42 – Title: Assessment of a Novel Imaging Method to Measure Nanomaterials Targeted to Smoke-Induced Lung Injury

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Introduction: Smoke inhalation injury contributes significantly to the pathologic state of burn victims by inciting an inflammatory response, increasing the risk for acute respiratory distress syndrome. Current treatment is largely supportive, with inhaled therapeutics showing inconsistent benefits. Peptide amphiphile (PA) nanofibers are useful drug delivery vehicles, as they can self-assemble and reach the distal airways through the microcirculation of the lung. PAs can be modified to target specific proteins, such as angiotensin-converting enzyme (ACE), which is located primarily on the vascular endothelium. We hypothesize that ACE-targeted PAs will localize to areas of lung injury and this localization can be quantified using light-sheet fluorescence microscopy (LSFM).

Methods: Targeted, backbone, and fluorescently labeled PA monomers were co-assembled. Nanofiber formation was confirmed using transmission electron microscopy (TEM). Male Sprague Dawley rats (n=7, 360-420g) were exposed to a consistent smoke density of 20-30% (total of 8 minutes) to induce smoke inhalation injury. Rats underwent tail vein injection of non-targeted (n=3) or ACE-targeted (n=4) PA nanofibers at 23 hours post smoke exposure, followed by euthanasia at 24 hours. Left lobe lung tissues were frozen and sectioned for hematoxylin and eosin (H&E) staining. Right lobe lung tissues were optically cleared for LSFM and were analyzed using Imaris imaging software, which produced 3D volumes based on fluorescence intensity. Statistical analysis was performed using Origin software.

Results: PA co-assemblies formed nanofibers at a range of molar ratios, with an average length of 340 nm and diameter of 10 nm as seen by TEM. H&E-stained lung sections from smoke-exposed rats were found to have interstitial edema and inflammatory cell infiltrates, consistent with lung injury. LSFM assessment of the lungs showed an average fluorescence volume of 3.5×10^5 μm^3 for rats injected with the ACE-targeted nanofiber and 9.0×10^3 μm^3 for rats injected with the non-targeted PA nanofiber. Fluorescence appeared to be randomly distributed throughout the lung parenchyma and did not appear to be in the bronchioles. After standardizing signal based on lung autofluorescence, analysis revealed >200-fold higher fluorescence in lungs of rats that received the ACE-targeted PA nanofiber as compared to the non-targeted nanofiber (1.2×10^{-5} vs. 2.8×10^{-7}, respectively, $p<0.05$).

Conclusions: Intravenously administered nanofibers localized to smoke-injured lungs via the circulation. Current methods to localize nanofibers include tissue sectioning, which can pose significant challenges due to sampling error. Using LSFM, the spatial distribution of nanoparticles within the lung can be studied efficiently and without significantly disrupting the tissue architecture. These data lay the foundation for modifying the PA with therapeutics to improve recovery after smoke-induced lung injury.