developed to regenerate or replace the nucleus pulposus. However, these kind of pre-formed devices have to remove the nucleus pulposus and then replace an artificial one to relieve the symptom of intervertebral disc degeneration. Recently, cell-based tissue engineering has provided a rational approach to regenerate active nucleus pulposus cells (NP cells) to restore intervertebral disc architecture and function. However, the source of autologous nucleus pulposus cells are limited and their functional state does not favor regeneration. Besides, nucleus pulposus cells grown in monolayer may result in fibroblast-like transformation. Thus, the 3D hydrogel co-culture system may be an alternative method to provide an adequate environment for nucleus pulposus cells proliferation, extracellular matrix production, cytokines secretion. In this study, we demonstrated that cell proliferation, total DNA and sulfated glycosaminoglycans synthesis of nucleus pulposus cells were significantly increased in the 3D hydrogel co-culture system. Furthermore, the extracellular matrix related gene expression and anabolism-related gene expression in 3D hydrogel co-culture system were significantly higher than other culture condition (such as monolayer culture or cultured in 3-D hydrogel without mesenchymal stem cells regulation).The gene expression of TIMP-1 and MMP-3 decreased in 3D hydrogel with mesenchymal stem cells co-culture system. This study suggests that the thermo-sensitive hyrogel could be an adequate material for nucleus pulposus cells proliferation and extracellular matrix production. Moreover, mesenchymal stem cells could regulate the isolated nucleus pulposus cells back to normal state through paracrine communications in the developed 3-D co-culture system.

O60 (EI0417)

THE POTENTIAL OF OSTEOGENIC CELL SHEETS CO-CULTURED WITH ENDOTHELIAL CELLS FOR BONE TISSUE ENGINEERING

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Objectives: Current strategies in the field of bone Tissue Engineering are limited by the use of scaffolds that present drawbacks such as cell necrosis at their bulk related to deficient vascularization after implantation. Cell sheet (CS) engineering has been proposed as a scaffold-free alternative for the regeneration of several tissues. This work proposes the use of this technology for bone regeneration by combining osteogenic CSs and endothelial cells.
Methods: Osteogenic CSs were fabricated by culturing male rat bone marrow cells (rBMSCs) in thermo-responsive culture dishes in osteogenic medium. Human umbilical vein endothelial cells (HUVECs) were seeded on the rBMSCs to create co-cultured CSs. The osteogenic CSs were recovered by lowering the temperature and then stacked on top of either a co-cultured or a similar osteogenic CS, and transplanted to female nude mice. Implants were recovered after 7 days and characterized by hematoxylin & eosin (H&E) and alizarin red (AR) stainings, immunohistochemistry for osterix, osteopontin, SRY (to identify transplanted male rat cells) and CD31, and calcium quantification.
Results and Discussion: H&E and AR stainings showed mineralized tissue formation in the implants both with and without HUVECs. Osterix and SRY immunostaining demonstrated the presence of host and donor osteogenic cells at the mineralization site showing recruitment of host osteogenic cells. HUVECs contribution to neo-vascularization was confirmed by identifying human CD31 cells in blood vessels. Furthermore, calcium quantification results showed a higher degree of mineralized tissue after the transplantation of the constructs with HUVECs.
Conclusions: This work confirmed the potential of transplanted osteogenic cell sheets for bone regeneration as well as the advantage of promoting cross-talk between osteogenic and endothelial cells for improved new tissue formation. The proposed approach avoids the constraints of scaffold use while successfully addressing the important issue of implant vascularization.

O61 (EI0330)

ENABLING TECHNOLOGIES FOR ORGAN PRINTING: TOWARD ORGAN BIOFABRICATION LINE

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Objectives: Organ printing is an emerging solid scaffold-free biofabrication technology or layer-by-layer additive bioprinting of functional 3D human tissue and organ constructs from self-assembling tissue spheroids. Bioprinter is a key tool for organ printing. It is becoming increasingly obvious that in order to bioprint human and organ constructs it is necessary to develop series of integrated automated robotic tools or an organ biofabrication line.
Methods: The scalable technique for tissue spheroid biofabrication employs micromolded recessed template in non-adhesive agarose hydrogel, wherein the cell suspension automatically loaded into the template self-assembles into tissue spheroids due to gravitational forces. Robotic bioprinter for the precise dispensation of tissue spheroids include three essential elements: X-Y-Z axis robotic precision position system, three automated biomaterial dispensers (two aseptic valve sprayers and one automated tissue spheroids dispenser) and computer-based software enabled operational control. The first two dispensers spray sequentially fibrinogen and thrombin and enable instant biofabrication of thin layers of fibrin hydrogel, whereas another robotic dispenser punches tissue spheroids into sequentially sprayed layer of fibrin hydrogel.
Results: It has been demonstrated that the use of micromolded recession in non-adhesive hydrogel, combined with automated cell seeding, is a reliable method for robotic fabrication of uniform size tissue spheroids at large scale. It has been also shown that the combination of hydrogel sprayers and tissue spheroids puncher enables to implement additive biofabrication of 3D tissue construct. The novel irrigation dripping tripled perfusion bioreactor with removable porous removable minitubes has been designed. Mathematical modeling and computer simulation demonstrated that proposed irrigation dripping circuit system will allow maintain viability of printed tissue constructs until the "build in" intra-organ branched vascular system will mature enough for initiation intravascular perfusion.
Conclusions: Thus, presented data strongly indicate that design and development of a fully integrated organ biofabrication line is a challenging but achievable goal.

O62 (EI0395)

MICROFLUIDIC ENCAPSULATION OF CELLS INTO SELF-ASSEMBLING XANTHAN-PHOSPOLIPID AMPHIPHILE FOR CELL THERAPY

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Objectives: We have investigated the synthesis of an amphiphilic polysaccharide, in which a phospholipid is attached to an anionic polysaccharide chain (xanthan gum), and its ability to self-assemble into capsular structures. Moreover, this work aimed to apply a microfluidic platform which can overcome inconveniences related with the heterogeneity of microcapsules produced by conventional systems. The properties and performance of the microcapsules were studied as well the ability of these self-assembled matrices to support the viability, function, and proliferation of encapsulated cells.
Methods: Xanthan gum was conjugated with 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) using carbodiimide chemistry to activate carboxylic groups of xanthan and coupling to amine groups of DOPE. The polysaccharide-lipid amphiphile was characterized by physico-chemical methods, such as ^{1}H Nuclear Magnetic Resonance, Fourier Transform Infra Red spectroscopy, X-Ray Diffraction, Circular Dichroism and Scanning Electron Microscopy. A microfluidic device was used to fabricate microcapsules with controlled size and shape. ATDC5 cells (a murine chondrocytic cell line) were encapsulated within the microcapsules and their metabolic activity and viability were investigated.
Results: The self-assembly of the amphiphilic polysaccharide in physiological ionic strength and pH resulted in the formation of stable hollow capsular structures. Using microfluidics, stable and homogenous microcapsules of xanthan-DOPE with average size around 300 µm were fabricated. ATDC5 cells were encapsulated within the capsules and remained viable and evidencing an increased cellular metabolic activity over 21 days of *in vitro* culture.
Conclusions: By combining self-assembly of xanthan-DOPE and microfluidic microencaspsulation we were able to fabricate microcapsules that provided an adequate environment for cells to survive and proliferate.