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Review

# Bioreactors to influence stem cell fate: Augmentation of mesenchymal stem cell signaling pathways via dynamic culture systems ☆

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ABSTRACT

*Background:* Mesenchymal stem cells (MSCs) are a promising cell source for bone and cartilage tissue engineering as they can be easily isolated from the body and differentiated into osteoblasts and chondrocytes. A cell based tissue engineering strategy using MSCs often involves the culture of these cells on three-dimensional scaffolds; however the size of these scaffolds and the cell population they can support can be restricted in traditional static culture. Thus dynamic culture in bioreactor systems provides a promising means to culture and differentiate MSCs *in vitro*.

*Scope of review:* This review seeks to characterize key MSC differentiation signaling pathways and provides evidence as to how dynamic culture is augmenting these pathways. Following an overview of dynamic culture systems, discussion will be provided on how these systems can effectively modify and maintain important culture parameters including oxygen content and shear stress. Literature is reviewed for both a highlight of key signaling pathways and evidence for regulation of these signaling pathways via dynamic culture systems.

*Major conclusions:* The ability to understand how these culture systems are affecting MSC signaling pathways could lead to a shear or oxygen regime to direct stem cell differentiation. In this way the efficacy of *in vitro* culture and differentiation of MSCs on three-dimensional scaffolds could be greatly increased.

*General significance:* Bioreactor systems have the ability to control many key differentiation stimuli including mechanical stress and oxygen content. The further integration of cell signaling investigations within dynamic culture systems will lead to a quicker realization of the promise of tissue engineering and regenerative medicine. This article is part of a Special Issue entitled Biochemistry of Stem Cells.



## 1. Introduction

Mesenchymal stem cells (MSCs) are a multipotent stem cell population present in bone marrow as well as other tissue including adipose and can be readily differentiated *in vitro* into osteoblasts, chondrocytes, and adipocytes as well as tenocytes and myoblasts [1–3]. Therefore, these cells are a promising therapeutic cell source for regenerative medicine therapies to replace and repair these tissues. Therapies involving MSCs include direct transplantation of an MSC population, growth factor loaded scaffolds for MSC recruitment, and implantation of scaffolds containing an *in vitro* cultured MSC population [4–7]. Successful *in vitro* culture of MSCs requires an understanding of the signaling pathways that cue both the proliferation and guided differentiation of these cells. During differentiation chemical, biological, and mechanical cues induce these cells to follow a specific pathway dictating if a cell remains multipotent or differentiates into a specific cell type. These cues signal the release and uptake of cytokines, hormones, and growth factors which induce dynamic signaling pathways and mediate cell fate. Key signaling cascades for MSC differentiation include mitogen activated protein kinase (MAPK), Wnt, and SMAD. These pathways are mediated by growth factors including bone morphogenic protein 2 (BMP-2), transforming growth factor β2 (TGF-β2), and fibroblast growth factor (FGF) (please see Table 1 for a complete list of abbreviations). Release of these growth factors is modulated by the environment of the cell including surrounding cell types, physical culture parameters, factors present in the media, and mechanical stimuli [8–14]. Thus the cell environment must be regulated during *in vitro* stem cell culture. Bioreactor systems represent an important tool to regulate this environment. Bioreactors provide controlled mechanical stimuli to the cell as well as regulating the cell culture medium. In that way, they provide a level of control of cell culture parameters perhaps not possible in static culture.

Bioreactors, extensively used in the culture of MSCs, include simple systems such as spinner flask and rotating wall bioreactors and more complicated systems including perfusion and dynamic loading bioreactors [15–28]. While spinner flasks and rotating wall bioreactors fail to provide full control of culture parameters, perfusion and dynamic

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**Table 1**
Abbreviations.

| | |
|---|---|
| ALP | alkaline phosphatase |
| BMP | bone morphogenic protein |
| BMSC | bone marrow stromal cell |
| BSP | bone sialoprotein |
| COX-2 | cyclooxygenase-2 |
| ECM | extracellular matrix |
| ERK | extracellular signal-regulated kinase |
| EP4 | prostaglandin E receptor 4 |
| FGF | fibroblast growth factor |
| FRZ | frizzled |
| HIF | hypoxia inducible factor |
| hMSC | human mesenchymal stem cells |
| JNK | c-Jun N-terminal kinases |
| MAPK | mitogen activated protein kinase |
| MSC | mesenchymal stem cell |
| NF-KB | nuclear factor kappa B |
| OC | osteocalcin |
| OPN | osteopontin |
| Osx | osterix |
| $PGE_2$ | prostaglandin $E_2$ |
| RTK | receptor tyrosine kinase |
| Runx2 | runt-related transcription factor-2 |
| siRNA | small interfering ribonucleic acid |
| TGF-β | transforming growth factor beta |

loading systems have been demonstrated to be very effective in MSC culture. These systems have been shown to enhance both MSC chondrogenesis and osteogenesis as well as increase proliferation of these cells. By perfusing media through a porous scaffold, bioreactors can provide homogenous nutrient and oxygen concentrations to cells. As nutrient deprivation and hypoxia often occur in static culture, the ability of bioreactor systems to deliver homogenous nutrient and oxygen concentrations to cells makes these systems a key part of an *in vitro* culture strategy. This review will focus on another advantage of these systems: the potential to mediate cell signaling pathways to direct MSC proliferation and differentiation. *In vitro* these signaling pathways can be potentially triggered by environmental cues including mechanical stress and oxygen content which can be controlled using bioreactor systems. Thus this review will attempt to answer the following questions: What aspects of dynamic culture affect MSC differentiation pathways? How can bioreactors be used to augment these pathways?

