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Ultrasound Enhance Drug Delivery Across Blood-Brain Barrier

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Abstract. To treat central nervous system (CNS) diseases, drugs need to be delivered into the brain and this has always been a challenge due to the blood-brain barrier (BBB). The BBB, which is a protective barrier of the brain, prevents pathogens from entering the brain. However, this also prevents the entry of 98% of drugs into the brain, which limits the treatment of most CNS diseases. To overcome this difficulty, many approaches to opening the BBB have been studied, one of which involves the use of ultrasound and microbubbles. By combining ultrasound with microbubbles, nonoperation can occur in vivo, which can increase the permeability of the BBB. After the BBB is opened, drugs can be delivered into the brain using nanocarriers. This increases the drug delivery efficiency across BBB. In this review, the mechanism by which ultrasound opens the BBB will be discussed. Furthermore, three nanocarriers that can be used to carry drugs across BBB after BBB opening using ultrasound will be introduced.

Keywords: Ultrasound, Drug delivery, Blood-brain barrier, nanoparticles

1. Introduction

1.1. Neurological diseases and BBB

Neurodegenerative diseases are characterized by neuronal loss and functional impairment. As shown in Figure 1, Parkinson's disease, a typical neurodegenerative disease, is caused by the progressive loss of midbrain substantia nigra as well as the formation of lewy bodies. Lewy bodies consist of misfolding alpha synuclein, which can induce inflammatory response and increase the release of reactive oxygen species (ROS), thereby damaging other functional neurons. The typical symptoms of Parkinson's disease include resting tremor, muscle rigidity and autonomic dysfunction. These symptoms are fatal to humans and the delivery of drugs to the CNS (central nervous system) as well as PNS (peripheral nervous system) is challenging due to the existence of blood brain barrier (BBB). BBB is composed of microvascular endothelial cells that form tight junctions by occluding and claudins. As a result, it is extremely difficult for substances to penetrate the BBB. Besides, these drugs often exhibit low permeability and poor draggability. Therefore, such drugs possess poor

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efficiency in drug delivery and treatment when not combined with advanced technologies. As a result, it is essential to gain a better understanding of the structure and biological features of BBB, while simultaneously design approaches to deliver drugs across it.

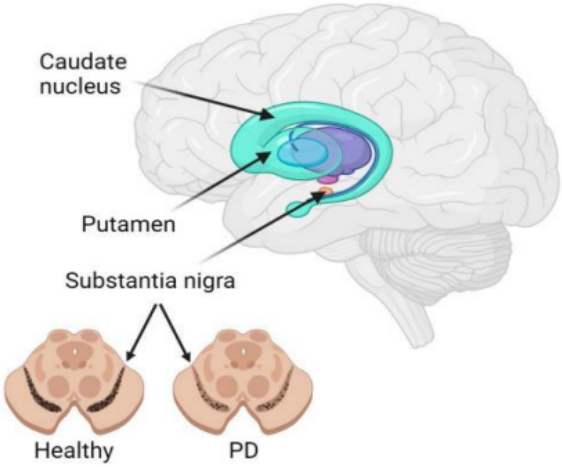


Figure 1. Image of brain with Parkinson's disease. Figure shows the difference between a healthy brain and brain with Parkinson's disease [1]

1.2. Structure of BBB

BBB is composed of a layered structure which consists of endothelial cells, basal membrane, mural cells, pericytes and astrocytic glial cells [2]. There are tight junctions between the endothelial cells, which forms a barrier. This barrier protects the brain from pathogens by limit the entering of large molecules into the brain.

1.2.1. Endothelial cells

Endothelial cells are located on the innermost side of the BBB. They constitute the core anatomic structure of the BBB. These cells exhibit numerous features which are critical to the function of the BBB. (1) Endothelial cells form enclosed structures through tight junctions and adherents' junctions.

This enables the BBB to generate trans endothelial resistance up to 1800 Ω/cm^2. This provides efficient limitation to paracellular transport [3,4]. (2) Endothelial cells possess limited vesicular transport capacity. This is key to reducing the number of solutes entering the central nervous system (CNS) [5]. (3) The interfaces of endothelial cells are negatively charged. This feature allows the cells to repel negatively charged compounds. (4) Endothelial cells have few apertures that allow free diffusion of substances. As a result, fast exchange of molecules between blood and tissue is limited. (5) The expression level of surface leukocyte adhesion molecules is extremely low. In this way, the quantity of immune cells entering the CNS is effectively limited [6].

1.2.2. Basement membrane

The basement membrane is the acellular component of the neurovascular unit. (1) It provides anchor points for its cellular components, thereby maintaining communication between cells and ensuring the stability of the microstructure of vessels. (2) The basement membrane is responsible for the polarization of endothelial cells. This is a crucial step for the correct localization of influx and efflux transporters and the formation of the lumen [7].

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1.2.3. Pericyte

(1) Pericytes are the key structures that control vesicular transport and induce the polarization of astrocytes by inducing Mfsd2a in endothelial cells. This process allows pericytes to guide and induce the adhesion of end-feet to endothelial cells. (2) Vascular pericytes can synthesize components of the extracellular matrix (ECM), including proteoglycans, collagen, elastin, and laminin. Therefore, they control the diameter of the capillaries and, consequently, the volume of blood flow in the brain [8].

