Combined Effects of BMP-2 and a Dynamically Stretched Culture Surface on Osteogenic Differentiation of C2C12 Cells

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INTRODUCTION
Murine myoblastic C2C12 cells have the ability to differentiate to either muscle, fat or bone cells depending on the environmental stimuli [1]. They therefore represent a valuable model system for the study of cell responses to mechanical stimuli and differentiation pathways pertinent to orthopaedic tissue engineering. In this study, our objectives were to investigate the osteogenic potential of combining exposure of C2C12 cells to bone morphogenetic protein (BMP-2) and to dynamic stretch of the culture surface. BMP-2 and its isoforms are known to induce differentiation of mesenchymal cells into osteoblasts, the bone forming cells [2]. Dynamic stretch was applied using a novel apparatus which is suitable for long-term cultures and cell population expansions [3]; therefore results may be directly translated into practical culture protocols for tissue engineering.

METHODS
C2C12 cells were acquired from American Type Culture Collection (ATCC) and cultured in DMEM (Cellgro) supplemented with 10% fetal bovine serum (FBS; Invitrogen) and 1% penicillin-streptomycin within a standard cell culture incubator. Prior to experiments, C2C12 cell populations were expanded on standard cell culture polystyrene to approximately 85% confluence on T-flasks.

During experiments, dynamic stretch was applied using a mechanical device which has been developed for performing cell population expansions on high extension culture surfaces (the Cellerator, Cytomec GmbH, Switzerland). The device has eight (8) arms, which open similar to the iris of a camera (Figure 1a: fully closed surface area 8 cm², 1b: fully opened surface area 113 cm²), and act to stretch a high extension silicone rubber culture surface which is attached to these arms. The device is motorized and interfaced to a PC for precise control of stretch protocols. The culture surface itself (Elasidis, Cytomec) is composed of polydimethylsiloxane (PDMS) moulded in the shape of a Petri dish. To promote cell adhesion and proliferation, collagen was covalently bound to the culture surface. Briefly, culture surfaces were activated by soaking in 30% sulfuric acid for 15 minutes at room temperature and then exposed to 1% 3-aminopropyltriethoxysilane (APTES) for 120 minutes. The device was then functionalized through exposure to 50µg/mL collagen type I overnight.

RESULTS
Bone-specific gene expression by C2C12 cells after BMP-2 exposure was significantly affected by dynamic stretch of the culture surface (Figure 2). Alpl, Colla1 and Ox2 were all significantly downregulated by stretch, while no significant change was observed in Runx2 expression levels (Figure 2).

DISCUSSION
These data suggest that although stretching does not affect the BMP-2 induced early chondro/osteogenic differentiation (as evidenced by Runx2), it strongly inhibits the differentiation of early common progenitors to osteoblasts (Alpl, Colla1 and Ox2). It is therefore important to clarify whether chondrogenic markers such as Sox9, Colla1, or aggrecan were upregulated by mechanical stretching. If yes, then this would suggest that stretching inhibits osteogenic differentiation and forces the common progenitors towards chondrogenic commitments. Future studies will be performed to elucidate the effects of dynamic stretch alone on these and other genes (also in the absence of BMP-2). Furthermore, it is possible that different stretch protocols may be more effective at activating or suppressing differentiation of C2C12 cells.

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REFERENCES