innervation to the hippocampus is critical for normal learning and memory and is severely degenerated in Alzheimer's disease. To understand the molecular events underlying this loss, we optimized a primary septal-hippocampal co-culture system that facilitates the study of central cholinergic synapses. METHODS/STUDY POPULATION: We developed an optimized in vitro septal-hippocampal co-culture system modified from previously published protocols. Briefly, hippocampal and septal tissue were harvested from embryonic day 19 (E19) Sprague-Dawley rats, digested with 0.1% trypsin, and an equal number of cells from each region plated onto coverslips coated with poly-D-lysine and laminin at a final density of 300 cells/mm2. We use immunostaining with validated primary antibodies and a fluorescent binding assay, together with confocal microscopy, to determine the structure of cholinergic synapses that are 1) native, 2) mammalian, 3) CNS derived, 4) comprised of physiological synaptic partners, and 5) developmentally mature. RESULTS/ANTICIPATED RESULTS: After DIV21, co-cultures maintained a healthy morphology. A subpopulation of neurons strongly expressed the cholinergic markers vesicular ACh transporter (vAChT), choline acetyltransferase (ChAT), and the high-affinity choline transporter (ChT1), whereas most neurons lacked vAChT expression and were presumably glutamatergic or GABAergic. The percentage of cholinergic neurons attained in the co-culture is ~5-7%. The size of these cholinergic neurons is strikingly similar to that reported for BFCNs in the intact brain (mean 30μm, range 18-43μm). All sampled cholinergic neurons (28/28 neurons) also expressed molecular machinery necessary for GABA release. Staining for a cholinergic postsynaptic marker shows that 63% of the contacts made with are synaptic. DISCUSSION/SIGNIFICANCE OF FINDINGS: Primary septal-hippocampal co-cultured neurons have not been exploited extensively in the field, perhaps due to the difficulty in maintaining such cultures for extended periods. Here, we optimized an in vitro septal-hippocampal co-culture system, a powerful tool to comprehensively analyze central cholinergic synapse formation and dysfunction.

Data Science/Biostatistics/Informatics

23255

Devices Engineered to Collect Exhaled Breath Condensate (EBC) and their Applications*

Alexander J. Schmidt[1], Eva Borras[1], Anh Nguyen[2], Nicholas J. Kenyon[2] and Cristina E. Davis[1]
[1]Department of Mechanical and Aerospace Engineering, University of California, Davis and [2]Department of Internal Medicine, University of California, Davis

ABSTRACT IMPACT: Human exhaled breath is rich in metabolomic content that represents pulmonary function and gas exchange with blood, which can provide insights into an individual's state of health. OBJECTIVES/GOALS: Human exhaled breath is rich in metabolomic content that represents pulmonary function and gas exchange with blood. It contains a mixture of compounds that offer insight into an individual's state of health. Here, we present two novel non-invasive breath sampling devices for use in basic medical practice. METHODS/STUDY POPULATION: The two breath samplers have a disposable mouthpiece, a set of inhale and exhale one-way flap valves to allow condensation of exhaled breath only, and a saliva filter. The housing is constructed out of Teflon®, a chemically inert material to reduce chemical absorbance. The first device condenses exhaled breath into a frozen condensate using dry ice pellets and the other is a miniaturized design that liquifies exhaled breath on a condenser surface with micropatterned features on a cooling plate. Both designs have individual strategic and analytical advantages: frozen exhaled breath condensate (EBC) has high retention of analytes and sample volume; EBC collected in liquid phase offers facilitated sample collection and device portability. RESULTS/ANTICIPATED RESULTS: We investigated if breath aerosol size distribution affects the types or abundances of metabolites. We modified the geometry of the first device to redirect aerosol trajectories based on size. The trapping of larger aerosols increases with filter length, thus altering the aerosol size distribution although no significant changes in the metabolite profiles were found. With the miniaturized device, metabolite abundances were measured in a small cohort of healthy control and mild asthmatic subjects. Differences among subjects were found, as well as main differences between control and asthmatic groups. All analyses of EBC were performed with liquid chromatography - mass spectrometry. Inflammatory suppression found in asthmatic subjects can be explained by prescribed daily use of inhaled corticosteroids. DISCUSSION/SIGNIFICANCE OF FINDINGS: Breath collection devices can be used in intensive care units, outpatient clinics, workplaces, and at home. EBC analysis has been used to monitor asthma and chronic obstructive pulmonary disease. It can be applied to infectious respiratory diseases (e.g. influenza, COVID-19) and for monitoring environmental and occupational chemical exposures.

30432

Novel insights from single-cell RNAseq analysis of the stromavascular fraction of human adipose tissue

Bingyao Wang[1], Gregory Smith[2], Frederique Ruf-Zamojski[2], Gyu Ho Lee[3], Stuart C. Sealfon[2], Jeanine Albu[2,4], Susan K. Fried[3] and Kalypso Karastergiou[4]
[1]Icahn School of Medicine at Mount Sinai, [2]Department of Neurology at Icahn School of Medicine at Mount Sinai, [3]Diabetes, Obesity and Metabolism Institute at Icahn School of Medicine at Mount Sinai and [4]Obesity and Metabolism Institute at Icahn School of Medicine at Mount Sinai

ABSTRACT IMPACT: Characterizing the cellular composition of human adipose tissue may contribute to the prevention and/or treatment of obesity-associated metabolic diseases. OBJECTIVES/GOALS: Our aims in this study were to use single-cell techniques 1. to characterize cell types within the stromavascular fraction of human adipose tissue, 2. to identify subsets of cells within each type (sub-clustering), 3. to identify gene sets and pathways that may provide information on the function and significance of each cell cluster. METHODS/STUDY POPULATION: Abdominal subcutaneous adipose tissue samples from n=6 healthy volunteers (1M, 5F, age 28-38 y, BMI 24.5-63.0 kg/m2) were collected by aspiration or during surgery. In 3 subjects, all females, paired femoral samples were also collected. After collagenase digestion approximately n=10,000 cells/sample were used for single-cell RNA sequencing using the 10X Genomics platform. After QC and downstream analysis, data were analyzed in Seurat v.3.1.5. We identified first different cell types and then subclusters in an unbiased fashion. Gene Set Enrichment Analysis (GSEA) was used for pathway analysis. RESULTS/

https://doi.org/10.1017/cts.2021.412 Published online by Cambridge University Press