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Mesenchymal stem cell (MSC) differentiation by Mechanical stress: Applications in the field of Musculoskeletal Tissue Engineering

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ABSTRACT

Mechanical forces have been implicated as a major factor responsible for *in vitro* mesenchymal cell lineage commitment[8] and are usually exploited in bone and other musculoskeletal tissue engineering through application of external loading, as has been attempted with other cells and tissues or by appropriate matrix design. The profile of protein expression that is induced in mechanically stimulated cells is specific to the type of mechanical stimulus delivered and a range of strain stimuli have been demonstrated to promote tissue specific differentiation. Thus mechanical forces generated intrinsically within the cell in response to its extracellular environment and extrinsic mechanical signals imposed upon the cell by the extracellular environment, play a central role in determining mesenchymal stem cell fate. However exact role of mechanical forces in mesenchymal stem cell differentiation is still far from clear and is the focus of the developing field of mechanobiology. Therefore an understanding of the mechanisms behind mechano induced MSC differentiation is essential.

KEY WORDS Mechanical stimulus, Mesenchymal lineages, Musculoskeletal tissue engineering

INTRODUCTION

The musculoskeletal system is made up of bones, muscles, cartilage, tendons, ligaments, joints and other connective tissues. Besides the primary functions like supporting the body, allowing movement and protecting vital organs, these tissues are also capable of withstanding tension due to the presence of closely packed parallel collagen fiber bundles. However when subjected to sudden or abnormally high strains, acute injuries may occur which may take more time to heal. This slow healing nature can be attributed to their avascularizaton i.e. lack of blood vessels.[1] As consequences, often the patient has to rely on surgical interventions and grafts to replace the lost tissues. For eg., autologous chondrocyte implantation (ACI), osteochondral autografts and allografts and in adverse situations, total joint replacements are available for treating cartilage defects. The limitations with these surgical techniques are donor site morbidity, pain, the availability, quality and quantity of graft materials.[2] Besides majority of studies using graft materials do not include cells as part of a treatment and depend only on the intrinsic capacity of the implanted material's bone-promoting properties to recruit surrounding cells which can delay the healing process. This has led to the exploration and development of novel methods of intervention for effective healing.

Tissue engineering – An emerging clinical field

The field of tissue engineering has gained more attention for tissue regeneration and replacement in several disease conditions. The advances in this field have resulted from interdisciplinary efforts of scientists in fields such as developmental biology, bioengineering, biomaterials science, stem cell biology and clinical medicine. The development and clinical trials of engineered tissues are going on for cardiac diseases and also for other tissues such as cornea and musculoskeletal systems. Tissue engineering utilizes the concept that functional or structural restoration of damaged or diseased tissues and organs is made through the implantation of combinations of cells, scaffolds and soluble mediators.[3] In this context the goal of a tissue engineer is to identify the best source of precursor cells that can replace or regenerate the damaged tissues and to develop an ideal scaffold that can replicate the biological environment at the molecular and macromolecular level.

Mesenchymal stem cells as a direct cell source for tissue engineering

During in-vivo healing process, the granulation tissue filling the site of injury is infiltrated by mesenchymal stem cells from the periosteum, the surrounding soft tissues such as muscle, the bone marrow and the neighboring cortical bone that eventually differentiate into osteoblasts, fibroblasts or chondrocytes and secrete matrix of differing biochemical compositions.[4] This has generated an interest on *in vitro* studies that have been directed to test the potentials of mesenchymal stem cells as a direct source of cells for *in vivo* tissue regeneration. Such studies on implantation of biomaterial substrate infused with mesenchymal stem cells at the site of injury have found to give beneficial healing effects.