1.2.4. Astrocyte

Astrocytes are the major glial cells of the NVU. (1) As shown in the figure 2, their stretching polar cell protrusions enclose the protrusions of the NVU and vessels simultaneously. This enables astrocytes to construct a connection between neuronal circuitry and vasculature, and to play a key role in the dynamic signal transduction of the CNS [9]. (2) Mature astrocytes maintain the stability of the BBB by improving the level of proteins responsible for tight junctions and secreting a variety of factors [9].

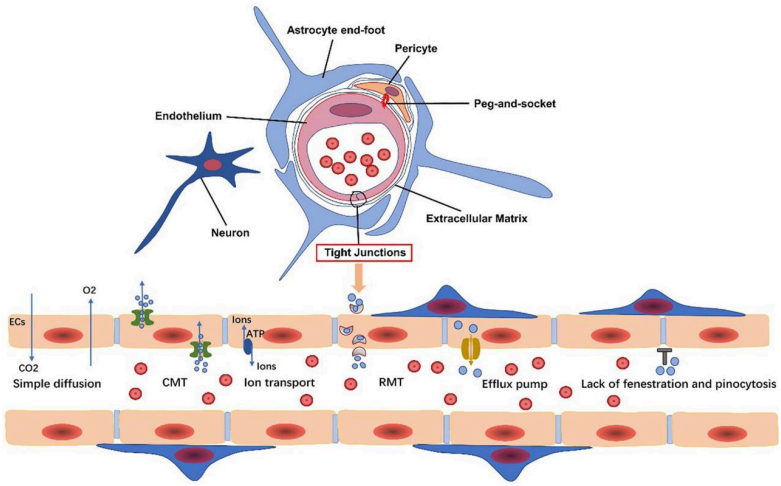


Figure 2. Blood-brain barrier anatomical structure. The upper part of the figure shows the structure of Blood-brain barrier. The lower part of the figure shows the specific structure of the tight junctions of Blood-brain barrier [10]

1.3. Traditional BBB delivery methods and their limitations

1.3.1. Receptor-mediated transcytosis

Receptor-mediated transcytosis involves the binding of ligands to receptors on endothelial cells. Several requirements must be met for this process to occur. (1) Receptors should be exclusively expressed on BBB endothelial cells. (2) Receptors must possess high transport capability mediated by selective ligands. (3) Receptors must be capable of mediate efficient endocytosis. However, to date, no ideal receptors meeting all these requirements have been identified [11].

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1.3.2. Trans nasal drug delivery to brain

Trans nasal drug delivery is an effective method because the olfactory region of the submucous space of the nose is contiguous with the cerebrospinal fluid (CSF) flow tracts around the olfactory lobe [12]. Therefore, drug molecules may enter the brain area directly. Nevertheless, two conditions must be met. (1) Drugs must penetrate nasal epithelial cells and be transported. (2) Drugs must penetrate the arachnoid membrane that separates the submucosal space of the nasal cavity from the cerebrospinal fluid cavity of the olfactory bulb [13]. Small lipophilic molecules can partially enter the cerebrospinal fluid after intranasal administration. However, most macromolecules and water-soluble molecules are not able to enter unless the nasal epithelial barrier is locally damaged. Therefore, the amount of drug delivered through this process is limited to a great extent [14].

1.4. Advantage of ultrasound microbubbles to open BBB

With the advancement of technology, new approaches to open BBB have emerged, one of which relies on ultrasound-controlled microbubbles. Figure 3 shows the process of BBB opening using microbubbles, which involves cavitation. Cavitation refers to the grow and collapse of microbubbles. In a liquid environment, a microbubble nucleus (void) can form. It can oscillate, grow and collapse in the presence of high-intensity ultrasound. The contraction and expansion of microbubbles can generate a push-pull mechanism, which can open the tight junctions of BBB. Furthermore, under high ultrasound energy, microbubbles will break. This produces secondary mechanical process. Both mechanisms can increase the permeability of BBB and allows the delivery of drug into brain [15].

The opened BBB can recover if the cavitation effect is controlled, thereby limiting damage to the brain. Therefore, this BBB-opening approach currently widely used and holds great potential.

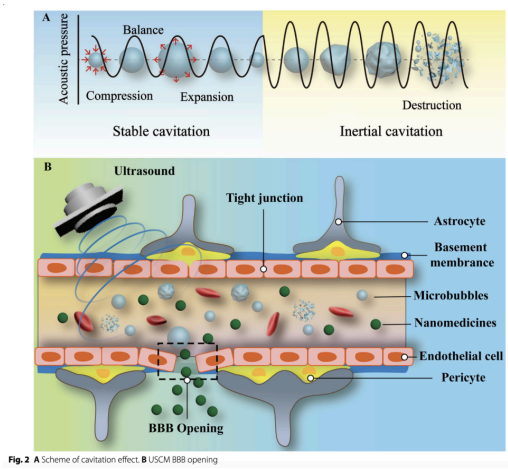


Figure 3. Image of cavitation effect opening BBB. Part A shows two types of cavitation. (1) Non-inertial cavitation in which the microbubbles compress and expand periodically without collapsing. (2) Inertial cavitation in which the microbubbles finally collapse during contraction phase. Part B shows the process of BBB opening. Through the cavitation process controlled by ultrasound, BBB will be successfully opened [15]

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1.4.1. Two types of cavitation

1.4.1.1. Non-inertial cavitation

Cavitation can be defined as the bubble formation. This can be illustrated by imagining the pulling of a piston containing water. When the piston is pulled at high speed, volume increases while temperature remains constant. In this way, liquid rapidly vaporize and bubbles are formed.

