Targeting transcytosis across Blood-Brain-Barrier

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ABSTRACT

The delivery of therapeutic agents into the Central Nervous System (CNS) across the blood brain barrier (BBB) has challenged scientists and clinicians for decades. One of the major routes through the BBB is via a specialized endocytotic mechanism known as Transcytosis BBB. During transcytosis, extracellular cargo is transported across small vessel cells of the brain within a membrane-vesicular carrier coated with specific receptors. This process is extremely regulated in order to maintain the environment of CNS both physically and chemically stable. [1] Existing studies are somewhat limited and much is unknown at the mechanistic level, such as cargo sorting and the dysfunctional aspects of transcytosis. In this project, in order to mimic the membrane-vesicular carrier, several formulations of self-assembled amphiphilic diblock copolymers (hydrophilic-hydrophobic copolymers) have been synthesized. The morphologies range from micelles to vesicles by controlling the copolymer molecular architecture. The main goal of our study is screening these nano-size polymersome for intracellular uptake kinetics. Afterwards, transcytotic-receptor modified polymersomes will be used to increase uptake kinetics enhancing also differential uptake by BBB endothelial cells.

Keywords: *Blood-Brain Barrier, Central nervous system, Nanotechnology, pH-sensitive Polymersomes, Targeted drug delivery*

Seeking a proper method to delivery therapeutic agents to central nervous system remains challenging in the world of pharmacology and medicine. Although many different formulations have been proven to be very effective as an intracellular vector, there are still many problems regarding biocompatibility, stability and effective targeting. The proposal for the project is to design and test biocompatible
polymersomes for cellullar delivery of different theraupetic agents that engineered to target the BBB transcytosis. Understandably the success of this will open several opportunities in the treatment of many different neurological disorders, such as Motor Neuron Disease (also known as Amyotrophic Lateral Sclerosis), Spinal Muscular Atrophy (SMA), Alzameir, Parkinson, and many others. Indeed the project is part of much larger interdisciplinary project that combine expertise from bioengineering, physics, chemistry with clinical neuroscience and neurology for generating new therapies for ALS, SMA and Parkinson.

1 BLOOD-BRAIN BARRIER AND TRANSCYTOSIS

1.1 Blood-brian barrier: The barrier of central nervous system

Blood-brain barrier was firstly observed by Paul Ehrlich in 1885 [2, 3], who described that as the water-soluble dyes injected into the circulatory system, all organs appeared to be stained with the exception of the brain and spinal cord. However the nature of the BBB was debated well into 20th century. Lina Stern was the pioneering scientist
who first used the term BBB refering to the hemato-encephalic barrier[4] The main function of the BBB is a protective one [5]that prevents the introduction of harmful blood-borne substance, and restricts the transportation of macromolecular cargo from blood to brain.

Anatomically, there are three main barrier layers that form the BBB. These are the endothelium of brain capillaries, the epithelia of choroid plexus (CP) and the arachnoid. At the interface between blood and brain tissue, tight junctions (TJ) form a continuous, circumferential (belt-like structure) barrier in epithelia and endothelial cells protecting them from the external environment [6]. More recently, the BBB has not only been considered as an anatomic barrier but also as a metabolic barrier (The blood-brain barrier/neurovascular unit in health and isease[7]. This is formed by the expression of degradative detoxifying enzymes to keep homeostasis of the brain. Among these, ecto-enzymes such as peptidases and nucleotidases and intracellular enzymes such as monoamine oxidase and cytochrome P450[8]. This restriction of traffic means that only small-uncharged molecules such as O2 and CO2 and small lipofilic molecules can pass through. The movement of substance across the BBB is either passive, driven by a concentration gradient from plasma to brain, with more lipid-soluble solutes entering more easily, or may be facilitated by passive or active transporters in the endothelial cell membranes.
Transporters in the endothelium (both luminal and abluminal membranes) limit the transcellular exchange to allow for nutrients to enter the BBB but not toxic molecules [9]. Nowadays it is believed that the BBB is a dynamic system, capable of responding to local changes and requirements, and able to be regulated via a number of mechanisms and cell types. Many chemical agents in the circulating plasma or produced by the local cells can effectively alter the permeability of this barrier. This can be produced in both ways, either impairing and hence opening the barrier function (i.e. bradykinin, histamine, serotonin, glutamate, purine nucleotides, pro-inflamatory cytokines and factors, complement proteins, prostaglandins leukotrienes and derivates and also free radicals) or tightening the barrier (i.e. steroids, elevated intracellular camp, adrenomedullin and noradrenergic receptors)[10, 11] As more is learned about BBB regulation, opportunities will emerge for targeting the brain endothelium to maintain functionality and to aid recovery from injury or infection, the BBB becomes a great target to develop strategies for drug therapy.

