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Review

Culture media for the differentiation of mesenchymal stromal cells

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ABSTRACT

Mesenchymal stromal cells (MSCs) can be isolated from various tissues such as bone marrow aspirates, fat or umbilical cord blood. These cells have the ability to proliferate in vitro and differentiate into a series of mesoderm-type lineages, including osteoblasts, chondrocytes, adipocytes, myocytes and vascular cells. Due to this ability, MSCs provide an appealing source of progenitor cells which may be used in the field of tissue regeneration for both research and clinical purposes. The key factors for successful MSC proliferation and differentiation in vitro are the culture conditions. Hence, we here summarize the culture media and their compositions currently available for the differentiation of MSCs towards osteogenic, chondrogenic, adipogenic, endothelial and vascular smooth muscle phenotypes. However, optimal combination of growth factors, cytokines and serum supplements and their concentration within the media is essential for the in vitro culture and differentiation of MSCs and thereby for their application in advanced tissue engineering.

1. Introduction

Since Owen and Friedenstein's work [1] it has been assumed that bone marrow contains special cells that can be expanded in vitro and differentiated into various mesoderm-type lineages, including bone, fat, cartilage, muscle, tendon, haematopoiesis-supporting stroma and vasculature [2–6]. We presently refer to these nonhematopoietic cells as mesenchymal stromal cell (MSC; Fig. 1) [7]. In addition, researchers have recently transdifferentiated MSCs into non-mesodermal cell types such as neuronal-like cells [8–11] and pancreatic cell progenitors [12–15].

To date, MSCs have been isolated not only from bone marrow but also from many other tissues and organs, including adipose tissue, umbilical cord blood, placental tissue, liver, spleen, testes, menstrual blood, amniotic fluid, pancreas and periosteum [16–20]. When cultured in vitro on polystyrene surfaces, MSCs reveal morphological heterogeneity. These cells can be narrow spindle-shaped, large polygonal or even cuboidal-shaped when growing into a confluent monolayer [21]. In adult human, MSCs lack the hematopoietic surface antigens, e.g. CD11, CD14, CD34 and CD45 [22]. Meanwhile numerous molecular markers have been found on MSC surface, but none of them is specific to MSCs. Despite this, molecules such as CD44, CD73, CD90, STRO-1 and CD105/SH2 [3,23–25] are still currently used to identify MSCs.

MSCs are considered to be nonimmunogenic since these cells have been transplanted into allogeneic hosts even without using any immunosuppressive drugs [22,26]. Furthermore, it has been reported that MSCs actually possess immunosuppressive properties by modulating the function of T-cells [27,28], dendritic cells and B-cells [29–32].

For the characterization of MSC plasticity, their ability to differentiate in vitro into osteoblasts, chondrocytes and adipocytes is currently treated as the gold standard. This, in combination with the advantages that MSCs have no immunogenicity and can be easily isolated from different tissues and expanded in vitro, enables MSCs to be a promising source of stem cells. Hence MSCs have

Abbreviations: 1,25-D3, 1,α25-dihydroxyvitamin D3; a2Col6, α chain 2 of type 6 collagen; ALP, alkaline phosphatase; asc, ascorbic acid; Asc-2-P, ascorbic acid 2-phosphate; AT, adipose tissue; BM, bone marrow; BM-MSCs, bone marrow-derived mesenchymal stromal cells; BMP, bone morphogenetic protein; BSP, bone sialoprotein; C/EBP, CCAAT-enhancer-binding proteins; cAMP, cyclic adenosine monophosphate; Col10, collagen type 10; Col11, collagen type 11; Col1a1, collagen type 1 alpha-1; Col2, collagen type 2; Col9, collagen type 9; CREB/p300, cAMP response element-binding protein/E1A binding protein p300; dex, dexamethasone; EC, endothelial cell; ECM, extracellular matrix; FABP4/aP2, fatty acid-binding protein-4; FCS, fetal calf serum; FGF, fibroblast growth factor; Gata-6, GATA-binding protein 6; GPDH, glycerol-3-phosphate dehydrogenase; IBMX, 3-isobutyl-1-methylxanthine; IGF, insulin-like growth factor; LDL, low-density lipoprotein; LPL, lipoprotein lipase; mRNA, messenger ribonucleic acid; MSC, mesenchymal stromal cell; OC, osteocalcin; ON, osteonectin; OPN, osteopontin; Osx, osterix; PDGF, platelet-derived growth factor; PPARγ2, peroxisome proliferation-activated receptor γ2; PRP, platelet-rich plasma; Runx-2, runt-related transcription factor-2; SCID, severe combined immunodeficiency; SM22-α, transgelin; SMMHC, smooth muscle myosin heavy chain; Sox9, SRY-related high-mobility group box 9; SRF, serum response factor; T3, triiodothyonine; TGF-β, transforming growth factor-beta; UCB, umbilical cord blood; UCWJ, umbilical cord Wharton's jelly; VEGF, vascular endothelial growth factor; vitD3, vitamin D3; VSMC, vascular smooth muscle cell; α-SMA, α-smooth muscle actin; β-GP, beta-glycerophosphate.