Under a low mechanical index, the oscillation of microbubbles is periodic, which means they oscillate symmetrically. Microbubbles expand and contract repeatedly under low sound pressure. During the gas inflow phase, microbubbles expand near the vascular walls, pushing open the endothelial cell layer. During the contraction phase, microbubbles contract, causing invagination of the vascular walls. This push-and-pull mechanism increases the possibility of opening tight junctions.

Expansion and contraction of microbubbles under the field of ultrasound form a fluid model, namely microstreaming. Microstreaming exerts shear stress on endothelial cells with a magnitude of up to thousands of pascals. This destroys the integrity of endothelial cells and thus increases the permeability of the BBB. The process of non-inertial cavitation is driven by the changes in pressure caused by ultrasound [16].

1.4.1.2. Inertial cavitation

Figure 4 shows the interaction of microbubbles with ultrasound, which explains how strong forces are produced by cavitation effects. When the mechanical index exceeds the threshold, microbubbles collapse vigorously during the contraction phase. In this process, the destruction of microbubbles exerts shear stress and releases

shock waves and jetting, together with high temperature and pressure. This process causes not only increased permeability of the endothelial cells but also their destruction, allowing larger drug molecules to enter the BBB. Inertial cavitation is dictated by the inertia of the surrounding fluid [16].

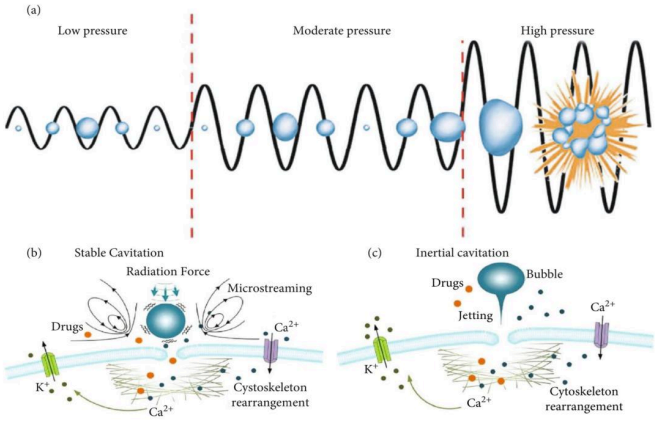


Figure 4. Interaction of microbubbles with ultrasound (a) Under low ultrasound pressure, microbubbles gently expand and shrink in a stable way (stable cavitation). But at higher pressure, they can violently collapse and break apart (inertial cavitation). (b) Stable cavitation, a typical form of non-inertial cavitation, creates forces like pushing and small fluid movements that can affect nearby cells. (c) Inertial cavitation creates strong jet-like forces. These forces can make small holes in cell membranes, change the cell's structure [17]

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1.4.2. Advantages of ultrasound microbubbles

As mentioned above, ultrasound microbubbles can induce two types of cavitation. Non-inertial cavitation is a milder process that increases the permeability of endothelial cells without causing structure damage. In contrast, inertial cavitation is much more aggressive, producing shock waves and microjets that can significantly disrupt endothelial structures, thereby forming larger channels for drug entry. Therefore, ultrasound microbubbles can regulate the size of the openings and the extent of BBB permeability. In this way, drug molecules of any size have the potential and possibility to cross the BBB and carry out treatments for neurological diseases.

1.4.3. Applications of ultrasound microbubbles

1.4.3.1. Ultrasound microbubbles in treating Parkinson's disease

Clinical applications of ultrasound microbubbles in treating Parkinson's disease have been reported. The process involves using ultrasound microbubbles to transiently open the BBB, followed by administration levodopa (L-Dopa). However, L-Dopa treatment may cause complications. To address these, several inhibitors, including catechol-O-methyl transferase (COMT) inhibitors, monoamine oxidase inhibitors (MAO-Is), and dopamine agonist inhibitors, are often co-administered [18-20].

1.4.3.2. Ultrasound microbubbles treating Alzheimer's disease

Two pathological proteins, amyloid-beta (Aβ) and Tau, are considered major contributors to Alzheimer's disease. Scanning ultrasound microbubbles were used in an experiment carried out over 5–8 weeks on middle-aged APP23 mice. Results showed that 75% of the mice exhibited plaque clearance, indicating removal of Aβ. This occurred by activating dormant brain microglia that took up the amyloid into their lysosomes and digested it [21].

In another study, scanning ultrasound microbubbles combined with a variety of Tau antibodies facilitated antibody removal into the brain, reducing Tau pathology in K3 and pR5 mice [22-25].