1.2 Transcytosis: Crossing cellular barrier

Transcytosis across BBB is one of the strategies for transport of macromolecules from one side of an endothelial cell to the other. The cargo is transported selectively between two environments without changing the unique compositions of those environments. Only few macromolecules can cross the BBB by transcytosis, small gas molecules and uncharged small lipids can pass freely. However, many macromolecules such proteins are necessary in the stroma of the brain and these have to get through the BBB by tightly controlled mechanisms. Blood-borne macromolecules that need to get through the BBB bind to specific receptors on the luminal side of BBB endothelium, they are then endocytosed and finally passed across the endothelium via exocytotic cytoplasmic receptor mediated transport delivering the cargoes in the interstitium of the brain.
An uncontrolled transcytosis in BBB might cause or consequence of various CNS disease such Parkinson's, Tourette's and Alzheimer's, could affect either spinal cord or brain. As CNS behaves the role of functionalise all the body's movement and brain activity, most of these diseases could be lead to a fatal illness. Traditional treatment for central nervous disease is either bran surgery or prescribed medications, which suffering both physical and biological side effect. The aim of this project is to design and select a proper vector targeting the BBB.

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2 POLYMERSOMES TARGETING BBB TRANSCYTOSIS

2.1pH-sensitive self-assembly polymersomes

It is well known that lipid membranes play an extremely important role in both structure and function of all type of cells and hence in all living systems, that form natural barrier to restrict the substance exchange between external environment (extra cellular) and internal environment (intra cellular). Particularly in endocytotic and transcytotic there are membrane

