Optimizing nanoparticle geometry to enhance drug delivery to the brain by the intrathecal route

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Statement of Purpose: Direct administration of substances to cerebrospinal fluid (CSF, i.e., intrathecal drug delivery) is a clinically established method for treatment of disease in the central nervous system (CNS). Our lab focuses on developing nanoparticle systems to enhance tolerability, distribution, and efficacy of intrathecally administered agents. Our data showed that IT administered nanoparticles circulate dynamically within the subarachnoid space but often fail to reach deeper parenchyma targets (1). Many studies have shown that CSF moves from the subarachnoid space into deep brain regions via paravascular spaces (PVS) that run alongside CNS vasculature (2). In PVS regions, CSF crosses the microperforated pial layers to reach the glia limitans, mainly made up of astrocytic endfeet, after which it enters the brain parenchyma (3). The exchange of CSF and interstitial fluid is regulated by Aquaporin-4 (AQP-4) water channels that are highly expressed on astrocyte endfeet encompassing cerebral vasculature (4). In this study, we hypothesized that shape- and surface property-optimized nanoparticles would be able to access the PVS to target AQP4, toward the long-term goal of designing nanoparticle systems capable reaching deep brain regions following intrathecal administration.

Methods:

Gold nanoparticles (AuNPs). All AuNPS were purchased from NanoComposix. AuNP with varying shape and size (15x40nm rod, 10nm and 100nm sphere) were delivered to the CSF of healthy mice via intrathecal cisterna magna administration. The distribution of AuNPs was observed and evaluated with a stereoscope *ex vivo*.

Modifying the shape of polystyrene FluoSphere (FS). Polystyrene FS with 100nm diameter were purchased from Invitrogen. 5-10% Polyvinyl alcohol (PVA) was dissolved in 75°C water, then 2% glycerol was added to plasticize the PVA film. FS were added to this PVA-glycerol mixture to a concentration of 0.04% wt/vol. After the PVA films containing FS were plasticized overnight, the films were soaked in toluene for 3h before stretching. The films were stretched until their lengths were double from their original lengths. Next, the films were dried at room temperature on the stretching apparatus for 24h. We soaked the stretched film in isopropanol for 24h to extract any remaining toluene. The films were then dissolved in PBS solution at 65°C and washed by centrifugation at 40°C with the same wash solution 10 times. The morphology of stretched FS were verified by transmission electron microscopy (TEM). Following this, unstretched and stretched FS were added into a cell culture of the human MDA-MB-231 breast cancer cell line. About 14-20ug of FS were incubated in the cell culture for 2hrs. After that, the cell culture was washed three times with PBS and processed for histology. The fluorescent signal of FS associated to nucleus and cytoplasm were observed and quantified under confocal microscopy.

Results:

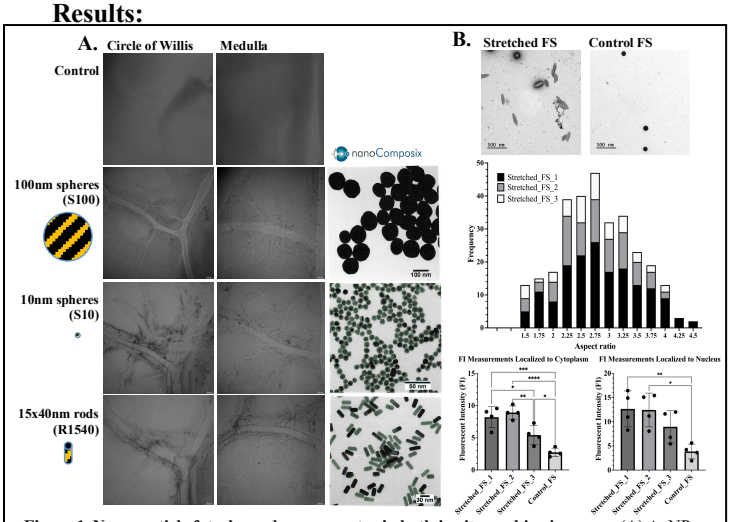

Figure 1. Nanoparticle fate depends on geometry in both *in vitro* and *in vivo* assay. (A) AuNPs were detected as dark puncti under stereoscopic illumination, which were not detected in brain tissues obtained from control subjects that did not receive AuNPs. Smaller spheres (S10) and rods (R1540) showed greater localization with paravascular tracks present on the surface of the brain. (B) A thin film stretching technique was utilized to stretch commercially purchase, polystyrene FluoSpheres (FS). Stretched FS possessed an average aspect ratio of 2.75. Following application to MDA-MB-231 cells, stretch FS were better internalized and exhibited greater nucleus localization than spherical control. All data are reported as mean plus/minus deviation for a minimum of 4 replicate measurements.

AuNPs were observed as dark puncti under stereoscopic illumination (Fig. 1A) compared to control brain sample. AuNPs with varying geometry showed different distribution in the SAS along the vessels (lighter background compared to the AuNP dark punti)

Stretched FS were captured under TEM (Fig. 1B). About 80% of FS were stretched with aspect ratio 1.5 to 4. There was still about 20% of FS in sphere shapes with aspect ratio under 1.25. The uptake of stretched FS were significantly increased compared to control FS in MDA-MB-231 cells, with distinct cytoplasmic vs. nuclear localization for control versus stretched FS.

Conclusions:

Rod-shaped AuNPs appeared to have greater localization with meningeal vessels compared to both large and small spheres. Modifying the geometry of spherical NPs enhanced the uptake of these NPs in human breast cancer cell culture. Nanoparticle size and geometry influence distribution in CSF *in vivo*, as well as impacting cellular uptake *in vitro*.

References:

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