1.4.4. Common materials used for ultrasound microbubbles

The material commonly used for making microbubbles is nanomaterials. Among nanomaterials, nanoparticles are most frequently used for drug delivery across the blood–brain barrier (BBB). Nanoparticles are typically 1-100 nm in diameter. Nanoparticles are usually used as carriers of drug after ultrasound opened BBB. This field has attracted extensive research attention. Nanocarriers are used to treat many different diseases. They can carry drugs and release them into distinct targeted tissues, so that other parts of the body will not be affected by the drugs [26]. In addition, nanoparticles enable controlled drug release, which can enhance therapeutic efficacy [27].

In most approaches to deliver drug across BBB, microbubbles are either loaded with nanoparticles or administered separately in combination with drug-loaded nanoparticles. In the first approach, the outer of microbubbles are loaded with nanoparticles combined with drugs. In the second one, microbubbles and nanoparticles with drugs are injected separately.

This review will focus on introducing nanoparticles used together with microbubbles for drug delivery across BBB. Two broad types of nanoparticles are commonly used, organic and inorganic [28]. They are nanoparticles commonly used in drug delivery across BBB. Organic nanoparticles and inorganic nanoparticles have big differences. Table 1. below concluded different properties of

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organic and inorganic nanoparticles. The stated properties include both biological and physical properties.

Table 1. Comparison between organic and inorganic nanoparticles. The left row are the properties of organic nanoparticles and the right row are properties of inorganic nanoparticles [29]

Organic nanoparticles	Inorganic nanoparticles
Biodegradable	Ease of surface functionalization
Biocompatible	Stability enhanced
Non-toxic	Improved magnetic properties
Non-stable	Toxic
Low reproducibility rate	Low biodegradability
Drug entrapment efficiency issue	Less biocompatible

2. Discussion

2.1. Gold nanoparticles

In the previous part, the mechanism of using ultrasound and microbubbles to increase the blood-brain barrier permeability was introduced. While temporary opening the BBB to permit drug entry is important in the central nervous system (CNS) disorders treatment, it is equally essential to consider strategies for delivering and distributing drugs within the CNS.

2.1.1. Gold nanoparticles properties

As introduced before, nanoparticles are used as the drug carriers. This section will focus on one specific type of inorganic nanoparticles. Inorganic nanoparticles can be easily functionalized and highly stable, which makes them a suitable nanocarriers for drugs. Many inorganic nanoparticles -- such as silver, copper, and gold, as well as the corresponding metal--are used in drug delivery. However, among all of them, gold nanoparticles have outstanding performance due to their six special properties as showed in Table 2. below. Therefore, AuNPs is the focus of this part.

Table 2. Properties of gold nanoparticles [29]

Easily produced	Surface coating	Biocompatible
Good uptake	High stability	Good loading capacity

2.1.2. Applications

To treat any CNS diseases, diagnosis is always the first step. There are different types of diagnosis method that involves the use of AuNPs, but the only one related to ultrasound is photo acoustic imaging (PAI). In PAI, AuNPs are using as contrast agents due their strong optical absorption ability. As shown in Figure 5, during diagnosis, contrast agent absorbs energy from the pulsed laser. Heat generated causes thermal expansion and produces pressure waves, which can be detected by a transducer [30].

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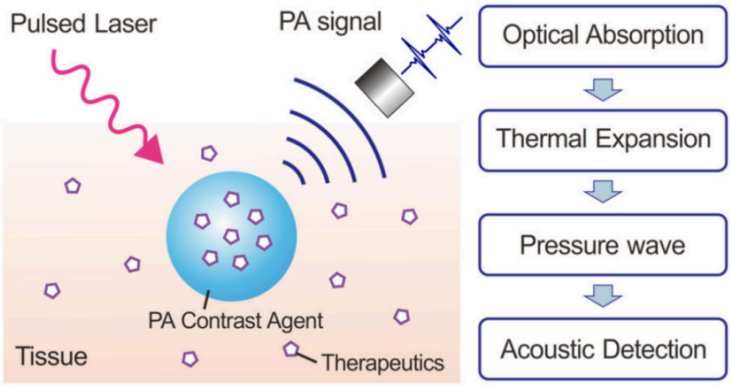


Figure 5. Mechanism of photoacoustic imaging. Laser is first absorbed by the PA contrast agent, which causes thermal expansion of agents. Then, pressure waves are produced and detected by transducer [30]

Other than diagnosis, AuNPs, as a nanocarrier, can also be used for drug delivery across the BBB. AuNPs can not only pass through BBB, but also be combined with ultrasound and microbubbles, which would increase the penetration number significantly [31]. There are different systems for the delivery of drug using AuNPs with microbubbles but only few of them actually combine AuNPs with microbubbles to form a conjugate. The more common approach used is to inject AuNPs with microbubbles separately [32]. In the second approach, microbubbles are first injected into the bloodstream. Focused ultrasound (FUS) then induces nonoperation via the microbubbles. This increases the permeability of BBB and the AuNPs injected next can move through BBB.

One example used a cisplatin–gold nanoparticle conjugate (GNP–Cis) with magnetic resonance–guided focused ultrasound (MRgFUS). MRgFUS was first combined with microbubbles injected into bloodstream to increase BBB permeability. GNP–Cis was then injected and crossed the BBB to reach the brain. The result indicated that conjugating cisplatin to AuNPs increased the amout of drug delivered [31].

Another study compared the number of AuNPs coated with PEG that penetrate the BBB with and without MRgFUS across BBB. The procedure was similar to that in the previous research. Results from this experiment showed that with MRgFUS, the AuNPs content in the brain was much higher than without MRgFUS [33].