## 2. Bioreactor systems for MSC culture

Many different bioreactors systems exist for the culture of mesenchymal stem cells including spinner flask [29–35], rotating wall [30,31,36–38], and perfusion [18,19,24,26–28,39–43] bioreactor systems (Fig. 1). Recent reviews have described the role of shear stress for bone tissue engineering [22,25] as well as detailing these systems [44–46]. All of these systems feature culture of MSCs in a three-dimensional environment.

Spinner flask culture consists of MSC containing scaffolds either suspended or free floating in a flask of culture media (Fig. 1A). The media is then circulated throughout the flask using a stir bar. Rotating wall bioreactors feature scaffolds placed between two concentric cylinders in culture media (Fig. 1B). While the inner cylinder remains stationary, the outer cylinder rotates moving the media in a circulatory manner. These systems have been shown to increase MSC proliferation and osteoblastic differentiation [32,35]; however, these systems lack the ability to regulate oxygen and shear stress throughout a scaffold. This is because these systems focus primarily on media mixing while exhibiting a small amount of shear stress to the outer regions of scaffolds. Media mixing ensures a homogenous oxygen gradient in the bulk media, but non-homogenous concentrations can result throughout the scaffold. Perfusion bioreactor systems have the ability to yield a more tight control over scaffold exposure to oxygen and shear stress. The basic perfusion bioreactor design features media pumped from a media reservoir through a tubing circuit, via a pump (Fig. 1C). Within the tubing circuit there is a growth chamber containing the scaffolds. In many perfusion bioreactor designs, a porous scaffold is used and is press fit into the growth chamber [15,28,39]. Media is then directly perfused through pores in the scaffold. An alternative type of perfusion bioreactor uses a modular design in which scaffolds are packed into a growth chamber [26,27,47–51]. In these designs, a collection of smaller scaffolds is cultured in a growth chamber and then can be implanted as one larger construct.

Perfusion bioreactor systems have been very effective for the culture of MSCs, being demonstrated to increase proliferation [26,52–54] , osteogenesis [15,24,28], and chondrogenesis [55]. These observed results are attributed to the ability of the systems to increase nutrient transport including oxygen and expose the cells to mechanical stimulus. When the effect of these two stimuli was independently evaluated, shear stress and mass transport were each shown to have an effect on human mesenchymal stem cell (hMSC) growth and osteoblastic differentiation [41]. In this study, shear and mass transport could be decoupled by changing media viscosity. In this way, it was shown that increasing shear from 0.05 to 0.15 dynes/$cm^2$ caused hMSCs to express higher levels of late osteoblastic markers osteopontin (OPN) and osteocalcin (OC) at 28 days. Increased flow rates (while keeping shear constant) also led to higher marker levels, but became inhibitory when the highest flow rate tested of 9 mL/min was reached. This demonstrates that MSCs cultured in perfusion systems respond to flow rates in two ways, through both changes in nutrient transport and shear stresses. Because of the multitude of factors influencing differentiation in bioreactor systems, the exact parameters influencing MSC differentiation may be difficult to discern. However, such studies decoupling these parameters can lead to a greater understanding of MSC culture in bioreactor systems.

## 3. Bioreactors to mediate shear stress

Another powerful mechanism by which bioreactor culture can augment stem cell signaling and fate is through exposure to mechanical stresses [5]. Shear has a dramatic effect on MSC differentiation and bioreactors have the capability to regulate shear in three-dimensional constructs [22,25,43]. A notable demonstration of this is the ability of bioreactor systems to influence osteoblastic differentiation of MSCs without typical osteogenic induction media including the glucocorticoid steroid dexamethasone [19]. In one study, MSCs cultured in a perfusion bioreactor without osteogenic supplements exhibited elevated levels of osteogenic markers compared to static culture. Based on alkaline phosphatase (ALP) and osteopontin levels in these studies, dynamic culture can be a strong inducer of MSC osteoblastic differentiation [19]. As a possible mechanism, human MSCs exposed to 12 dynes/$cm^2$ of shear stress show an upregulation of ALP expression dependent on p38 and extracellular signal-related kinase (ERK) activation [56]. These two signaling mechanisms of the mitogen activated protein kinase (MAPK) pathway described in detail later in the review may provide a mechanism for shear stress regulation of MSC osteoblastic differentiation. In addition to the presence and magnitude of shear, the particular shear regime may also influence stem cell fate [57,58]. When rat bone marrow stromal cells (BMSCs), containing a heterogeneous population of MSCs, were differentiated into immature osteoblasts and exposed to a continuous shear stress of 2.3 dynes/$cm^2$ the cells underwent a rapid phosporalization of ERK and p38. When this shear was delivered intermittently, the phosporalization was delayed. Synthesis of prostaglandin $E_2$ ($PGE_2$) however was increased with intermittent flow, hypothesized to be a result of signaling molecules being permitted to accumulate during breaks in the flow regime. Following just 24 hours of stimulation, cells expressed higher levels of osteoblastic differentiation markers 13 days later; however the flow regime did not affect these markers. Thus shear may have a powerful effect on MSC differentiation after just a short regime, and the nature of the shear regime could have an outcome