428d Intermittent Fluid Flow Alters Mechanotransductive Signaling and Osteoblastic Differentiation of Bone Marrow-Derived Progenitor Cells

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Bone marrow stromal cells (BMSCs) are a promising ingredient for the development of engineered bone tissue due to their propensity to develop the osteoblastic phenotype in vitro. Further, these cells are mechanosensitive and shearing flow has been shown to stimulate synthesis of osteocalcin and osteopontin, and deposition of mineral in vitro. Initiation of fluid flow activates a series of biochemical markers of mechanotransduction and alters cell morphology, gene expression, and phenotypic behavior. However, an extended duration of continuous flow may undermine osteoblastic differentiation by diminishing intercellular contacts and detaching cells. We postulate that an intermittent flow strategy may more efficiently initiate mechanotransductive signaling and provide quiescent periods for the reestablishment of intercellular and cell/substratum contacts.

To test this hypothesis intermittent (5 min flow/5 min no flow) and continuous fluid flow strategies were compared for their ability to activate biochemical markers of mechanotransduction, maintain cell attachment, and stimulate osteoblastic differentiation. Using parallel-plate flow chambers, BMSCs were exposed to a shear stress of 0.25 Pa for 24 hrs on day 6 (after the addition of osteoinductive factors), and then analyzed for cell number. Concurrently, the recirculating medium was analyzed for prostaglandin E2 and vascular endothelial growth factor. Separately, a set of cell layers were exposed to shearing flow for 24 hrs, then cultured under static conditions for an additional 13 days, and finally analyzed for accumulation of osteopontin. Lastly, a set of cell layers were exposed to fluid flow for 1 hr and analyzed for phosphorylation of the MAP kinase ERK.

The results of this study demonstrate that differing patterns of mechanical stimulus alter mechanotransductive signaling pathways and modulate gene expression and osteoblastic differentiation.