A third study examined BBB penetration of DNA–Au nanoparticles of different sizes using ultrasound, which showed that DNA-Au nanoparticle of size 6.3±0.7nm exhibited the best penetration [34]. Collectively, these experiments showed that gold nanoparticles combining with microbubbles and ultrasound can carry more drugs across BBB than other approaches.

After the AuNPs are delivered to the brain, it is important to release the drugs. Figure 6. shows two main different methods of drug release, one is intrinsic and another is extrinsic. Intrinsic method mainly depends on the biological stimuli inside the targeted tissue or body parts. Different from intrinsic method, extrinsic method depends only on the external stimuli. Drugs release are controlled by light and ultrasound [35].

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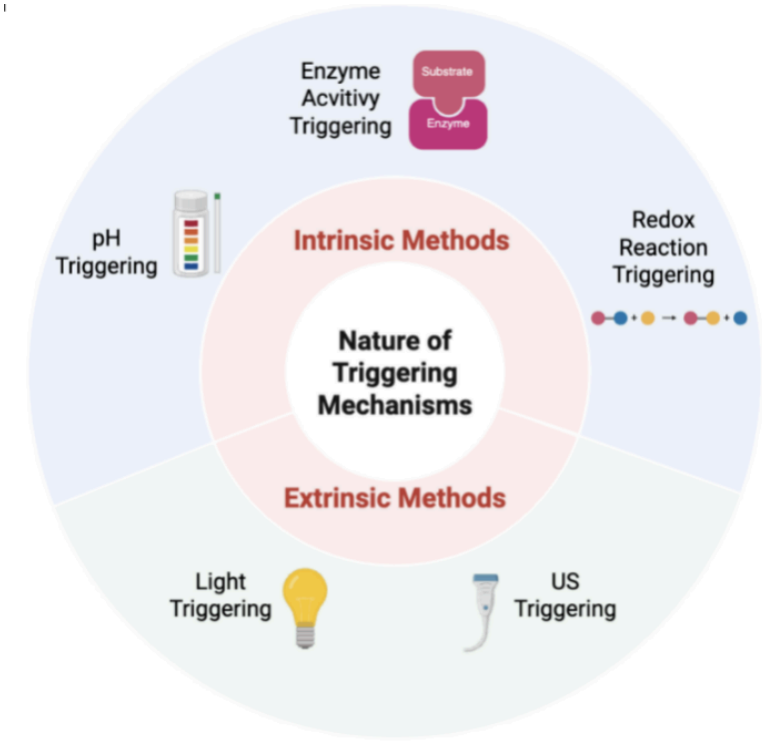


Figure 6. Types of drug release triggering methods. Intrinsic Method: pH triggering, enzyme activity triggering and redox reaction triggering. Extrinsic Methods: light triggering and ultrasound triggering

There are a few challenges for the application of this approach in human body. On challenge concerns determining particle size. AuNPs with size smaller than 5.5nm is renal clearable [36] and AuNPs with size smaller than 40nm have longer blood circulation time [37]. Therefore, there are many factors that need to be considered when designing the AuNPs

Furthermore, the toxicity of AuNPs is also a critical concern in application. The toxicity of AuNPs depends on the size, surface charge shape and surface chemistry. Experiments shows that larger AuNPs causes higher mortality rate than smaller ones [38]. In addition, AuNPs that have surface positively charged are more toxic than negatively charged [39]. AuNPs with higher toxicity can damage human body. Thus, AuNPs used to deliver drug need to have the lowest toxicity to minimize the adverse effect of this approach of drug delivery.

2.2. Polymers

2.2.1. The introduction and relative issue about BBB

The blood-brain-barrier (BBB) serves as the interface between systemic circulation and the central nervous system (CNS). Specifically, BBB is a barrier as a combination of the capillary vessels. As these fine vessels, under physiological condition, the BBB can inhibit the passage to administered the drug molecular into the CNS, while inhibited the disease cells into it. Normally, BBB allow only highly lipophilic and relatively small molecules into the CNS. Thus, polymers have different type of functions as approaches to drive drug molecules easier though BBB, like the use of hyaluronic acid (HA) can combine with receptors in the surface of BBB to achieve targeting delivery.

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For some brain diseases and tumors, opening the BBB is necessary for effective treatment. An example is Glioblastoma multiforme (GBM), one of the most common and deadly primary brain tumors.

Even the patients with treatment only have 12 to 14 months of survival period. Temozolomide (TMZ) is the standard chemotherapeutic agent for GBM [40], but its efficacy is limited by the BBB; combining TMZ with polymeric carriers can improve delivery and inhibit tumor growth.

Therefore, some composite or special polymers can pass through the BBB together with therapeutic drugs by wrapping them up. They can also be chemically modified to adjust their molecular structure, or bind to certain receptors. This way, they can extend the time the drugs are released in the human body, improve the targeted delivery of the drugs, and make them more easily recognized by the BBB.

2.2.2. Experiments and the compare of different polymers

HA has low toxicity and, through receptor-specific binding to CD44 on the surface of BBB endothelial cells, enters the CNS via receptor-mediated endocytosis without disrupting BBB structure. Its favorable safety profile highlights the potential of HA for long-term administration in chronic brain diseases.

