Characterizing Ferrofluid Permeability Across the Blood-Brain Barrier Model

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Statement of Purpose: The blood-brain barrier is known as a sanctuary that separates somatic circulating blood from the cerebrospinal fluid in the central nervous system (CNS). Previous research has demonstrated that small lipid-soluble-molecules which have a molecular weight less than 600 Da can be transported across the blood-brain barrier, suggesting a pathway to design novel nanoparticles which can either be inhibited or promoted to cross the blood brain barrier[1]. As there are concerns about both the delivery of drugs across the blood-brain barrier and the accumulation of SPIONs (SuperParamegnetic Iron Oxide Nanoparticles)in the body, our study established an in vitro blood-brain barrier model to test the ability of a new type of magnetic materials (ferrofluids or FF) to pass through the blood-brain barrier. In this specific case, we used an immortalized cell line, b.End3 cells, in our blood–brain barrier model and tested several variations of magnetic nanoparticles in an effort to both increase blood-brain barrier passage (for neural drug delivery applications) and decrease blood-brain barrier passage (to minimize toxicity). Results provided significant promising evidence that a combination of bioactive ligands (eg., BSA or collagen et al.)used during the *in situ* synthesis of ferrofluids controlled whether they cross the blood brain barrier.

Methods: According to a patented process[2], five ferrofluids were synthesized by incubating a ferrous/ferric salt solution in phosphate-buffered saline supplemented with the additives of interest such as collagen, poly(vinyl) alcohol (PVA) and/or bovine serum albumin (BSA) using ammonium hydroxide under highly alkaline conditions. After synthesis, ferrofluids were centrifuged for a stability test so that the supernatant byproducts could be washed away. Dynamic light scattering and TEM were used to characterize their diameter and zeta potential was used to characterize the charge of those ferrofluids. An in vitro blood-brain barrier model based on b.End 3 cells (ATCC) was then used to test the permeability of the various nanoparticles which were GGB (ferrofluid synthesized using glycine, glutamic acid and BSA), GGC (glycine, glutamic acid and collagen), GGP (glycine, glutamic acid and PVA), BPC (BSA, PEG and collagen) and CPB (collagen, PVA and BSA). For this, nanoparticles were diluted 1:19 with HBSS (Sigma) and then added to inserts (Sigma) for 2 hours. After 2 hours, a 100 µL solution was taken from each well and full spectrum absorbance was used to determine the iron concentration that passed through the blood brain barrier model. Each experiment was conducted in triplicate and repeated at least three times[3].

Results and Discussion: Results showed that the highest permeability was obtained from CPB and the lowest permeability was obtained from GGB. Also, ferrofluids synthesized using a combination of collagen and BSA generally had higher permeability than those synthesized using glycine and glutamic acid (Figure 1). These results suggested that for nanoparticles that need to be delivered through the BBB (i.e., for treating nuerological diseases), FF should be coated with collagen while, on the other hand, FF should be coated with glycine and glutamic acid to keep the nanoparticles from penetrating the BBB (i.e., for whole body MRI imaging to decrease brain toxicity).

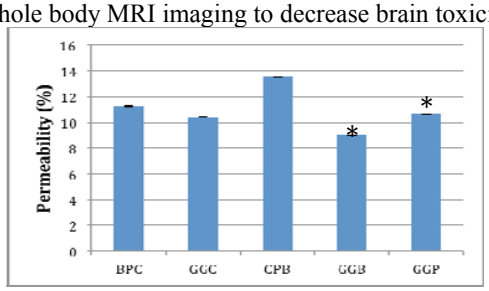

Figure 1. Blood–brain barrier permeability by sample. Data are shown as the mean ± SD. N = 3. *P<0.01 compared with *P < 0.01 compared with respective samples.
Abbreviations: GGB (glycine, glutamic acid and BSA), GGC (glycine, glutamic acid and collagen), GGP (glycine, glutamic acid and PVA), BPC (BSA, PEG and collagen) and CPB (collagen, PVA and BSA).

Conclusions: An in vitro model of the blood-brain barrier was established using b.End3 cells. As the permeability decreased with increasing exposure to serum-free medium, the model was confirmed by comparing the permeability of FITC-dextran in serum-free medium with previous research. With the successfully established model, the permeability of five magnetic nanoparticles ferrofluids was examined. The present results suggest a possibility to manipulate magnetic nanoparticle penetration across the blood–brain barrier by control bioactive coatings. Such data lay the foundation for the modification of ferrofluids to be either coated with collagen to pass through blood-brain barrier, or to be coated with glycine and glutamic acid to avoid penetration. In addition, comparing to a previous study that focused on characterizing nanoparticles with different combination of PVA, BSA, glutamic acid and collagen coating, we can further affirm that as coating candidates, collagen or PVA itself with low concentration, or combined with glutamic acid or BSA, should have a good permeability through the blood-brain barrier.

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