Self Contained Bioreactor For Bone Regeneration

Pratima Labroo, MS¹, Ching-wen Li, PhD², Himanshu Sant, PhD¹, Bruce Gale, PhD¹, Jill E. Shea, PhD¹, Jayant Agarwal, MD¹

¹University of Utah, Salt Lake City, UT, ²National Ching Hsing University, Taipei City, Taiwan

PURPOSE: The current gold standard for the repair of segmental bone defects is autologous bone grafts or flaps, however these are limited by donor site morbidity, limited graft tissue availability, and potential for complications at the harvest site. ASCs (adipose derived stem cells) have potential to aid bone regeneration due to their ability to differentiate into osteoblasts. BMP-2 (bone morphogenetic protein) has been shown to induce osteogenic differentiation of ASCs and enhance bone growth. We present a bioreactor for bone regeneration consisting of three main components: (i) a biodegradable PLLA scaffold seeded with ASCs, (ii) a drug reservoir made of PLLA and (iii) a controlled drug diffusion system for sustained release of BMP-2 to repair segmental bone defects. The drug reservoir is attached to the porous polymer scaffold seeded with ASCs and is capable of delivering growth factors for prolonged periods.

METHODS: The components for the drug delivering setup were made from poly-lactic-acid (Purac Biomaterials) using solvent casting method. The device consists of two concentric tubes and a reservoir in between the tubes that stores BMP-2. Six 180 μm diffusion holes were drilled into the inner tube by pulsing a laser cutter. In vitro release tests were performed over a 30-day period. The concentration of BMP-2 released from devices was determined with an enzyme linked immunosorbent assay (R&D systems). The PLLA scaffolds were manufactured by a salt leaching method. ASC seeded scaffolds were inserted in the inner tube. We first evaluated the dosage(s) of BMP-2 that allows for the differentiation of ASCs into osteogenic cells using RT-PCR. ASC seeded devices were then loaded with BMP-2 and in vitro osteogenic differentiation of the ASCs was evaluated by direct delivery of BMP-2 from the drug reservoir to the seeded scaffold.

RESULTS: The RT-PCR data showed increased expression of osteogenic markers such as ALK and Runx-2 in ASCs treated with 100ng/ml BMP-2 compared to no BMP-2 (p<0.05). Alizarin Red staining results showed that we were able to achieve osteogenic differentiation of ASCs with 100ng/ml BMP-2/day and the percentage of mineralization was greater in the PLLA scaffold compared to ‘no scaffold’ (p<0.05). The results for drug release kinetics showed that the drug delivery setup test was effective in achieving controlled local release of BMP-2 for 30 days. The results of the sealed (no hole) devices (n=2) validated sealing techniques for the drug-release reservoirs. The diffusion tests (n=6) indicated that 6x180 μm holes allowed for a sustainable and controlled BMP-2 release in the range of 50–100ng/ml/day for 30 days.

CONCLUSION: The bone bioreactor was able to release BMP-2 for 30 days in a controlled manner. The ASC seeded PLLA scaffold exposed to 50-100ng/m/day of BMP-2 produced significantly higher mineralization as compared to no scaffold. Further in vivo testing will be done to demonstrate that directly delivering BMP-2 from our reservoir to the ASC seeded scaffold enhances bone regeneration.

The Mechanical Environment Modulates Lymphatic Tube Formation In Vitro

Gene K. Lee, MD, MPH, David P. Perrault, BS, Yi-Chen Wu, MS, Josephine Y. Fang, PhD, Sun Young Park, MS, Bo Han, PhD, Young-Kwon Hong, PhD, Alex K. Wong, MD, FACS

University of Southern California, Los Angeles, CA

PURPOSE: Clinical lymphedema can be tempered by exogenous administration of pro-lymphangiogenic molecules such as VEGF-C or retinoic acids at the time of lymphatic injury. In this study, we sought to explore the effect of three-dimensional mechanical environment on VEGF induced lymphangiogenesis. Improved understanding of growth factor induced lymphangiogenesis will aid clinical translation of novel therapies against lymphedema.