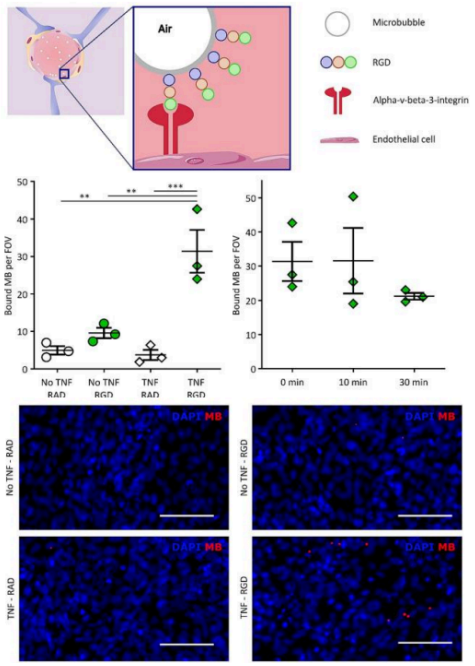


Figure 7. Binding of RGD-MB to inflamed endothelial under flow condition. In figure (a): Schematic diagram of the binding between RGD-modified microbubbles (RGD-MB) and integrin receptors on endothelial cells; In figure (b): the binding amount of RGD-MB in the inflamed group is significantly higher than that in the control group; In figure (c): MB bound to inflamed endothelium were placed in an environment with capillary flow speeds and incubated for 0, 10, or 30 minutes respectively, followed by observation; In figure (d): Specific binding of RGD-MB to inflamed cells under fluorescence microscopy [41]

PHPMA is a kind of polymer that has the ability of controllable opening through physical and chemical synergy. As shown in figure 7, RGD-modified microbubbles (RGD-MB) can specifically bind to inflamed endothelial cells — a property that can be combined with polymer-mediated BBB

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opening strategies. In one study, by the cavitation effect of microbubbles and ultrasound, the BBB is opened, and the experimental data is measured by trans endothelial electrical resistance (TEER), which measures the electrical resistance across a cell monolayer: a small current is applied and the voltage is recorded to calculate resistance. Lower TEER values indicate weaker tight junctions and higher BBB permeability.

In the experiment, ultrasound triggering of fluorescently labeled PHPMA led to a decrease in TEER, indicating a transient increase in BBB permeability. On the contrary, this temporary opening can avoid permanent damage to BBB, and the timing of drug release can be accurately controlled by adjusting the parameters of ultrasound, which is more controllable. Also, the penetration amount is positively correlated with the change range of TEER. For example, when the ultrasound power is 0.5MPa, TEER decreases by 30%, and the fluorescence intensity increases by 2.1 times as shown in Figure 8 [41].

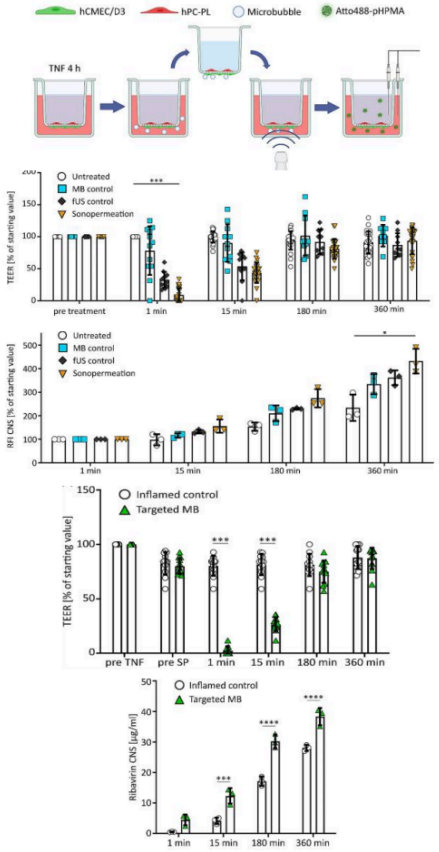


Figure 8. Sono permeation (in vitro BBB) and RGD-MB-induced son permeation (inflamed BBB)

In figure (a): use TNF to induce an inflamed blood-brain barrier (BBB) model, then add microbubbles (MB) and Atto488-pHPMA, and finally treat with focused ultrasound (FUS); In figure (b): the BBB trans endothelial electrical resistance (TEER) ratios of the untreated, MB control, FUS control, and Sono permeation groups at different time points, which intuitively shows the impact of each group on BBB barrier function; In figure (c): The value of Atto488-pHPMA nanocarriers passing through the BBB into the CNS was determined by fluorescence intensity. After 360 minutes, the son permeation (SP) treatment group showed a significant difference from all control groups; In figure (d): Compares BBB-TEER ratios of inflamed control and targeted MB groups after and begin TNF and son permeation, observing targeted MB's effect on inflamed BBB opening; In figure 5: the

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detection values of the inflamed control group and the targeted MB group at different time points, which can reflect the changes in substance levels in the BBB after son permeation treatment.

