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Mechanoregulation of cell phenotype for musculoskeletal tissue engineering

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INTRODUCTION: For tissue engineering of musculoskeletal tissues, e.g. cartilage and bone, it is often difficult to harvest enough mature differentiated cells, without substantial morbidity. Hence, the recruitment of progenitor cells to form the desired tissue type is crucial. In addition to biochemical factors and gene therapy, biophysical signals have also proven to alter cell differentiation and proliferation. As such signals may be induced through load bearing in vivo, or in bioreactors in vitro, mechanical stimulation may be applied over long durations and adjusted during tissue regeneration.

It is well-documented that both chondrocytes and osteoblasts respond to various mechanical signals, e.g. fluid flow, strain and hydraulic pressure. Moreover, in vivo experiments have demonstrated that mechanical conditions play a crucial role in bone regeneration, where progenitor cells differentiate into fibroblasts, chondrocytes and osteoblasts. Theoretical models have been developed to mathematically describe the regulatory role of biophysical stimuli in tissue differentiation (e.g. Prendergast 1997), and when incorporated into simplified computational adaptive models have been shown to often corroborate with temporal and spatial tissue distributions during bone healing (Lacroix 2002, Isaksson 2006). However, because bone healing is a complex biological phenomenon and adaptive computational models are sensitive to many unknown cellular and biological processes, which are simplified, the validity of mechanoregulation theories are still debated.

This study investigates the validity of one established mechanoregulation theory in a simple tissue engineering system for the purpose of defining mechanical signals which may aid in the future stimulation of chondrogenic and osteogenic cells from progenitor cells.

METHODS:

Fibrin scaffold FEA: Cylindrical fibrin hydrogel specimens were prepared and allowed to equilibrate in normal saline at RT. Unconfined stress relaxation tests were then conducted. The specimens were sequentially compressed between polished stainless steel and glass surfaces at 2, 4, 6, 8, 10, 15 and 20% strain, held till equilibrium for each step. Reaction force was recorded throughout, and the equilibrium radial expansion of the disc at the glass surface was imaged. Equilibrium modulus, E, and Poisson's ratio, ν, were directly calculated.

A poroviscoelastic axisymmetric finite element model of the fibrin specimens was created consisting of user defined elements with an Upper Convected Maxwell viscoelastic constitutive formulation of the solid matrix (Baaijens 2005). This formulation describes the material behavior of the fibrin with 5 parameters: E, ν, relaxation time, λ, viscoelastic shear modulus, G_v, and permeability, κ. The values of the latter three material parameters were found by fitting the stress relaxation reaction load measurements at each strain level to the finite element model simulations using a multidimensional unconstrained nonlinear minimization algorithm.

In vitro progenitor cell stimulation: Bone marrow mesenchymal stem cells (BMSCs) were isolated from the tibiae and femora of Wistar rats. The adherent cells were expanded up to the third passage in monolayer culture with αMEM and 15% FCS. 10^7 cells/ml were cast into fibrin cylinders.

The fibrin/cell constructs were placed individually into chambers that held the fibrin cylinder between two polished stainless steel platens. The chamber design allowed continuous circulation of culture media (αMEM, 1% ITS, 1% aprotinin, 10 mM βGDP, 60 mg/l ascorbic acid) around the outer cylindrical surface. After 3 days without loading, the chambers were placed in a multi-unit pneumatic actuator and dynamically loaded under displacement control. Control construct were collected at this stage (day 0 controls). Constructs were cyclically-loaded for 2h daily, for 7 and 14 days, in uniaxial unconfined compression (see below for strain magnitudes). The constructs then remained in the chambers with circulating media for 7 more days without load.

