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Low frequency mechanical stimulation inhibits adipogenic differentiation of C3H10T1/2 mesenchymal stem cells

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ABSTRACT

Oscillatory mechanical stimulation at relatively high frequencies (0.1 Hz) has been shown to inhibit adipogenic and promote osteogenic differentiation of mesenchymal stem cells. However, for physiological interpretations and ease of implementation it is of interest to know whether different rates of mechanical stimulation can produce similar results. We hypothesized that relatively low frequency mechanical stimulation (0.01 Hz) can inhibit adipogenic differentiation of C3H10T1/2 mouse mesenchymal stem cells, even in a potent adipogenic differentiation medium. C3H10T1/2 cells were cultured in adipogenic medium under control (non-mechanically stimulated) conditions and under oscillatory surface stretch with 10% amplitude and 0.01 Hz frequency for 6 h per day for up to 5 days. Cell population was assessed by counting and adipogenic differentiation was assessed by real-time quantitative PCR (qPCR) analysis of peroxisome proliferator-activated receptor gamma (PPARγ) and fatty acid binding protein 4 (FABP4) after 3 and 5 days. Involvement of the ERK signaling pathway was assessed by Western blot. Low frequency mechanical stimulation significantly decreased expression of PPARγ after 3 days and FABP4 after 3 and 5 days versus non-stimulated culture. ERK signaling was decreased in mechanically-stimulated culture, indicating a role in the inhibition of adipogenic differentiation.

Application of this study: Low frequency mechanical stimulation may provide a technically simple means for control of mesenchymal stem cell differentiation in cell-based therapies, particularly for inhibition of differentiation toward undesired adipogenic lineages.

1. Introduction

Mesenchymal stem cells (MSCs) are pluripotent cells, which are of interest for studying events pertinent to development and tissue engineering because of their ability to differentiate along myo-, adipo-, osteo- and chondrogenic lineages (Caplan and Bruder, 2001; Pittenger et al., 1999). In order to direct differentiation of stem cells to specific lineages, a variety of different cytokines and chemokines may be used. Adipogenic differentiation is induced in expanded MSC cultures by treatment with 1-methyl-3-isobutylxanthine, dexamethasone, insulin and indomethacin (Fève, 2005; Fink and Zachar, 2011; Nagai et al., 2007; Pittenger et al., 1999). MSCs differentiating along this lineage are characterized by decreased proliferation rate, lipid sequestration in fat bodies and increased expression of peroxisome proliferator-activated receptor gamma (PPARγ) and fatty acid binding protein 4 (FABP4). Since adipogenic differentiation of MSCs occurs at the expense of osteogenic and chondrogenic lineage specificity, it is of importance to understand the control of adipogenesis for tissue engineering applications.

Mechanical stimulation can modulate the effects of cytokine stimulation of mesenchymal stem cells, and may play an important role in influencing both lineage selection and progress toward terminally differentiated phenotypes (Rui et al., 2011a; Shi et al., 2011). Recent studies have demonstrated that lineage selection in differentiating MSCs can be strongly influenced by oscillatory stretch of a silicone rubber culture surface at 2% amplitude and a frequency of 0.17 Hz (Sen et al., 2008). Interestingly, mechanical stimulation promotes osteocyte lineage selection at the expense of adipogenic differentiation under these conditions, suggesting relationships between diseases such as osteoporosis and obesity and their modulation by physical exercise (Sen et al., 2008). To date, mechanotransduction experiments have emphasized a relatively limited range of frequencies (0.1–1 Hz) of oscillatory stimulation of cells in culture (David et al., 2007; Tanabe et al., 2008; Tanabe and Nakayama, 2004; Turner et al., 2008). However, mechanical activity in vivo covers a much wider range of frequencies (from diurnal variations of loading at roughly 0.0001 Hz to kilohertz frequency components associated with step changes in loading). Furthermore, gene-level responses can be rapid and sustained after only a single cycle of mechanical stimulation (Turner et al., 2008), indicating that a very wide range of mechanical inputs might meaningfully be

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applied for directed differentiation of MSCs. It is therefore important to more fully characterize the frequency-dependent effects of oscillatory mechanical stimulation on MSC differentiation in order to elucidate mechanotransduction mechanisms and pathways, and to identify the range of mechanical conditions which might be used to direct MSC differentiation in clinical applications.

Recent work has also highlighted the benefits of culture of MSCs on high-extension surfaces for reasons other than application of mechanical stimulation (Majd et al., 2009). Culture on a continuously expanding surface allows MSCs to be maintained at high density as they grow, but with significantly reduced exposure to degradative enzymes due to decreased regularity of passaging (Majd et al., 2011, 2009). Under these conditions MSCs proliferate more quickly than in standard cultures while maintaining a pluripotent MSC character and avoiding the emergence of undesired fibrotic phenotypes expressing large amounts of alpha-smooth muscle actin (Majd et al., 2011). It is therefore possible that continuous expansion culture could be combined with oscillatory mechanical stimulation to provide culture conditions under which pluripotent MSCs can proliferate rapidly and then be directed along desired differentiation pathways. This could be achieved largely through relatively simple and inexpensive manipulation of culture surface extension and with decreased need for cytokine stimulation.

