Comparison of liquid versus dry aerosol drug delivery in a 3D printed avian trachea and mainstream bronchi model

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OBJECTIVES/SPECIFIC AIMS: This study investigates the process configuration parameters involved in targeted drug delivery to the avian respiratory system. Previously, direct intratracheal aerosol delivery in an avian model using a commercial atomizer was found to result in delivery of a high portion of the total dose into one lung lobe. We hypothesize that controlling process configuration will decrease the asymmetric distribution. METHODS/STUDY POPULATION: A 3D printed model of an avian trachea and mainstream bronchi was constructed to create a representative model for direct instillation of aerosols. Construction of the model respiratory tract included the trachea and the first mainstream bronchi bifurcation to measure left/right (L/R) distribution of aerosol delivered. Both liquid aerosol delivery (LAD) using a custom-built dry powder insufflator device were tested. Two experimental variables were controlled: (1) retraction distance from the carina and (2) centering of device shaft in the lumen of the trachea. Measurement of device efficiency (dose delivered to the 3D model as a fraction of total dose), aerosol delivery efficiency (dose captured at L/R bifurcations as a fraction of total dose), and aerosol lateralization (L/R) was conducted. RESULTS/ANTICIPATED RESULTS: The aerosol delivery efficiency for both LAD and DAD devices (73.9% (95% CI: 68.2–79.2) and 73.4% (95% CI: 55.5–91.3), respectively) did not have an appreciable difference. However, the LAD device had a higher efficiency as compared with the DAD device. The L/R distribution for the DAD device was found to be highly dependent on both retraction distance and shaft centering. Appreciable improvement in the L/R distribution was seen using the DAD device by increasing the retraction distance distal to the carina.

DISCUSSION/SIGNIFICANCE OF IMPACT: The use of targeted drug delivery to treat pulmonary pathologies requires a careful design, manufacture, and therapeutic positioning of devices. In particular, clinically relevant animal models and treatment regimes requires a sound understanding of the physical processes controlling aerosol distribution in the respiratory system. By using a simulated respiratory model, many of the physical parameters of drug delivery can be tested before using a live animal model. This is especially important from an animal welfare perspective as well as an animal subject availability aspect.

Neuropilin-2 is expressed by activated alveolar macrophages and negatively regulates allergic airway inflammation

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OBJECTIVES/SPECIFIC AIMS: Allergic asthma is a chronic lung disease driven by inappropriate inflammatory responses against inhaled allergens. Neuropilin-2 (NRP2) is a pleiotropic transmembrane receptor expressed in the lung, but its role in allergic airway inflammation is unknown. Here, we characterized NRP2 expression in lung immune cells and investigated the effects of NRP2 deficiency on airway inflammation. METHODS/STUDY POPULATION: NRP2 expression by lung immune cells from NRP2 reporter mice was determined by flow cytometry. NRP2 expression by human alveolar macrophages (AM) from healthy individuals was determined by miRNA analysis and flow cytometry. Airway Inflammation in NRP2-deficient mice was assessed by bronchoalveolar lavage (BAL) cytokology and inflammatory gene expression in lung tissue.

RESULTS/ANTICIPATED RESULTS: NRP2 expression in lung immune cells was negligible under steady-state conditions. In contrast, inhalational exposure to lipopolysaccharide (LPS) adjuvant dramatically induced NRP2 expression in AM, as 63.3% of AM from LPS-treated mice were NRP2+ compared with 1.5% of AM from control mice. Ex vivo treatment of human AM with LPS resulted in a 1.5-fold and 2.6-fold increase in NRP2 mRNA and surface protein expression, respectively. Compared to lerrnate controls, NRP2-deficient mice had greater numbers of BAL leukocytes and increased lung expression of the T helper type 2 cytokines IL-4 and IL-5. Furthermore, NRP2 deficiency resulted in stochastic development of allergic airway inflammation, as spontaneous airway eosinophilia was detected in 25% (2/8) of NRP2-deficient mice compared with 0% (0/8) of littermate controls. DISCUSSION/SIGNIFICANCE OF IMPACT: NRP2 is expressed by activated human and murine AM and suppresses the spontaneous development of allergic airway inflammation. These findings suggest that NRP2 may play a key role in allergic asthma pathogenesis, and could prove to be an important therapeutic target in patients with asthma and other allergic diseases.

