maturation, and depression, suggesting that pubertal risk factors may relate more closely with emotion-regulation and self-referential processing deficits.

3221

Optimization of chondrogenesis on 3-dimensionally printed porous tissue bioscaffolds for auricular tissue engineering

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OBJECTIVES/SPECIFIC AIMS: This study's aims are to optimize the isolation and growth of chondrocytes from pig auricular cartilage; to identify the ideal seeding conditions onto 3D printed auricular bioscaffolds to maximize chondrocyte growth; and to investigate what quantity and types of host tissue can grow on the bioscaffold. Primary outcomes will include comparisons between different seeding conditions in various objective measures of bioscaffold growth and survival as listed in the methods section. Secondary outcomes will include continued optimization of bioscaffolds to minimize extrusion rates and maximize morphologic and histologic similarity to human auricular cartilage. METHODS/STUDY POPULATION: For chondrocyte-seeded scaffolds, cartilage will be collected from freshly harvested porcine auricular tissue and digested in type II collagenase. Chondrocytes derived from the harvest will be seeded into auricular PCL scaffolds using a type I collagen/hyaluronic acid composite gel, which has been previously shown to support chondrogenesis. For scaffolds containing cartilage, punch biopsies will be collected and embedded in specific areas of the scaffold previously shown to experience excessive stress/strain compared to the rest of the construct. From there, five of each chondrocyte-seeded bioscaffolds, chondrocyte-unseeded bioscaffolds, and cartilage-containing bioscaffolds will be implanted into athymic rats. Total follow up will be for six months, with outcomes as measured by clinical assessments, morphologic measurements, radiological imaging, histological analysis, biomechanical evaluation, and photodocumentation. Once these measures are obtained, we will work closely with Dr. Myra Kim, an adjunct professor with the Biostatistics Department, to appropriately analyze differences between the models. RESULTS/ANTICIPATED RESULTS: We believe that while all scaffolds (chondrocyte-seeded, chondrocyte-unseeded, and cartilage-containing) will be structurally sound, the chondrocyte-seeded scaffolds and cartilage-containing scaffolds will exhibit improved soft tissue coverage and have lower exposure and fracture rates. Additionally, between the two, we posit that there will not be appreciable differences histologically, radiologically, or morphologically. DISCUSSION/SIGNIFICANCE OF IMPACT: Auricular reconstruction is a geometrically complex and technically challenging problem. Reconstruction hinges on the physical characteristics of the deformity, patient preferences, and reconstructive materials available. The current gold standard for auricular reconstruction uses autologous rib cartilage as foundational support for overlying soft tissue and these techniques involve freehand carving of the cartilage, requiring high levels of technical skill. Harvesting the materials for this procedure is invasive, and the outcomes of the surgery are largely variable and sometimes undesirable. As alternatives, implantable scaffolds including those made from high density porous polyethylene (commercially referred to as MedPor) have been investigated. However, many of these have proven inadequate due to factors including infection, extrusion, and morphologic and biomechanical dissimilarity from native tissue. 3D printing represents an exciting new avenue through which to address many of these difficulties. Our group has previously demonstrated the successful design, production, and implantation of 3D-printed models: in auricular reconstruction, we have demonstrated the successful creation and implementation of a 3D printed ear scaffold into an athymic rodent model. We now turn our attention to optimization of seeding of our ear scaffold with chondrocytes derived from porcine auricular cartilage or with cartilage punch biopsies, all while maintaining emphasis on regulatory feasibility. With success in this arena, we will be able to provide a much less invasive and technically challenging alternative to the current gold standard, create patient-specific bioscaffolds which are more form fitting and individualized, and provide children with ear malformations better alternatives and treatments for their conditions.

3270

Patient Attitudes Survey to Guide Development of a Cell Replacement Device for the Management of Type 1 Diabetes

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OBJECTIVES/SPECIFIC AIMS: This study aims to estimate patient attitudes and receptiveness towards stem cell-based cell replacement devices to management Type 1 Diabetes. The primary outcomes of this study are mean response values to questions interrogating patient attitudes, knowledge, and receptiveness. METHODS/STUDY POPULATION: A RedCap survey was generated for this study. 100 participants will be drawn from Mayo Clinic Rochester, MN patients living with Type 1 Diabetes. RESULTS/ANTICIPATED RESULTS: Response values will be used to estimate broader patient attitudes. DISCUSSION/SIGNIFICANCE OF IMPACT: The response values of this survey will help inform future directions of cell replacement device development. Additionally, understanding patient attitudes may be useful in crafting more effective education strategies.

3094

Production of Engineered Cardiac Tissue for Disease Modeling

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OBJECTIVES/SPECIFIC AIMS: Cardiovascular diseases (CVD) is the leading cause of death worldwide in both men and women due to lack of cardiac regeneration after disease or damaged is caused. There are many challenges to studying CVD since native cardiomyocytes cannot be cultured in vitro. With the advancements in biomaterial and pluripotent stem cells research, scientists are now able to produce engineered cardiac tissue models in vitro that mimic the native myocardium. This study shows our methods for producing engineered cardiac tissue with potential applications in cardiac regeneration, disease modeling, and scalable production. METHODS/STUDY POPULATION: In this study, human induced pluripotent stem cells (hiPSCs) were combined with two different photocrosslinkable hybrid biomaterials, poly (ethylene)- glycol fibrinogen (PF) and gelatin methacrylate (GelMa), in various tissue geometries to form 3D human engineered cardiac tissues (3D-hECTs). To study