PEG-b-PMBSH [42] has the ability to target and enhance BTB penetration. The system of boron polymer combined with microbubbles and focused ultrasound (FUS) [43,44]: it uses the adsorption of cationic microbubbles with tumor vascular endothelium, and combines FUS to locally open the blood-brain tumor barrier (BTB), so as to realize the concentrated distribution of boron drugs in GBM. We can compare its similarities with PHPMA (both rely on ultrasound-microbubble synergy) and differences (it targets the tumor microenvironment, more suitable for local treatment of brain tumors).

2.2.3. Limitations and challenges

The receptor binding of HA may be affected by the tumor microenvironment (like, high expression of CD44 in GBM may enhance the binding, but low expression in healthy brain tissue may reduce efficiency); PHPMA and PEG-b-PMBSH rely on ultrasound equipment, their penetration depth and positioning accuracy may decrease in large animal models.

In experiments, the in vitro BBB model can't fully simulate the in vivo hemodynamics and neural regulation. TEER measurement only reflects the integrity of tight junctions, need to combine with other indicators to evaluate together.

Explore the combination of HA's receptor targeting and ultrasound-microbubble controllable opening (like HA-modified PHPMA), to balance safety and

For PHPMA, can optimize ultrasound parameters (such as focusing depth) to adapt different brain

Refer to the prospect in the second document, promote the application of ultrasound equipment and clinical treatment (like chemotherapy), solve the problem of difference in ultrasound penetration efficiency between human body and animal models.

2.2.4. Conclusion

In general, three polymers—hyaluronic acid (HA), PHPMA, and PEG-b-PMBSH—provide a range of treatment options for crossing the blood-brain barrier (BBB) or blood-tumor barrier (BTB). HA achieve low-toxicity delivery through receptor mediation, PHPMA enable controllable opening of the BBB via ultrasound-microbubble synergy, and PEG-b-PMBSH target the tumor microenvironment to enhance local drug accumulation. Experimental data has all confirmed their potential. Although there are problems such as affected by the tumor microenvironment, relying on ultrasound equipment, and limitations of in vitro models, the three polymers respectively provide new ideas for drug delivery in brain diseases (such as glioblastoma) from the perspectives of physiology, controllability, and targeting, and expected to promote clinical translation and application in the future.

2.3. Lipid

Curcumin is a polyphenol compound extracted from the rhizoma and has shown beneficial effects in treating neurological disorders. Several studies are carried out which corroborate the antioxidant property of curcumin, which would help clear out the reactive oxygen species (ROS) in the brain. In addition, curcumin can also combine with the alpha synuclein by hydrophobic interaction so the lewy body can swiftly target by phagocytes and become easier to be cleared out. Nevertheless,

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curcumin has some defects such as low bio-availability and poor solubility. To increase its solubility, the melt-crystallization method is used: curcumin is mixed with poly (ethylene glycol) (PEG) and heated above its melting point, then rapidly quenched (e.g., liquid nitrogen) to form a solid dispersion. PEG is a hydrophilic polymer that disrupts curcumin crystallization and maintains it in an amorphous state. As shown in the figure 9, the Lipid microbubbles are then made by double emulsion evaporation process. The brief process is to mix chemicals including distilled water, ammonium carbonate, PLGA, DSPC, PEG, DCL and follow by emulsification, homogenization as well as centrifugation. After this process, hydrogen bond or hydrophobic interaction with the curcumin is formed in the microbubble. The hydrophobic functional groups of the curcumin will then be masked and the hydrophilic character of curcumin will increase [45].

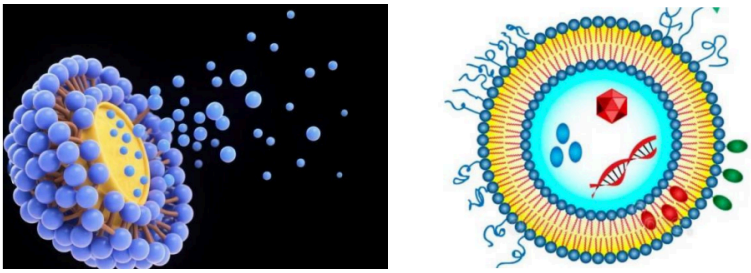


Figure 9. The structure of lipid microbubbles [46]

Besides, if more precise target of the brain is required, receptor molecules can be conjugated to the lipid shell. There are generally two ways of modification including the biotin-avidin linking and a direct covalent bond. In biotin-avidin technique, the bio-tinplated ligand is coupled to a bio-tinplated microbubble via an avidin bridge [47]. However, the immunogenicity of avidin limits translation to humans, making the method of using covalent bond a more desirable choice. Certain intensity of LIFU (low-intensity focused ultrasound) can precisely induce stable cavitation inside the brain. Furthermore, a more advanced method of treatment are discovered. A Liposome can be used to transport mRNA to target parts in the brain by surrounding the mRNA inside the microbubble. Enzymes including degrading enzymes as well as inflammatory enzymes which cannot be made inside the brain or passed through the BBB originally can then be delivered through the BBB by combining with mRNA and Ultrasound induced liposome bubbles. During the experiment, the head of the experimental mice will be placed on ultrasonic gel and irradiated by certain intensity of ultrasound. High intensity focused ultrasound is often used to temporarily open the blood brain barrier while delivering mRNA. The Evans blue extravasation as well as the luciferase protein expression (code by mRNA) can be used to identify the extent of BBB opening [48]. The experimental data shows that both extravasation of Evan's blue and expression of the luciferase protein increases within the first three hours after exposing under the focused ultrasound and then gradually deceases. This phenomenon represents the success in temporarily opening the blood brain barrier to provide enough time for substances including drugs to penetrate the BBB. As shown in figure 10, the experiment found out that high intensity focused ultrasound at about 1.5kw/cm^2 is the most efficient to open the BBB without harming other parts of the mice brain (no hemorrhage or edema). This approach and method achieve exogenous protein expression which is more effective in producing certain proteins used to resist neurodegenerative diseases such as Parkinson's disease [49].

