Improved throughput of gold nanoparticle localization and imaging in the brain through the development of a novel SEM-STEM technique

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A major hurdle to the improved treatment of neurological disorders is the successful delivery of active drug to the brain parenchyma. The low accumulation of drug in the parenchyma is the result of the blood brain barrier (BBB), preventing most compounds from passing into the brain. While the presence of the BBB negates to use of passive uptake mechanisms such as the enhanced permeability and retention (EPR) effect commonly associated with tumors, active targeting can be utilized to improve uptake in to the brain. A popular target for brain drug delivery is the transferrin receptor which is highly expressed in brain endothelial cells. In this work, the uptake of transferrin receptor targeted gold nanoparticles (AuNPs) across the blood brain barrier in healthy mice was characterized.

Transmission electron microscopy (TEM) is a powerful tool to directly image AuNPs within brain tissue and facilitate their precise localization with respect to the BBB. While TEM enables locating AuNPs in both the capillaries and parenchyma, this requires considerable time and effort due to the relative rarity with which these events occur. The bulk of the injected AuNPs accumulated in the spleen and liver with only about 0.1% ID/g making it to the brain. Of this amount, even less reached the parenchyma with the majority accumulating in the vasculature. Identifying these "rare" events requires imaging increasingly larger volumes of tissue in the electron microscope, a cost and time inefficient methodology. To improve throughput, we developed a novel SEM-STEM technique to prescreen tissue sections on TEM grids for the presence of AuNPs.

The SEM-STEM technique utilizes an 8 sample STEM detector in a FEI Quanta 200 FEG-SEM operated at 15 keV. SEM-STEM has a number of attributes that allow for a higher throughput relative to TEM. First, lower accelerating voltages result in higher contrast from the AuNPs permitting imaging with shorter dwell times and lower magnifications. Second, the interconnected scanning coils and stage allows for both image rotation and single frame stage translation which facilitates quick and efficient scanning of the entire sample. Finally, the ability to load 8 samples simultaneously decreases sample loading times, mitigates the risk of a bad grid, and enables the use of a control grid known to contain AuNPs.

Utilizing this technique over 2,000,000 m^3 of brain tissue was analyzed for the presence of low affinity antibody targeted AuNPs (L-AuNPS) at ~68000 μm^3/hr, figure 1(A). This is a 6-fold improvement from traditional TEM at ~11,000 μm^3/hr. 356 L-AuNPs were located within the brain, at 11.9 AuNPs/hr, with the bulk located in capillaries, figure 1(B). From the same tissue analyzed in TEM, figure 1(C), 51 L-AuNPs were found at 2.0 AuNPs/hr. The TEM imaging rates were comparable for high affinity antibody targeted AuNPs (H-AuNPs) as well at ~11,000 μm^3/hr and 2.1 AuNPs/hr, figure 1(D).

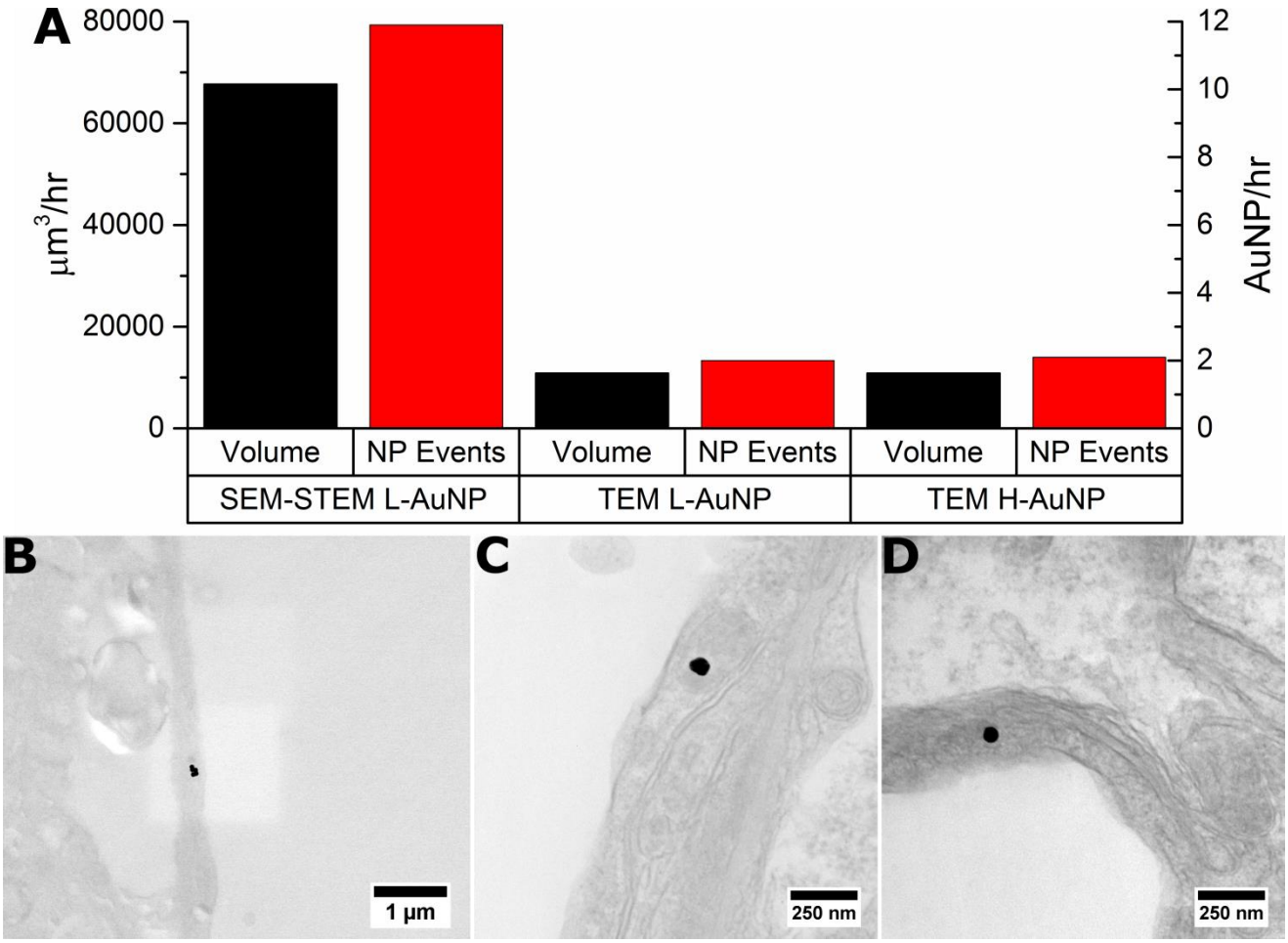

Figure 1: (A) Chart showing the improved throughput achieved through the SEM-STEM technique. (B-C) SEM-STEM and TEM micrographs showing L-AuNPs in brain capillaries for both imaging techniques. (D) TEM micrograph of H-AuNPs also located within a capillary.

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