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Relative impact of form-induced stress vs. uniaxial alignment on multipotent stem cell myogenesis

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ABSTRACT

Tissue engineering strategies based on multipotent stem cells (MSCs) hold significant promise for the repair or replacement of damaged smooth muscle tissue. To design scaffolds which specifically induce MSC smooth muscle lineage progression requires a deeper understanding of the relative influence of various microenvironmental signals on myogenesis. For instance, MSC myogenic differentiation has been shown to be promoted by increases in active RhoA and FAK, both of which can be induced via increased cell–substrate stress. Separate studies have demonstrated MSC myogenesis to be enhanced by uniaxial cell alignment. The goal of the present study was to compare the impact of increased peak cell–substrate stresses vs. increased uniaxial cell alignment on MSC myogenic differentiation. To this end, MSC fate decisions were compared within two distinct multicellular "forms". A "stripe" multicellular pattern was designed to induce uniaxial cell alignment. In contrast, a second multicellular pattern was designed with "loops" or curves, which altered cell directionality while simultaneously generating regional peak stresses significantly above that intrinsic to the "stripe" form. As anticipated, the higher peak stress levels of the "loop" pattern were associated with increased fractions of active RhoA and active FAK. In contrast, two markers of early smooth muscle lineage progression, myocardin and SM-α-actin, were significantly elevated in the "stripe" pattern relative to the "loop" pattern. These results indicate that scaffolds which promote uniaxial MSC alignment may be more inductive of myogenic differentiation than those associated with increased peak, cell–substrate stress but in which cell directionality varies.

1. Introduction

Regenerative medicine is a potential approach to repairing or replacing diseased or damaged smooth muscle tissue when current treatment methods fail. In particular, tissue engineering strategies based on multipotent stem cells (MSCs) hold significant promise owing to the greater regenerative capacity of MSCs relative to adult, differentiated muscle cells. However, the design of scaffolds with properties which "optimally" promote MSC lineage progression toward smooth muscle fates requires a deeper understanding of the relative influence of various microenvironmental signals on myogenesis than currently exists.

Recent studies have shown that multicellular organization or form is a critical determinant of MSC fate decisions [1]. Different multicellular forms appear to influence cell behavior in part via the distinct cell–substrate stress patterns supported by particular forms [1,2]. Indeed, increased cell–substrate stress has been associated with higher levels of active RhoA [2] and activated focal adhesion kinase (FAK) [3], both of which have been linked to increases in muscle-specific gene expression [4–6]. Separate studies have demonstrated that multicellular forms that promote uniaxial cell alignment induce MSC expression of muscle-specific genes [7]. The present study was therefore designed to compare the impact of increased form-induced stress with that of increased uniaxial cell alignment on MSC smooth muscle lineage progression.

To this end, the fate decisions of mouse NIH/3T3 MSCs were compared within two distinct multicellular forms. Although NIH/3T3 cells are commonly referred to as "fibroblasts", they were derived from the murine embryonic mesoderm and have the capacity to differentiate into a range of cell types, including adipocytes [8,9], muscle cells [10,11] and osteoblasts [12]. These cells were seeded onto either "stripe" or "loop" patterns formed on substrates of "myogenic" stiffness. The "stripe" pattern was designed to induce uniaxial cell alignment. In contrast, the "loop" pattern was designed with curves intended to alter cell directionality while simultaneously generating peak, cell–substrate stresses significantly above

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