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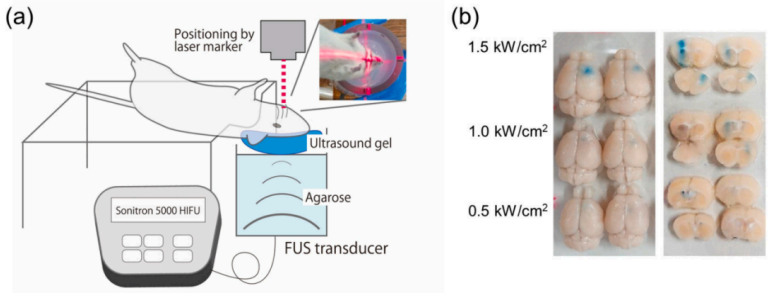


Figure 10. (a) is the procedure of the experiment. The head of the mice was placed on ultrasonic gel. Laser marker is used to position the mice head. It is then irradiated by ultrasound with various intensities using FUS transducer. (b) is the data of the experiment. The brain of mice under different intensities of ultrasound including 0.5kW/cm2, 1.0kW/cm2 and 1.5kW/cm2. Under different intensity, the opening of BBB is distinct [48]

To sum up, the curcumin treatment by using the melt-crystallization method and lipid microbubbles greatly improved the solubility of curcumin which then increased its bio-availability as well as cavitation stability. This approach raises the opportunity to successfully treat neurodegenerative diseases like Parkinson's disease as well as Alzheimer's disease. It acts as a model to provide a platform for other drugs to develop new ways of treating CNS more effectively. Drug-loaded lipid-shelled microbubbles prepared via the melt-crystallization route have stiffer shells than conventional lipid microbubbles. These characteristics make it more stable and easier to be monitored in the brain, as indicates by its stable emission of harmonic waves. Microbubbles perform best under the high-intensity focused ultrasound when frequency is around 3.5 MHz and the intensity is 1.5kw/cm^2. At these settings, cavitation-related damage to brain tissue is reduced. The research also found out that higher cavitation doses increase the probability that the drug will be delivered through the BBB more effectively in a greater amount. Furthermore, the combination of lipids surrounding microbubbles as well as the focused ultrasound radiation augments the permeability of BBB so it can open temporarily to allow mRNA passing through. As a result, exogenous protein expression in the brain parenchyma increases significantly.

Using lipids combining microbubbles and liposome to deliver substances through the BBB has remarkable achievement in the clinical experiments. However, SEM/TEM imaging of microbubbles remains challenging, because dehydration and freezing can distort their native size and shape. Besides, when designing or marking the surface of the lipids surrounding microbubbles, it is hard to confine the final target into a short area and it impose potential hazards on surrounding tissues and cells. It is necessary to have more experiments on how to maximize the targeting of microbubbles to treat neurodegenerative diseases by coupling with receptors like glycolipids and glycoproteins. In addition, it is crucial to ameliorate medical diagnosis and analysis while opening the BBB to prevent undesirable microorganisms or drugs passing through. It is promising to discover more chemicals and drugs that can combine with focused ultrasound as well as lipids microbubbles to treat neurodegenerative diseases.

3. Future outlook

Although ultrasound microbubble technology is currently acknowledged to be a method that is effective for BBB opening and is applied in clinical treatment, this method still faces challenges. For certain disease treatments, inertial cavitation is applied for the entry of macromolecular drugs or large amounts of drugs. However, the destruction caused by inertial cavitation takes a long period of

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time to recover. Meanwhile, inflammation can occur. Therefore, looking for solutions for destruction and inflammation is crucial in the future.

Moreover, achieving precise localization can be difficult for ultrasound, as the network of the BBB is complex. If ultrasound is generated at locations that are not affected by disease, additional destruction and injury can occur. So, precise localization will have to be achieved.

4. Conclusion

In conclusion, ultrasound enhance drug delivery across BBB include mainly two steps. The first step is to inject microbubbles into bloodstream and expose them to focused ultrasound, which causes sonoporation. This increases the permeability of BBB and allows larger molecules to pass through. The second step is to inject nanoparticles that carries drug into the body and let them enter the brain across BBB. Therefore, drugs are successfully delivered into the brain and can be used to treat CNS diseases. Nanoparticles or nanocarriers that are made up of gold, polymer and lipids can all be used as drug carriers.

However, there are still some challenges for this approach that needs to be overcome in the future. First of all, this way of delivery can cause tissue damage and even neurological deficit if treatments are done many times [50]. In addition, focused ultrasound with high intensity can cause local tissue damage and other side effects. Therefore, more tests experiments need to be done before applying this approach in clinical field and protocols need to be set to ensure the safety [51].

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