Properties of Mesenchymal stem cells

The therapeutic potential of MSCs stems from the fact that they can be induced to differentiate *in vitro* into various types of cells. MSCs are readily expandable in culture and retain their multipotential characteristics with expansion. The transplantation of *ex vivo*-expanded allogeneic MSCs has shown little immunogenic responses *in vivo*. Besides, these cells can be isolated from a wide variety of tissue sources, not necessary that it should be isolated from specific sites. Some of the diseases where autologous bone marrow-derived MSCs have been used include fracture nonunion, osteogenesis imperfecta, bone metabolic diseases and demonstrated bone formation and limb function recovery in patients.[5, 6] Similarly injection of *in vitro* expanded autologous MSCs as a potential cell therapy reported an increase in the cartilage and meniscus volume on MRI, as well as increased range of motion and decreased modified VAS (visual analogue scale) pain scores.[7] Further, direct delivery of MSCs that can be induced to differentiate into matured cells is also suggested to reduce the need to depend solely on intrinsic cells migrating to a wound site.

The mechanical environment in the load bearing tissues

Cells in the musculoskeletal tissues are constantly subjected to a wide range of mechanical loads such as gravity, tension, stiffness, compression, pressure and shear stress. Therefore the architecture of these load bearing tissues is more optimized for its mechanical environment rather than to biochemical environment. The cell adapt to changes in their mechanical environment to maintain the homeostasis.[8] This mechanical homeostasis with cells responding to and interpreting growth factors and other biochemical signals within the context of mechanical forces provide an environment that defines its structure and function.

Mechanical forces and *in vitro* mesenchymal cell lineage commitment

Mesenchymal stem cells are responsible for the formation and maintenance of load-bearing tissues of the musculoskeletal system.[8] The mechanical forces that are acting upon the matured musculoskeletal cells may also act on MSCs which might play critical roles in the development and regeneration of mesenchymal tissues and also in diseases in which there are deficiencies in the regulation of tissue formation.

Mechanical forces have also been implicated as a major factor responsible for *in vitro* mesenchymal cell lineage commitment (**Table 1**) and are usually exploited in bone and other musculoskeletal tissue engineering through application of external loading, as has been attempted with other cells and tissues or by appropriate matrix design. Cyclic strain is a potent regulator of *in vitro* skeletal tissue differentiation from MSCs. The profile of protein expression that is induced in mechanically stimulated cells is specific to the type of mechanical stimulus delivered, and a range of strain stimuli have been demonstrated to promote tissue specific differentiation. Exposure to cyclic compression enhanced chondrogenesis of MSCs by an upregulation of chondrogenic markers type II collagen and aggrecan[9, 10] Applying a range of tensile mechanical stimuli on cells cultured on deformable substrates indicated pattern of differentiation that was dependent on the stimuli conditions. Such studies involve culturing of cells on an elastomeric membrane, which is usually coated with a variety of adhesion molecules, such as collagen 1, fibronectin or laminin. Varying the orientation of strain (uniaxial vs. biaxial), the magnitude, frequency and duration of dynamic tensile strain regimes and the nature of the modified substrate surface resulted in different

lineages. MSC differentiation into osteocytes was enhanced on application of cyclic tensile strain, typically at magnitudes ranging from 0.4% to 5%.[11,12,13] Increasing the levels of tensile strain favored differentiation toward a tendon/ligament-type phenotype or myogenic differentiation.

Attempts to differentiate MSCs into different mesenchymal lineages have also relied largely upon models where MSCs are seeded on two or three dimensional (3D) constructs and subject to various external forces such as tensile or compressive strains. Most of these studies focused on the effect of static or dynamic tension on matrix production and gene expression patterns of candidate tissue markers. In one such dynamic compression studies using alginate and fibrin-polyurethane composites has demonstrated *in vitro* chondrogenesis through alterations in the expression of cartilage-specific genes such as SOX-9, collagens II, X and aggrecan. Intracellular calcium signaling was also activated.[14] Compression using higher dynamic frequencies and higher compression amplitudes was able to induce significant expression of collagen type II and aggrecan when compared to lower amplitude/lower frequency conditions which exhibited a tendency towards the expression of osteogenic markers (osterix, collagen I). Application of ligament-like multidimensional mechanical strains to the undifferentiated cells embedded in a collagen gel over a period of 21 days up-regulated ligament fibroblast markers, including collagen types I, III and tenascin-C. The mechanical stimulation also showed statistically significant cell alignment and density and resulted in the formation of oriented collagen fibers, all features characteristic of ligament cell. This indicates that MSCs can be induced into specific lineages by specific strain applications.