We hypothesized that relatively low frequency mechanical stimulation, which can readily be applied using hardware for continuous expansion cultures, can influence lineage selection in differentiating MSCs. Specifically, we investigated the inhibition of adipogenesis by oscillatory culture surface stretch, which was a prominent feature in previous studies using higher frequency mechanical stimulation. The C3H10T1/2 cell line was used, which represents pluripotent MSCs, which can be differentiated into multiple lineages including adipocytes (Yamaguchi, 1995). Mechanical stimulation was applied in the presence of strongly adipogenic culture medium, and the potency of low frequency mechanical stimulation in competition with chemical cytokines was assessed.

2. Materials and methods

2.1. Cell culture and adipogenic differentiation medium

The cell line C3H10T1/2 (ATCC CCL-226; American Type Culture Collection, Location) was maintained on 10 cm diameter petri dishes in growth medium consisting of Dulbecco's modified Eagle medium (DMEM; Invitrogen, Burlington, ON) supplemented with 10% fetal bovine serum (FBS; Invitrogen) and 1% Penicillin–Streptomycin (Invitrogen) at 37 °C in a humidified atmosphere of 5% CO_2 in air until at least passage 5. Cells from passages 5–15 were seeded on modified (as below) high extension silicone rubber (HESR) culture dishes and maintained for an attachment period of two days. Seeding was performed using 10^5 cells on HESR dishes expanded to 20 cm^2. On the first day following the attachment period (experimental day 1), medium was switched to an adipogenic differentiation medium (DMEM, 5 μg/ml insulin, 0.1 μM dexamethasone and 50 μM indomethacin, 1% pen/strep). Cells were then maintained in adipogenic medium for 3–5 days. A time zero control was established using 10^5 cells seeded on two wells of a 6-well culture dish (total surface area 19.6 cm^2) for the same attachment period; time zero control cells were never exposed to adipogenic medium.

2.2. Preparation of silicone rubber culture surfaces

High extension silicone rubber culture dishes (Cytomec GmbH, Spiez, Switzerland) were modified to promote cell adhesion. First, with HESR dishes in their unstretched state (8 cm^2 culture surface area), culture surfaces were coated with 30% sulfuric acid for 15 min, then washed thoroughly with deionized water. Then surfaces were silanized with 1% (3-aminopropyl) triethoxysilane for 2 h at 70 °C and washed thoroughly again. Surfaces were then functionalized with 5% (wt/vol) glutaraldehye for another 15 min and washed. Next, surfaces were sterilized by rinsing with 70% ethanol, washed with phosphate buffered saline (PBS), mounted in a mechanical device (below), expanded to 20 cm^2 culture surface area and coated with protein by incubation with 50 μg/mL collagen type I in PBS overnight. On the next day, HESR surfaces were washed again with PBS prior to cell seeding (Majd et al., 2009).

2.3. Mechanical stimulation

Mechanical stimulation was applied using an iris-like device (Cytomec) within which HESR culture dishes were mounted (Majd et al., 2009) (Fig. 1A–C). Approximately uniform expansion and contraction of the culture surface was applied by opening or

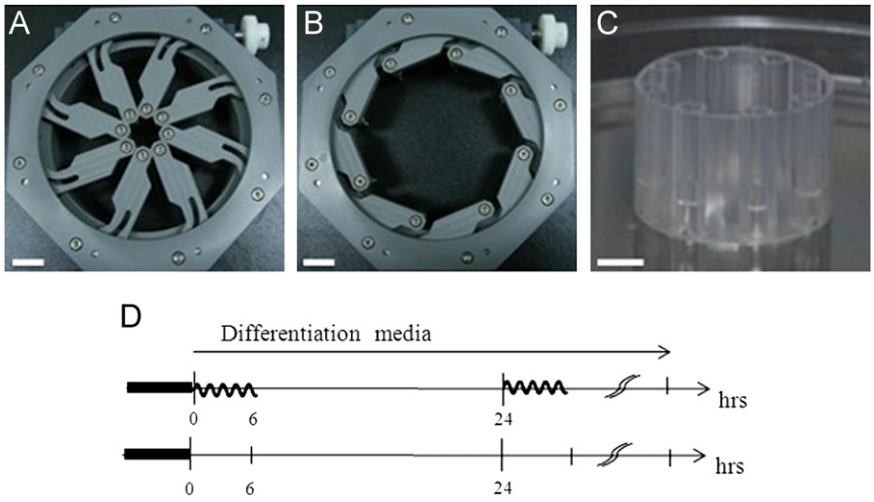

Fig. 1. Experiments were conducted using an iris-like mechanical device, which can stretch a high extension silicone rubber culture surface from 8 to 113 cm^2. (A) Iris-like device in the fully closed position at 8 cm^2. Scale bar represents 2 cm. (B) Iris-like device in the fully open position at 113 cm^2. Scale bar represents 2 cm. (C) Close-up of the HESR petri dish prior to mounting in the iris-like device. Scale bar represents 1 cm. (D) Mechanical stimulation was applied to C3H10T1/2 cells in adipogenic medium on HESR dishes on experimental days 1–5. Unstimulated controls were maintained under identical conditions.