can be an effective way to accelerate the development prevention strategies because computational methods are relatively inexpensive and much more scalable than in vivo approaches.

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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 commercial atomizer and dry aerosol delivery (DAD) 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 at as 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 pathogens 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.

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Effects of anoxia on viability and differentiation of human cardiosphere-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 infarcted 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 assessed in 16 hours of anoxia. Following incubation, hCDCs were assayed for viability using calcein dye and immunostained for CD31, a marker of endothelial differentiation. RESULTS/ANTICIPATED RESULTS: hCDCs incubated for 8 hours in anoxia in both models equally demonstrated increased survival up to 30% when compared with cells incubated in normoxia. However, in contrast to hCDCs alone, hCDCs with TH-hydrogel additionally demonstrated increased differentiation into endothelial cells in both anoxia and normoxia. We found that hCDCs alone were able to upregulate CD31 only when subjected to 16 hours of anoxia. DISCUSSION/SIGNIFICANCE OF IMPACT: We demonstrate a new, previously unknown response of hCDCs to anoxia. This induces increased viability and differentiation of hCDCs into endothelial cells. The differentiation in anoxia was time dependent and could be expedited with use of TH-hydrogel. Anoxic preconditioning of hCDCs together with the TH-hydrogel system may improve the therapeutic potential of stem cell transplantation following MI.

2215

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 mRNA analysis and flow cytometry. Airway inflammation in NRP2-deficient mice was assessed by bronchoalveolar lavage (BAL) cytology 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 littermate 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.

2217

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 assay was run under a dim-light condition based on recorded rod b-waves in larval fish (Moyano *et al.*, 2013) and the minimum cone activation threshold in mice (Cachafeiro *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 (1.802e-05 μ W/cm^2 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 displayed an attenuated VMR (0.121 ± 0.041 cm) to the dim-light offset as compared with the control larvae (0.2751 ± 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, Welch two-sample *t*-test, *p*-value = 1.4e4). It 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

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the degeneration. DISCUSSION/SIGNIFICANCE OF IMPACT: These results suggest that the attenuated Q344X VMR is a result of the rod photoreceptor death. This behavioral phenotype can be taken advantage of to develop a drug screening assay. Future steps will screen chemical libraries to identify compounds that ameliorate the rod degeneration. Compounds that prevent degeneration are expected to result in a significant increase in locomotion in response to the dim visual stimulus.

2229

Development of an angiogenic proteoglycan mimic to accelerate ischemic diabetic foot ulcer repair