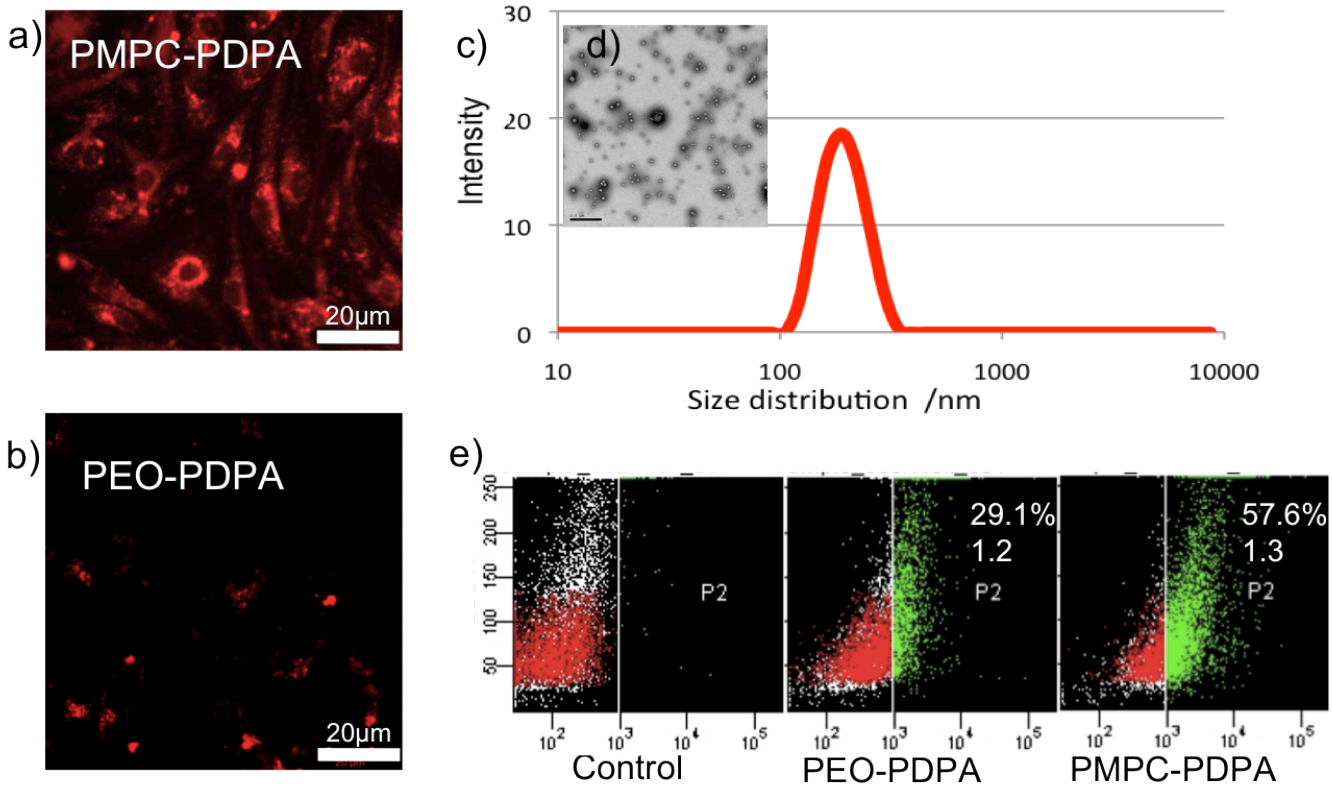

Figure 1 a) and b): b.End3 (Mouse brain endothelium)cells incubated with Rhodamine-PMPC-PDPA and Rhodamine-PEO-PDPA show the difference of cellular uptake . c) Size distribution of PMPC-PDPA polymersomes. d) Transmission Electron Microscopy (TEM) show PMPC-PDPA polymersomes. e) Flow cytometry show the b.End3 cellular uptake of PEO and PMPC polymersomes.

vesicular (receptor-mediated/non receptor-mediated) process involved.

In the previous work in our group, for the purpose of bio-mimicking the properties and structure of natural lipid membrane, we have synthesised various amphiphilic di-block polymers [12,13], by controlling the hydrophilic and hydrophobic molecular mass the polymer that can be able to self-assemble into membrane and eventually form vesicular morphology. The size of the vesicular polymersomes arranges from 100-200nm, that neither greater could recognized by phagocytosis by the cell immune system nor smaller to avoid excretion via the kidneys; more specifically, the formed polymersomes triggered by pH change: for the most common used two di-block polymer PMPC-PDPA and PEO-PDPA, as their pKa of 6.4 is within the range of endocytic pH, the size and pH-switchable nature of polymersomes makes them ideal for the delivery of therapeutic agent and provide the possibility of allowing application to the body via direct injection into the blood circulation. We have already proved that these polymersomes can be avidly uptake by almost any eukaryotic cells through endocytosis and deliver a large variety molecules and macromolecules[14]: within the acidic endosomes they disassemble leading to endosomal membrane destabilization and consequent release of their cargo into cell cytosol.

2.2 Micro phase separation of blended polymersomes

Interestingly in our previous work, [15] we not only found the polymersomes are excellent candidates for intro-cellular delivery, and also we found the PMPC polymersomes bind and internalized with the cell strongly and weakly cell interaction for PEO polymers. However, the hybrid polymersomes formed from the blended copolymer mixture do not follow this trend. The previous study reveals the blended formulations allow the formation of nanometre-sized domains on the surface of polymersomes. It is believe that the formation of domains due to micro phase

segregation between the thermodynamically immiscible PMPC and PEO chains

2.3 polymersomes targeting transcytosis

For the propose of seeking an ideal model of transcytosis to across the blood brain barrier, the PMPC/PEO hybrid polymersomes posses unique properties: long time circulation in blood and fast uptake by brain blood vessels. Several well studied transcytosis receptor such as RVG, angiopep-2 will be used and conjugated with PMPC-PDPA co-block polymers, in order to specialise the cellular uptake by transcytosis by the endothelium cell of brain blood capillaries

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