Mesenchymal stem cell (MSC)-seeded collagen constructs are currently being used to repair patellar tendon defect injuries in the rabbit model. These cell-assisted repairs though show improved mechanical properties such as increased tolerance to the maximum force and stiffness at the wound sites, significantly differ from the normal values. Thus these implants are usually are not strong enough to resist the peak in vivo forces. One way to overcome this is to precondition the implants to their *in vivo* environment by mechanical stimulation prior to transplantation.[15] Such preconditioning was found to have a beneficial role.

CONCLUSION

Mechanical forces generated intrinsically within the cell in response to its extracellular environment and extrinsic mechanical signals imposed upon the cell by the extracellular environment, play a central role in determining MSC fate. Applying a range of mechanical stimuli on cells cultured on deformable substrates indicates different pattern of differentiation that was dependent on the stimuli conditions. Mechanical conditioning may be applied within a tissue engineering context to stimulate *in vitro* biosynthesis by cells seeded on two dimensional (2D) or three-dimensional (3D) scaffolds prior to implantation. *In vitro* application of mechanical load has a beneficial effect on the quality and quantity of the generated tissue and may be an important factor in musculoskeletal tissue engineering. However exact role of mechanical forces in mesenchymal stem cell differentiation is still far from clear and is the focus of the developing field of Mechanobiology. Therefore an understanding of the mechanisms behind mechano induced MSC differentiation is essential.

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S. No	Cells Studied	Force	Strain magnitude and frequency	Observed patterns	Cell lineage	Ref
1	MSCs on collagen- or elastin-coated membranes	Cyclic Equiaxial strain	10%, 1 Hz	Reduced levels of SM alpha – actin and SM-22a	No differentiation	Park *et al.,* 2003
		Cyclic uniaxial	10%, 1 Hz	Increased expression of SM a-actin and SM-22a and Collagen type I expression	MSC differentiation into smooth muscle cells (SMC)	
2	Human Mesenchymal cells	Dynamic compressive loading	Frequency – 0.17 Hz, Amplitude – 10 MPa	Increased expression of Collagen type II, Aggrecan and TGF Beta 1	MSC differentiation into Chondrogenic lineage	Tägil and Aspenberg, 1999
3	Rabbit BMMSCs in agarose cultures	Cyclic compressive loading	Frequency – 1 Hz Amplitude – 10% strain	Increased expression of Collagen type II, Aggrecan and TGF Beta 1	MSC differentiation into Chondrogenic lineage	Bahuleyan *et al.,* 2009
4	Mesenchymal cells	Static compression	-	No significant changes	-	Elder *et al.*, 2000, 2001
5	Human osteoblastic precursor cell line (hFOB 1.19) in three-dimensional type I collagen matrices	Cyclic uniaxial strain	Frequency – 1 Hz Amplitude – 1% strain	Expression of histone H4, core binding factor 1, alkaline phosphatase, osteopontin, osteocalcin, and collagen type I (Col I)	MSC differentiation into Osteogenic lineage	Ignatius *et al.,* 2005
6	MSCs	Cyclic tensile strain	2.5% 0.17 Hz	Expression of osteogenic markers Cbfa1, collagen type I, osteocalcin, and BMP2	MSC differentiation into Osteogenic lineage	Kearney et al., 2010

7	**Human bone marrow stromal cells (BMSC) cultivated with (D+) or without (D-) dexamethasone**	**Cyclical mechanical stretching**	**8% 1 Hz**	**Increased ALP and OC levels Upregulated Col I and III, Cbfa1 expression**	**MSC differentiation into Osteogenic lineage**	**Jagodzinski *et al.,* 2004**
8	**Bovine bone marrow cells**	**Translational Rotational strain**	**10%, 2 mm 25%, 90º 0.0167 Hz**	**Up-regulated ligament fibroblast markers, including collagen types I and III and tenascin-C, significant cell alignment and density formation of oriented collagen fibers**	**MSC differentiation into Ligament lineage**	**Altman *et al.,* 2001**

Table 1: Different modes of mechanical strain induces different responses