials, retinal pigment epithelial transplantation and treatment of spinal cord injury were reported. Cell doses per treatment can range between 50,000 and 6 billion cells. The current 2-dimensional tissue culture platform can be used when low cell doses are needed and it becomes impractical when doses above 50 million are needed. This demand for future cell therapy has reinvigorated interests in the use of the microcarrier platform for generating stem cells in a scalable 3-dimensional manner.

Microcarriers developed for culturing adherent cell lines in suspension have been used mainly in vaccine production and research purposes. Since MSCs grow as monolayers similar to conventional adherent cell lines, adapting MSCs to a microcarrier based expansion platform has been progressing rapidly. On the other hand, establishing a robust microcarrier platform for hPSCs is more challenging as these cells grow in multilayer colonies on extracellular matrices and are more susceptible to shear stress.

This review describes properties of commercially available microcarriers developed for cultivation of anchorage dependent cells and present current achievements for expansion and differentiation of stem cells. Key issues such as microcarrier properties and coatings, cell seeding conditions, medium development and improved bioprocess parameters needed for optimal stem cell systems are discussed.

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1. Introduction

In this review, we cover the two broad classes of anchorage dependent stem cells (human mesenchymal and pluripotent stem cells) with the greatest promises for cell therapy success in clinical trials. Both of these cell types are universally grown in 2 dimensional (2D) cultures. However for large scale production, there is a shift towards 3 dimensional (3D) suspension cultures, in particular with the use of microcarriers (MCs) in bioreactors. This article begins with reviews on these two cell types, their growth requirements, use in clinical trials and potential applications. MC technology and their usage in stem cell expansion and differentiation are subsequently depicted. Challenges still to be overcome are also highlighted, as the production of large doses of cells becomes necessary for late stage clinical trials and commercialization.

2. Multipotent and pluripotent stem cells: source, mode of growth and applications in clinical trials

2.1. Sources of multipotent/mesenchymal stem cells

Multipotent or mesenchymal stem cells (MSCs) are attracting increasing interest for possible application in cell therapies. MSCs encompass a broad range of anchorage dependent fibroblast-like cells which can be obtained from bone marrow aspirates, skeletal muscle connective tissue, human trabecular bones, adipose tissue, periosteum, fetal blood and liver, and umbilical cord blood, as reviewed by Oh and Choo (2011). Homogeneous MSCs can also be derived from human embryonic stem cells (hESCs) (Lian et al., 2007; Olivier et al., 2006). MSC express the CD29, CD44, CD73, CD90, CD105 and primitive Stro-1 markers (Dominici et al., 2006). They can proliferate in vitro and differentiate into mesoderm-type lineages, including osteoblasts, chondrocytes, adipocytes, myocytes and vascular cells. Due to this ability, MSC provide a versatile source of progenitor cells for research and clinical applications in the field of tissue regeneration.

2.2. Mode of growth

MSCs are typically grown on plastic tissue culture dishes as monolayers with no additional coatings (Fig. 1A and C). Basal media supplemented with fetal calf serum between 5 and 10% is widely utilized, but its use in the context of clinical applications is associated with several risks such as viral and prion transmission (Bernardo et al., 2007; Govindasamy et al., 2011; Shahdadfar et al., 2005) or immunological reactions (Selvaggi et al., 1997; Tuschong et al., 2002). Several new serum free media such as MesenCult®-XF Medium (Stemcell Technologies), StemPro® MSC SFM (Life Technologies), MSC Nutristem® XF Medium (Biological Industries), BD Mosaic™ (Becton Dickinson) in conjunction with surface coatings with proprietary extracellular matrices are now becoming available from companies such as Life Technologies, StemCell Technologies, Biological Industries and Becton Dickinson.

2.3. Differentiation capabilities

The tri-lineage differentiation capability of MSCs into osteoblasts, adipocytes, and chondrocytes has been evaluated by many groups. Osteogenesis requires MSCs to be incubated with β-glycerol-phosphate, ascorbic acid-2-phosphate, dexamethasone and fetal bovine serum. MSCs should reveal osteoblastic morphology together with high expression of alkaline phosphatase and calcium deposition. To view osteoblast generation, Von Kossa staining is a technique which subjects cell cultures to silver nitrate solution. Calcium is then reduced by light and silver deposits generated, which can be visualized by microscopy (Chase et al., 2010). For adipogenesis, MSC cultures are incubated with isobutylmethylxanthine to form adipocytes with lipid vacuoles. This process is induced by nuclear receptor, PPAR-γ, transcription factors and fatty acid synthetase. Lipid vacuoles are detected by oil red O staining; a fat soluble-oil for staining lipid and fat in culture sections (Chase et al., 2010). Chondrogenesis is performed in a 3D culture pellet, with a serum-free nutrient medium and transforming growth factor-β3 (TGF-β3). Under such conditions, MSCs quickly change their fibroblastic appearance and express cartilage-specific matrix-layers filled with glycosaminoglycans. Toluidine blue indicator, a polychromatic dye, is used to stain for glycosaminoglycan-containing components (Chase et al., 2010; Zhang et al., 2011). In addition, such differentiated MSCs can generate type II collagen, another cartilage component (Zhang et al., 2011).

Other than these three lineages, MSCs are believed to be able to differentiate into myoblasts, cardiomyocytes and even neurons. Formation of cells of non-mesodermal origin may be a result of a phenomenon known as “stem cell plasticity”, a transdifferentiation process in which organ-specific stem cells are no longer restricted to forming the differentiated cell types of the tissue where they reside (Lakshmipathy and Verfaillie, 2005).

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