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tissue growth and contraction, image and video analysis was performed at specific timepoints. To analyze differentiation efficiency and cell population, flow cytometry was performed using cardiac markers. To evaluate gene expression, qPCR was performed using pluripotency and cardiac specific primers. RESULTS/ANTICIPATED RESULTS: Direct cardiac differentiation of encapsulated hiPSCs resulted in synchronously contracting 3D-hECTs in both biomaterials and all tissue geometries. Spontaneous contractions started on Day 7 and increased in velocity, frequency, and synchronicity over time. 3D-hECTs had high cell viability with > 70% of cells positive for cardiac markers. Engineered tissues showed appropriate temporal changes in gene expression over time with pluripotency gene expression decreasing and cardiac gene expression increasing. DISCUSSION/SIGNIFICANCE OF IMPACT: This study shows the potential for direct differentiation of encapsulated hiPSCs to produce physiologically relevant engineered cardiac tissues. Resulting 3D-hECTS showed features of mature myocardium with appropriate cardiomyocyte populations, mechanical motion, and gene expression. Using this platform, we are able to produce engineered cardiac tissue in a variety of biomaterials and tissue geometries to study new therapeutics, mechanism of disease, and scalable tissue culture.

3300

Progesterone receptor alters lipid biology in luminal breast cancer

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OBJECTIVES/SPECIFIC AIMS: These studies seek to evaluate hormonal regulation of luminal breast cancer lipid metabolism and to identify targetable progesterone-mediated changes in lipid biology that contribute to therapeutic resistance in breast cancer. METHODS/STUDY POPULATION: Established and patient-derived luminal breast cancer cell lines, which express ER and PR, were used for this study. RNA transcript and protein expression levels were evaluated by qRT-PCR and immunoblot, respectively. Broad scale lipidomics of progesterone-treated cells was conducted via ultra-high pressure liquid chromatography-mass spectrometry (UHPLC-MS) through the UCD Skaggs School of Pharmacy Mass Spectrometry Core. RESULTS/ANTICIPATED RESULTS: Data mining of previously published microarray data of CK5+ and CK5− syngeneic cancer sublines revealed that CK5+ cells have increased expression of lipid processing genes, including LPL and PPARG. As progestin treatment induces a subpopulation of cells to turn on CK5 expression in luminal breast cancers, UHPLC-MS-based lipidomics analysis will expose whether modulation of the lipid landscape occurs in all cells with progesterone treatment, or whether this phenomenon is heightened specifically in CK5+ cells. I also expect that ER+ breast cancers with progestin induced-altered lipid content, such as lipid droplet formation, will evade therapy-induced death. DISCUSSION/SIGNIFICANCE OF IMPACT: There are numerous approved and developmental therapeutics targeting lipid biology. By determining if progestins alter lipid metabolic genes specifically in CK5+ CSCs, which are endocrine resistant, strategies may be devised to target these resistant cells using combination therapy in conjunction with existing therapies to prevent tumor recurrence.

3326

Radiofrequency Renal Denervation Prevents Further Progression of Hypertension and Decreases Renal Medullary Fibrosis in One-year-old Spontaneously Hypertensive Rats (SHR)

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OBJECTIVES/SPECIFIC AIMS: We have reported that radiofrequency renal denervation (RF-RDN) in SHR at 20-weeks of age, decreased blood pressure (BP) and fibrosis in kidney cortex and medulla when rats were sacrificed at 6 months. However, whether RF-RDN can have similar benefits in older rats remains unknown. This study examined whether performing RF-RDN in older rats also has a beneficial effect on BP and renal fibrosis. METHODS/STUDY POPULATION: Baseline systolic and diastolic BP (SBP/DPB) was measured (telemetry) in nine-month-old SHR and Wistar Kyoto rats (WKY). Groups of rats then received bilateral RF-RDN or Sham-RDN (SHR-RDN, n=9; SHR-Sham, n=10; WKY-RDN, n=5; WKY-Sham, n=8). Rats were then sacrificed at 12-months of age. Kidneys were harvested, sectioned, and assessed for fibrosis by Masson's trichrome stain. A pathologist, who was blinded to treatment groups, evaluated each kidney section for fibrosis. RESULTS/ANTICIPATED RESULTS: Compared to SHR with Sham-RDN, RF-RDN prevented a further increase in systolic and diastolic BP from baseline (9-month) in SHR as they aged to 12-months (SHR-Sham mmHg: 9-month 193±4/127±4; 12-month 207±3/142±5; SHR-RDN mmHg: 9-month 197±3/132±2; 12-month 197±4/132±3). RF-RDN did not alter SBP or DBP in aged WKY. One-year-old SHR with prior Sham-RDN showed extensive renal fibrosis in kidney cortex and medulla. In contrast, RF-RDN significantly decreased renal fibrosis in the medulla, but not cortex. There was no fibrosis in kidneys of age matched WKY. DISCUSSION/SIGNIFICANCE OF IMPACT: These findings suggest that RF-RDN may be a potential therapy for halting progression of hypertension and decreasing medullary fibrosis in the aged population.

3293

Region Specific Dysregulation of Dopaminergic Signaling in Mice Displaying Excessive Over-Grooming

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OBJECTIVES/SPECIFIC AIMS: The objective of this study was to determine if dopamine signaling is altered in a mouse model displaying excessive self-grooming and further elucidate the potential utility of compounds targeting the striatal DA system in modulating repetitive behaviors. METHODS/STUDY POPULATION: Here, we report studies using fast-scan cyclic voltammetry (FSCV) in mice lacking the postsynaptic protein SAP90/PSD95-associated protein (SAPAP3 KO mice) as well as control littermates. Rodent self-grooming provides a behavioral output with which one can monitor repetitive, self-directed, patterned behavior that has great translational value to OCD-like disorders. Total time spent grooming was monitored in SAPAP3KO mice and control littermates. To further examine the role of DA in regulating repetitive grooming behaviors the magnitude and kinetics of DA transients were assessed using

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