Jenny Lin and Alyssa Panitch

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 synergism with VEGF by evaluating (1) endothelial cell (EC) migration and proliferation, (2) EC VEGF receptor activation, (3) EC tubule formation in collagen scaffolds, and (4) angiogenesis from a chick chorioallantoic membrane (CAM assay) growing into the scaffold, reflecting the ability of the collagen scaffold to integrate into existing vasculature. The next main goal is to develop and characterize an MMP-degradable nanoparticle system for controlled release of VEGF. Future work will evaluate in vivo effects of VEGFp-DS-SILY bound to a 3D collagen scaffold on ischemic wound repair in a combined excisional wound/bipedical dorsal skin flap rat model. METHODS/STUDY POPULATION: Peptide hydrazides are conjugated to the free carboxylic acid functional groups on dermatan sulfate using EDC chemistry. We added a 3 amino acid spacer (-Gly-Ser-Gly) to the C-terminus of the established QK sequence before the hydrazide functional group and refer to this modified QK as "VEGFp." VEGFp, SILY, and *N*-terminal biotinylated versions were synthesized using standard Fmoc solid-phase peptide synthesis protocols and purified using reverse phase HPLC. Coupling efficiencies of peptides to dermatan sulfate were determined spectroscopically at 280 nm measuring the aromatic residues (Trp or Tyr) using a NanoDrop system. Dermatan sulfate with 1 or 4 VEGFp peptides coupled were termed DSV1 and DSV4, respectively. After further conjugation with SILY, we will blend this VEGFp-DS-SILY with unmodified DS-SILY to a total 10 μM to test increasing densities of VEGFp. To verify that the collagen-binding properties of VEGFp-DS-SILY are not compromised by the addition of VEGFp, we will use a streptavidin-HRP system to detect bound biotinylated VEGFp-DS-SILY on collagen-coated plates by established protocols. DSV1 and DSV4 were tested for their effects on endothelial VEGFR2 phosphorylation using an MSD ELISA-type assay and endothelial proliferation using an MTS assay. Cell migration was monitored using an ORIS assay where cells are grown to confluence around a silicone stopper that is then removed to allow cells to migrate inward. Tubulogenesis was evaluated by examining tubule formation on matrigel. Finally, in vivo angiogenesis will be evaluated using a chorioallantoic membrane assay. For extracellular VEGF release, hollow MMP-degradable thermoresponsive nanoparticles [NIPAM, 5 mol% 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS), 1% Acrylic Acid (AAc), 2 mol% MMP-degradable peptide diacrylate, and potassium persulfate initiator] will be synthesized around noncross-linked polymer cores. The cores will then be diffused out through the shell by dialysis prior to drug loading. SILY (and some biotinylated SILY for visualization) will be conjugated with EDC chemistry for targeting nanoparticles to collagen. NPs size and zeta-potential will be measured on a Malvern Zetasizer. VEGF will be loaded into NPs by co-incubating a loading solution of 1 μg/mL VEGF with 1 mg of NPs, incubating overnight at 4°C. VEGF loading and release will be measured by ELISA. Biological activity of the released VEGF from particles will be determined on ECs using assays similar to those outlined previously. RESULTS/ANTICIPATED RESULTS: Preliminary data have verified the synthesis and purification of SILY and VEGFp (QK-Gly-Ser-Gly-hydrazide), as well as an *N*-terminal biotinylated version, through mass spectrometry and reverse-phase HPLC, respectively. For proof-of-concept, we have verified binding of VEGFp to the VEGF receptor 2 using a ForteBio Blitz interferometry instrument. In addition to support based on published reports showing retained bioactivity of QK after conjugation using other spacers, our preliminary data suggests that VEGFp still binds to VEGF receptor 2, albeit with decreased affinity like QK as compared with VEGF. Circular dichroism also shows that VEGFp has retained its α-helical structure necessary for bioactivity; however it appears that it has some uncoiling when conjugated to dermatan sulfate. We hypothesize that varying densities of VEGFp conjugated to the decorin mimetic (DS-SILY) will modulate the degree of angiogenic activity and synergy with VEGF. We determined that we can achieve ~70% VEGFp conjugation completion to dermatan sulfate after 3.5 hours. We have quantified VEGFR2 phosphorylation after 5 minute treatments by using phospho-specific antibodies and an ELISA-type protocol in a mesoscale discovery system. Preliminary data with human umbilical vein endothelial cells shows that VEGFp exhibits synergism with VEGF at levels similar to QK. DSV1 and DSV4 data suggests synergy with VEGF, although free-peptides and engineered compounds alone did not show effects similar to VEGF in the conditions tested. Prelminary data with 30 minute treatments suggests 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 slightly increased proliferation of dermal microvascular endothelial cells (HMVECs) after 60 hours incubation. However, incubation with dermatan sulfate and DSV 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 (10 μM QK, 10 μM VEGFp, 1 μM DSV4, and 10 μM DSV4) than the 10 ng/mL 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 tubule 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 treatment will increase the number of vessels formed on the CAM. Preliminary data using a Fluoraldehyde assay indicates that loading of ~300 ng VEGF per mg of nanoparticles can be achieved. We expect that using an 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.

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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 recolonized by gavage for 3-days with intestinal microbiota from ethanol-fed or pair-fed animals. Following recolonization 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 immunology, intestinal permeability, as well as, liver damage and inflammation. RESULTS/ANTICIPATED RESULTS: We found

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