Mechanoregulation corroboration: Prior to in vitro experiments, the fitted parameter values as well as boundary conditions for the in vitro experiments were input into the FE model of the fibrin constructs. For a variety of realizable loading conditions, the distribution of stimulated cell phenotype in the fibrin according to the mechanoregulation theory based on octahedral shear strain and interstitial relative fluid velocity (Prendergast 1997) was predicted. The MSC seeded fibrin constructs were then loaded under selected loading conditions similar to that used in the FE model. Finally, the mechanoregulation theory predicted cell phenotype was compared to the gene expression profile (RT-PCR: loaded, n=8/ε; unloaded, n=12) and histology assessment (IHC: CD105, n=2) in the core of the fibrin construct from the in vitro experiments.

RESULTS:

Average Poisson's ratio was 0.15 (range 0.11-0.19). Equilibrium modulus did not differ between 2-10% ε (0.02 MPa), but was lower for 15-20% ε (0.01 MPa). A good fit between measured and simulated force data was found for all strain levels (ave. R^2=0.94, range 0.87-0-97). The optimization procedure was found to yield a unique solution independent of initial values. λ and G_v were found to vary linearly with ε whereas κ was similar for the 2-10% ε range (1.24×10^{-12} m^4/Ns)).

Various loading waveforms were explored with the FEM of the fibrin cylinders in the bioreactor. As the shear strain and fluid velocity were similar for the range of ν and κ found above, constant values were used. A trapezoidal waveform (100 μm/s ramp-up, 5 s hold, 100 μm/s ramp-down, 5 s hold) was finally selected. With this waveform, steady state was reached in 10-15 cycles for the range of strain magnitudes explored. As expected, shear strain was constant throughout the carrier whereas fluid velocity differed and had the highest gradients at the free outer surface. This led to a radial distribution of mechanical stimuli, but at some strain magnitudes all or the majority of the construct was predicted to have a homogenous cell phenotype. The strain magnitudes of 2, 5 and 10% were predicted to stimulate osteogenic, chondrogenic and fibrogenic cells respectively at the central core of the fibrin carrier.

The isolated and expanded (2 passages) MSCs were checked for their multi-potentiality. MSCs when grown in osteogenic media (Sakai 2003) for 21 days in monolayer developed mineralized nodules as confirmed by ^{45}C assay and von Kossa staining. When grown in chondrogenic media (Sakai 2003) embedded in fibrin for 14 days, aggrecan and collagen-II mRNA were up-regulated.

The MSCs when loaded into the fibrin carrier were found to be well distributed throughout the carrier (qualitatively checked on H&E stained images) and did not change in cell viability over the 17 day culturing period (calcein AM stain compared quantitatively at day 3 and day 17).

After 7 days of loading: only mRNA levels of OPN was significantly up-regulated 4x with 2% strain compared to 0% strain. There was no difference in coll-I mRNA levels between different strain magnitudes. Aggrecan mRNA levels tended to increase with increasing strain levels. Coll-II mRNA levels were similarly slightly up-regulated for 2, 5 and 10% strain. SOX9 was only slightly up-regulated for 10% strain.

CD105 was highly expressed in cells before loading (day 0) and after 7 days at 0% strain. At strain levels 2 and 5% after 7 days of loading, CD105 was also partially expressed at a much lower level. CD105 expression for 10% strain was very low but still detectable.

All results after 14 days of loading are pending.

DISCUSSION:

Surprisingly a simple biphasic or poroelastic description of fibrin at a concentration in the range used for tissue engineering constructs was not valid. Especially the peak reaction loads and pressures were quite inaccurate. In contrast, a poroviscoelastic description was more accurate in describing the entire transient and equilibrium response. Curve fitting to calculate parameter values was very robust.

MSCs maintained their phenotypic cell marker for up to 17 days of culture at 0% strain. Although gene expression changes were modest after 7 days of stimulation, cells exposed to 2% strain were more osteogenic and those at 5 and 10% were more chondrogenic. No fibrogenic cell phenotype tendency was observed after 7 days of stimulation.

However, 7 days of stimulation may not be sufficient as some cells continued to express an MSC phenotypic cell marker. This will be resolved with further duration of stimulation.

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