A transgenic retinitis pigmentosa zebrafish model for drug discovery

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OBJECTIVES/SPECIFIC AIMS: Retinitis pigmentosa (RP) is a hereditary retinal degeneration disease that affects ~1 in 4000 individuals globally, and there are currently no effective treatment options available. In order to identify potential drug treatments, we optimized our existing a behavioral assay around a transgenic zebrafish carrying a truncated human rhodopsin transgene [Tg(rho: Hsa.RH1_Q344X)]. This line was also crossed with the Tg(-3.7rho:EGFP) reporter for rod visualization. The Q344X larvae experiences significant rod photoreceptor death by 7 days postfertilization (dpf) (Nakao et al., 2012). METHODS/STUDY POPULATION: To assess the vision of the Q344X zebrafish, the VMR was run under a dim-light condition based on recorded rod-b-waves in larval fish (Ployano et al., 2013) and the minimum cone activation threshold in mice (Cashafie et al., 2010). Specifically, Q344X and control larvae at 7 dpf were placed into a 96-well plate and acclimated to a dim-light source (1.807e-05 Μ W/cm² at 500 nm) for 1 hour. The VMR was tracked and quantified during light offset. The total distance traveled was averaged and analyzed at 1 second poststimulus. Retinas were dissected from Q344X and control larvae and whole-mounted to validate the rod degeneration in the Q344X model. RESULTS/ANTICIPATED RESULTS: We found that the Q344X larvae delayed an attenuated VMR (0.13 ± 0.041 cm) to the dim-light offset as compared with the control larvae (0.275 ± 0.038 cm) (two-sample t-test; p-value = 4.619e-14, n = 19). Analysis of whole-mounted retinae indicated significant rod degeneration at 7 dpf compared with controls (control: 87 rods/ retina, Q344X: 9.3 rods/retina). This is unlikely that the cones of the zebrafish contributed to this VMR since the light intensity of the assay was below the cone detection threshold of mice. As the only apparent difference between the 2 groups of larvae is significant rod degeneration, it can be concluded that the behavioral phenotype was a result of with use of TH-hydrogel. Anoxia preconditioning of hCDCs together with the TH-hydrogel system may improve the therapeutic potential of stem cell transplantation following MI.

Effects of anoxia on viability and differentiation of human cardiospheres-derived cells

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OBJECTIVES/SPECIFIC AIMS: A major limitation of cardiac stem cell transplantation following myocardial infarction (MI) is poor retention of cells in the ischemic microenvironment. Our study aims to better understand and promote the survival and differentiation of human cardiosphere-derived cells (hCDCs) in anoxia, a feature of infected myocardium. METHODS/STUDY POPULATION: We previously demonstrated that TGFβ1 and heparin-containing hydrogels (TH-hydrogel) can promote murine CDC survival. In this study, hCDCs were incubated in either normoxia or anoxia for 8 hours with and without TH-hydrogel. In addition, hCDCs without TH-hydrogel were subjected to 16 hours of anoxia.

DISCUSSION/SIGNIFICANCE OF IMPACT: The differentiation in anoxia was time dependent and could be expedited because computational methods are relatively inexpensive and much more can be an effective way to accelerate the development prevention strategies than in vivo approaches.
Development of an angiogenic proteoglycan mimic to accelerate ischemic diabetic foot ulcer repair

