Differences in fetal bovine serum affect the responsiveness of cells to mechanical loads

applications in refilling drug depots in cancer therapy, wound healing, and drug-eluting vascular grafts and stents.

Glycocalyx Integrity Influences Nanoparticle Uptake by Endothelial Cells

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Atherosclerosis is a precursor of cardiovascular disease, a leading cause of global mortality. Mechanisms of endothelial cell (EC) dependent atherosclerosis are not fully understood. The EC surface sugar coat–the glycocalyx (GCX)–may play an important role, since it is shed in atherosclerosis. GCX may be a possible therapeutic target if it can be regenerated. Nanoparticle-based regeneration to treat cardiovascular disease is becoming very popular, and we wish to study how GCX alterations may impair or amplify the effect of regenerative agents delivered to sites of atherosclerosis via nanoparticles. To demonstrate how nanoparticle-based drug delivery is impacted by GCX conditions, we expose rat fat pad EC (RFPEC) with intact GCX to ultra-small PEGylated gold nanoparticles. The RFPEC with intact GCX do not exhibit any nanoparticle uptake. In contrast, RFPEC with protein deficient and collapsed GCX retain some nanoparticles. RFPEC with enzymatically degraded GCX heparan sulfate, the most abundant component of the GCX, retain a more substantial number of nanoparticles. In another case, after enzymatic heparan sulfate degradation, we induce GCX regeneration by adding heparan sulfate to the culture media for its incorporation into the GCX. HS regeneration results in restoring blockage of nanoparticle entry into RFPEC. This work indicates that the GCX integrity and composition does influence nanoparticle uptake by EC. We look forward to further elucidating how glycocalyx mediates the action of nanoparticle drug carriers, especially those that are under development for cardiovascular disease treatment. Funding: Northeastern University and IGERT Nanomedicine Science & Technology Program at Northeastern University (NSF/DGE-096843).

Differences in Fetal Bovine Serum Affect the Responsiveness of Cells to Mechanical Loads

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Nowadays, the end-point of a cell culture in bone tissue engineering (BTE) is the acquisition of a well mineralized extracellular matrix. The biological performance of BTE relies on evaluation of the cell capacity to proliferate and to produce extracellular matrix by quantification of gene expression and by histology or calcium quantification assays. Micro-computed tomography (micro-CT) allows monitoring of BTE mineral constructs in a non-destructive manner. Although fetal bovine serum (FBS) is commonly used as supplement in cell cultures, its high composition variability between different brands and batches leads to differences in the experimental outcomes. Nevertheless, only few studies have focused on a systematic investigation of the differences. While we have recently reported the influence of FBS type on matrix mineralization under static culture conditions, it is still unknown how FBS affects cells in dynamic cultures. Different FBS types were used to differentiate human mesenchymal stem cells down the osteogenic lineage under dynamic spinner-flask bioreactors. Opposite to static culture conditions, differences in FBS affected the responsiveness of cells to differentiate under mechanical loads. Although all FBS types upregulated the expression of bone-specific genes, differences in the osteogenic differentiation stage were observed among the different FBS. Accordingly, micro-CT analysis only showed mineral deposition for cultures in an advanced differentiation stage.

Thus the selection of the FBS type is crucial for the success in the acquisition of BTE constructs. The combination of micro-CT with molecular biology techniques will benefit efforts to optimize scaffolds design and cell culture conditions for scaling-up the BTE constructs.

3D Reconstitution of Brain Stromal Microenvironment

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Neural stem cells (NSCs) reside in a specialized microenvironment termed the ''Neural stem cell niche'', which maintains the capacity of both self-renewal and differentiation of NSCs through various microenvironmental cues involving growth factors, small molecules, extracellular matrix (ECM), cell-cell and cell-ECM interactions, and brain vasculature. To increase our knowledge of the mechanisms governing the behaviors of NSCs, it should be required to comprehensively understand their instructive stromal microenvironment.

To investigate microenvironmental regulation on NSCs' behavior and homeostasis, vascular niche on ECM was formed in 3D. The 3D reconstitution dramatically presents NSC behavior under various microenvironments. For example, brain vasculature enhanced NSCs' self-renewal and at the same time, also regulated their differentiation fate. The microfluidic study enabled various types of interactions, on spatial proximity, chemical/physical interactions and interstitial molecular transport.

Multiscale and Multidisciplinary Analysis of Rat Bone Health Following In Utero Vitamin D Deficiency

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Bone mechanical competence is derived from a number of size, material and structural components, which are directed by the bone biology environment. Previously we have described an integrated approach to interrogate these components within a bone sample to predict strength. In this investigation, we utilise this approach together with computational modelling to analyse a rat model of *in utero* vitamin D deficiency (VDD). Femora from 21 day old male rats were analysed ($n=5$ for control and VDD groups) for osteogenic gene expression by RT-qPCR, microarchitecture and bone mineral density by μCT scanning, fracture toughness by notched bend testing and overall bone strength by three-point bend testing. μCT scans were used to generate FE models to predict bone strength computationally. Femora from VDD background rats were found to have reduced midshaft area when compared to controls ($p=0.03$) despite no detected difference in bone volume. FE modelling predicted lower compressive failure forces for the VDD femora ($p=0.04$). This was confirmed experimentally in the three-point bend data, where VDD femora failed at lower loads compared to controls ($p=0.04$). No differences were found within osteogenic gene expression, BMD, fracture toughness or cortical thickness. These results show how *in utero* VDD causes reduced bone health in male rats at 21 days of age and indicates how mechanical function of bone can be predicted. These data and approach can be used to inform and target bone regenerative therapy and scaffold tissue formation to key components of bone health.

Micro-RNA Plasmid Loaded Nanoparticles Efficiently Modulate Transforming Growth Factor-beta1 Expression in Healing Intrasynovial Flexor Tendons: An *In Vitro* and *In Vivo* Study

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