METHODS: Human dermal lymphatic endothelial cells (LEC) were isolated from human foreskin. The cells were expanded in traditional 2D culture in EBM in LEC media with 15% fetal bovine serum supplement and 1% Ampicillin/Gentamycin. Transglutaminase-crosslinked collagen hydrogels (Col-Tgel) were prepared with a 12% gelatin stock gel washed with PBS, and further diluted for various
gel concentrations. Mechanical tests were carried out with an indentation test, and the deformation distance of the gel construct was used as an indicator of relative gel stiffness. A total of 5 different concentrations were used with relative stiffness including: <1, 5, 9, 18 and 25 kPa. The 3D gels were cultured as a single droplet on a 48-well suspension cell culture plate with exchange of fresh media and 100ng/mL of VEGF-A/C every 2–3 days. The gels were directly observed daily under the light microscope and recorded.

CONCLUSIONS: The 3D hydrogel served as the interstitial substrate to support LEC tube formation under different mechanical properties. Our experimental results showed a high density of tube formation in LECs cultured in ~18 kPa Col-Tgels beginning as early as 48 hours after plating. Shorter and early stages of tubule structure formation was also visible in the ~9 kPa gel. Col-Tgels with stiffness lesser than 9 kPa or greater than 18 kPa were not conducive to lymphatic tube formation. Lymphatic tube formation was similarly seen in the 18 kPa gels cultured with LEC media alone without soluble cytokines, but only after a 72–96 hour lag compared to the gels with cytokine treatment (images not shown). Our results show that the 3D environment plays an integral role in both cell-cell and cell-extracellular matrix (ECM) interactions. LECs respond not only to soluble factors associated with the ECM, but also the biomechanical cues for tubulogenesis.

P31

Surgical Factors Associated with Prolonged Hospitalization After Reconstructive Spinal Surgery

Hannah Carl, BS, Devin Coon, MD MSE, Nicholas Calotta, BA, Rachel Pedriera, BA, Justin Sacks, MD MBA

Johns Hopkins University School of Medicine, Baltimore, MD

PURPOSE: Posterior trunk reconstruction following oncological resection is increasingly possible as a result of advances in spinal instrumentation, reconstructive approaches, and perioperative critical care. Extensive cases often require a muscle flap or complex closure to obliterate dead space. Postsurgical wound complications and subsequent reoperations can lead to neural injury, higher hospital costs, and longer hospitalizations. We aim to identify risk factors that are associated with increased length of stay (LOS) for patients receiving flaps to close a spinal defect.

METHODS: A single institution, retrospective cohort study was performed on all patients from 2002–2014 who received a muscle flap to close a spinal defect. Medical and perioperative variables that were significantly associated with LOS (p<0.05) in univariate analysis were included in a stepwise regression model.

RESULTS: A total of 288 cases were included in this study. In terms of tumor etiology, 34.3% of the cohort had primary spinal tumors, 22.6% had metastatic spinal tumors, 28.5% underwent hardware revision or spinal fusion, and the rest had benign lesions or other rare tumors. Presence of instrumentation, pre-operative chemotherapy, wound dehiscence, CSF leak, partial/total flap loss, and medical morbidity occurrence were all independently associated with increased LOS in a combined multivariate model (p<0.02 for each of the six variables). Importantly, Kaplan-Meier analysis demonstrated that post-operative wound dehiscence increased length of stay by twelve days (median LOS 11 [95% CI 9–14] versus 23 [95% CI 14–28]).

CONCLUSIONS: Spine tumor resections often create large cavitary defects that necessitate the use of muscle flaps for closure. Patients who have received adjuvant chemotherapy, require instrumentation, or those who develop specific wound-related or medical complications are at an increased risk for prolonged hospitalization following spinal reconstruction. Thus, with knowledge of the effects of these complications, simple interventions can be employed to enhance the safety of the post-operative period and reduce the financial burden associated with unnecessarily long post-operative admissions.

P33

Validation of Vectra 3D Imaging for Quantitative Volumetric Measurement of Upper Extremity Lymphedema

Mark J. Landau, PhD, Jennifer S. Kim, BA, Ketan M. Patel, MD

Keck School of Medicine of USC, Los Angeles, CA

BACKGROUND: Secondary lymphedema of the arm is a complication of lymph node removal in cancer surgery. Limb volume measurements are considered the gold standard in evaluating outcomes in upper limb lymphedema. However, current techniques for volume measurement are limited by a lack of sensitivity to localized changes. The aim of this study was to