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OBJECTIVES/SPECIFIC AIMS: This project aims to synthesize an angiogenic decorin mimic (VEGFp-DS-SILY) with varying densities of QK and characterize its angiogenic potential and synergy with VEGF by evaluating (1) endothelial cell (EC) migration and proliferation, (2) EC VEGF receptor activation, (3) EC tube formation in collagen scaffolds, and (4) angiogenesis from a chick chondrocytic cell line (CAM assay) after 60 hours comparing treatments by using phospho-specific antibodies and an ELISA-type protocol in a mesoscale discovery system. Preliminary data with human umbilical vein endothelial cells (HMVECs) that VEGFp binds to VEGF receptor 2, albeit with decreased affinity like QK as compared to control the rate of angiogenesis and synergy with VEGF. We determined that the peptides and compounds may require longer exposures to induce activation, as they may have slower binding rates. In contrast, prolonged stimulation with VEGF causes a sharp increase in receptor activation, peaking around 10 minutes and decreasing significantly by 30 minutes. Peptides QK and VEGFp both increased proliferation of normal murine aortic endothelial cells (ECs) after 60 hours compared to control. DSV4 caused significant cell death after 24 hours in reduced growth factor media, likely due to sequestering of growth factors. It is possible that VEGFp-DS-SILY may better stimulate proliferation since it would be presented as a surface bound proteoglycan mimic, rather than as a soluble factor. HMVECs migrated farther for all treatment groups (100 µM QK, 10 µM VEGFp, 1 µM DSV4, and 10 µM DSV4) than the 10 nmol/L VEGF positive control, although more cells migrated in response to VEGF. This may be accounted at least in part by the more pronounced proliferation induced by VEGF. Migration will also be tested in 3D culture within a collagen gel. We are currently testing a 2D matrigel system for tubulogenesis. We have found that 10 µM DSV4 forms qualitatively more well-defined tubules than the untreated control on reduced growth factor matrigel. However, we were not able to quantify the improved tubule formation and are still troubleshooting the tube analysis. After seeding ECs and culturing for 4, 8, and 12 hours, cells will be fluorescently stained with anti-CD31 and imaged for 3D tubule formation. CAM assay angiogenesis growing into a collagen scaffold. In brief, fertilized chicken embryos are incubated for 2 days before exposing the CAM. VEGFp-DS-SILY bound to a collagen gel will be placed onto the CAM. Some treatment groups will receive additional VEGF to investigate synergistic effects. Light microscope images of angiogenesis into the collagen gel coated with VEGFp-DS-SILY, taken every day from days 10 to 13, will reflect the ability of the collagen scaffold to integrate into existing vasculature and 3D angiogenic potential of VEGFp-DS-SILY with or without VEGF. We expect that VEGFp-DS-SILY will increase the number of vessels formed on the CAM. Preliminary data using a Fluorodehydro assay indicates that loading of ~300 ng VEGF per mg of nanoparticles can be achieved. We expect that using a MMP-degradable peptide diacrylate crosslinker will allow nanoparticles to degrade in protease-rich environments like the chronic wound bed and release VEGF. Adjustments to the formulation, such as crosslinker density, may need to be modified to control the rate of VEGF release. DISCUSSION/SIGNIFICANCE OF IMPACT: We expect that our angiogenic decorin mimetic will lead to a novel treatment to accelerate healing of ischemic diabetic foot ulcers, thereby reducing the need for limb amputation and mortality rate of diabetic patients. We anticipate that the diabetes research and regenerative medicine communities will (1) gain a platform for targeted delivery of growth factors, (2) understand the dependence of vascularization within 3D collagen constructs on VEGFp densities and VEGF receptor activation in controlling the degree of angiogenesis, and (3) gain the benefits of controlled angiogenesis in ischemic diabetic wound healing.

The impact of alcohol dysbiosis on host defense against pneumonia

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OBJECTIVES/SPECIFIC AIMS: Alcohol consumption perturbs the normal intestinal microbial communities (alcohol dysbiosis). To begin to investigate the relationship between alcohol-mediated dysbiosis and host defense we developed an alcohol dysbiosis fecal adoptive transfer model, which allows us to isolate the host immune response to a pathogenic challenge at a distal organ (ie, the lung). This model system allowed us to determine whether the host immune responses to Klebsiella pneumoniae are altered by ethanol-associated dysbiosis, independent of alcohol use. We hypothesized that alcohol-induced changes in intestinal microbial communities would impair pulmonary host defenses against K. pneumoniae. METHODS/STUDY POPULATION: Mice were treated with a cocktail of antibiotics daily for 2 weeks. Microbiota-depleted mice were then reconstituted by gavage for 3 days with intestinal microbiota from ethanol-fed or pair-fed animals. Following reconstitution groups of mice were sacrificed prior to and 48 hours post respiratory infection with K. pneumoniae. We then assessed susceptibility to Klebsiella infection by determining colony counts for pathogen burden in the lungs. We also determined lung and intestinal immunity, intestinal permeability, as well as, liver damage and inflammation. RESULTS/ANTICIPATED RESULTS: We found...