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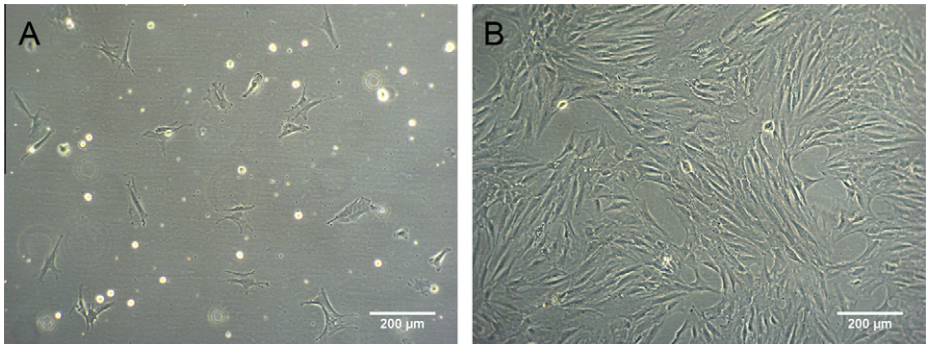

Fig. 1. Morphology of human immortalized single cell-derived BM-MSCs at different stages of confluency (A: 10%; B: 90%). Cells were cultured in α-MEM containing 1% penicillin/streptomycin and 10% FCS (BM-MSCs, bone marrow-derived MSCs).

been used in the therapy of diseases such as extended osseous defects [33], acute myocardial infarction [34], leukemia [35] and diabetes [36]. In addition, the homing capability endows MSCs with further potential applications. For example, MSCs may be used for supporting tissue regeneration [37], correcting congenital disorders (e.g. osteogenesis imperfecta [38]) and controlling chronic inflammatory diseases [39,40], and have even employed as vehicles for the delivery of biological agents [22] and as probes in the biocompatibility test of new implant materials. A prerequisite for the therapeutic application of MSCs is to develop efficient and standardized protocols so that MSCs can be induced to differentiate along the way as required. Therefore, we here present an overview of the optimized protocols for MSC differentiation towards the osteogenic, chondrogenic, adipogenic, endothelial and vascular smooth muscle phenotypes.

2. Osteogenic differentiation

2.1. Background

Bone diseases are major socioeconomic issues. The World Health Organization has acknowledged this fact by declaring the years 2000–2010 "The Bone and Joint Decade". The development of innovative bone-healing strategies is a prerequisite for the successful treatment of a variety of patients suffering from local bone defects caused by trauma, tumour, infection, degenerative joint disease, congenital crippling disorders or periprosthetic bone loss. Furthermore, bone graft material is frequently needed for spinal fusion, joint revision surgery, corrective osteotomy procedures and bone reconstruction in the field of oral and maxillofacial surgery. Bone grafting is one of the most common orthopaedic procedures with autologous bone graft providing osteoinductive growth factors, bone-forming cells and structural support for new bone ingrowth. However, the use of autologous bone graft is associated with the disadvantages of limited graft availability and donor site morbidity, e.g. pain, infection, pelvic fractures or neurovascular injury. The implantation of sterilized bone allograft material – usually derived from femoral heads during joint replacement procedures – as a widely used alternative bone-filling material may result in failure rates of up to 30% due to insufficient osseointegration of the graft, requiring further surgical intervention [41]. Insufficient bone-healing therefore remains a challenging issue. In this context, innovative cell-based strategies using MSCs are promising for both site-specific and systemic bone regeneration.

2.2. Morphology and differentiation markers

When being differentiated into osteoblasts, MSCs transform from a fibroblastic to a cuboidal shape, produce extracellular matrix (ECM), mainly composed of collagen type I, and in a later stage form aggregates or nodules that can be stained positively by alizarin red and von Kossa techniques. Increased expression of alkaline phosphatase (ALP; Fig. 2A) and calcium accumulation are observed in MSCs during osteogenic differentiation [3,22]. The enzymatic activity of ALP as well as the calcium content can be quantified by colorimetric assays [42]. At the molecular level, osteogenic differentiation of MSCs is controlled by interactions between distinct hormones and transcription factors.

Runt-related transcription factor-2 (Runx-2) effectuates the expression of bone-specific genes, e.g. osterix (Osx), collagen type 1 alpha-1 (Col1a1), osteocalcin (OC) and bone sialoprotein (BSP), by binding to the promoters of these genes [43–48]. Generally, Runx-2, ALP, Col1a1, transforming growth factor-beta 1 (TGF-β1), osteonectin (ON) and bone morphogenetic protein-2 (BMP-2) are known to be early markers of osteoblastic differentiation, whereas OC and osteopontin (OPN) are expressed later in the differentiation process [49–52].

2.3. Differentiation protocols

The classical method for osteogenic differentiation of MSCs in vitro involves incubating a confluent monolayer of MSCs with combinations of dexamethasone (dex), beta-glycerophosphate (β-GP) and ascorbic acid (asc) for several weeks. In addition, combinations of vitamin D3 (vitD3), transforming growth factor-beta (TGF-β) and bone morphogenetic proteins (BMPs) are used for osteogenic differentiation. In the following these supplements will be described in detail.

Dex is a synthetic glucocorticoid and has been reported to be an essential requirement for osteoprogenitor cell differentiation in MSCs [53,54]. While MSCs that were cultured in basal medium without osteogenic supplements express increased levels of ALP, they fail to express mineralized ECM as well as other osteogenic markers such as Col1 [55]. Although the precise mechanisms of action of dex on stem cell differentiation and skeletal function are not known, it is supposed that dex induces transcriptional effects. In rat osteoblast-like cells, for instance, dex induces transcription of BSP by binding on a glucocorticoid response element in the promoter region of the BSP gene [56]. On the other hand, dex improves the expression of the β-catenin-like molecule TAZ (transcriptional coactivator with PDZ-binding motif) as well as integrin α5, which both promote osteoblastic differentiation of MSCs by activating Runx-2-dependent gene transcription [57,58]. However, glucocorticoids in supraphysiological amounts have deleterious effects on bone in vivo, resulting in inhibition of osteoblast function [59]. In a study by Walsh et al. MSCs were cultured in the presence and absence of dex at concentrations between 10 pM and 1 μM for up to 28 days [60]. The authors suggest that the critical effective

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