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REVIEW ARTICLE

Nanotechnology in Blood Brain Barrier: A Review

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ABSTRACT

Delivery of drugs to brain is still a major challenge. Successful delivery across the blood brain barrier has only been achieved in some cases, for example pro-drugs; alternative approach is delivery to the brain by using nanoparticles. Nanotechnology is expected to bring revolutionary changes in the field of life sciences including drug delivery, diagnostics, neutraceuticals and production of biomaterials. Liposomes can also be used for delivery of drug to the brain through the blood brain barrier (BBB). The review focuses on blood brain barrier, nanoparticles and their preparation, and mechanism of nanoparticles mediated drug transfer across blood brain barrier, application of nanoparticles as biomarkers, and liposomes and their manufacturing.

Keywords: Nanotechnology, Blood brain barrier, Polymer, Nanoparticles.

INTRODUCTION

The challenge in treating most brain disorders is overcoming the difficulty of delivering therapeutic agents to specific regions of the brain by crossing the blood-brain barrier (BBB). This barrier – a tight seal of endothelial cells that lines the blood vessels in the brain – is a physiological checkpoint that selectively allows the entry of certain molecules from blood circulation into the brain. The problem for scientists is that the BBB does not differentiate what it keeps out. BBB strictly limits transport into the brain through both physical (tight junctions) and metabolic (enzymes) barriers.

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With very few exceptions, only nonionic and low molecular weight molecules soluble in fat clear the BBB. For instance, alcohol, caffeine, nicotine and antidepressants meet these criteria [1]. However, large molecules needed to deliver drugs do not. Thus, while the BBB naturally evolved in order to protect the brain from invasion of various circulating toxins and other harmful molecules, it also serves as a major impediment towards the brain-specific delivery of various diagnostic/therapeutic molecules needed for combating various neuronal disorders.

The blood brain barrier represents a formidable obstacle for a large number of drugs, including anticancer agents, peptides and nucleic acids. As consequence this barrier effective treatment of many severe and life

threatening diseases such as brain tumors, Alzheimer's disease, Parkinson's disease and other neurological disorders [2].

BLOOD BRAIN BARRIER

The blood brain barrier (BBB) is a separation of circulating blood and cerebrospinal fluid (CSF) maintained by the choroid plexus in the central nervous system (CNS). Endothelial cells restrict the diffusion of microscopic objects (bacteria) and large or hydrophilic molecules into the CSF, while allowing the diffusion of small hydrophobic molecules (O_2 hormone and CO_2). Cells of the barrier actively transport metabolic products such as glucose across the barrier with specific proteins.

The blood brain barrier in its original meaning is formed by a complex system of endothelial cells, pericytes as well as basal lamina disconnecting the cellular system. In addition to its physical barrier properties the blood brain barrier is considered to be a metabolic barrier. While immunological properties are displayed by endothelial cells together with the surface molecules, which play a key role in pathological conditions such as inflammation, tumor, and angiogenesis and wound healing [3, 4].

Many attempts have been made to overcome the blood brain barrier to transport drugs across it. The most frequent and successful attempts entail chemical modification of drugs or opening of blood brain barrier by osmotic methods. Liposomes were also investigated to deliver drugs to the brain. An alternative approach is the employment of the nanoparticles. They consist of macromolecule materials in which the active principle is dissolved, entrapped, encapsulated or to which the active principle is absorbed or attached. Nanoparticles can be used therapeutically, as drug carriers or as adjuvant in vaccines [5, 6-7].

This "barrier" results from the selectivity of the tight junctions between endothelial cells in CNS vessels that restricts the passage of solutes. At the interface between blood and brain, endothelial cells and associated astrocytes are stitched together by these tight junctions, which are composed of smaller subunits, frequently dimers, that are transmembrane proteins such as occludin, claudins, junctional adhesion molecule (JAM), ESAM and others. Each of these transmembrane proteins is anchored into the endothelial cells by another protein complex that includes zo-1 and associated proteins.

The blood-brain barrier is composed of high density cells restricting passage of substances from the bloodstream much more than endothelial cells in capillaries elsewhere in the body. Astrocyte cell projections called astrocytic feet (also known as "glia limitans") surround the endothelial cells of the BBB, providing biochemical support to those cells. The BBB is distinct from the similar blood-cerebrospinal fluid barrier, a function of the choroidal cells of the choroid plexus, and from the blood-retinal barrier, which can be considered a part of the whole [8, 9 - 10].

HISTORY

Paul Ehrlich was a bacteriologist studying staining, used for many studies to make fine structures visible. When he injected some of these dyes (notably the aniline dyes that were then popular), the dye would stain all of the organs of an animal except the brain. At the time, Ehrlich attributed this to the brain simply not picking up as much of the dye.

However, in a later experiment in 1913, Edwin Goldmann (one of Ehrlich's students) injected the dye into the spinal fluid of the brain directly. He found that in this case the brain would become dyed, but the rest of the body would not. This clearly demonstrated the existence of some sort of compartmentalization between the two. At the time, it was thought that the blood vessels themselves were responsible for the barrier, as no obvious membrane could be found. The concept of the blood-brain barrier (then termed hematoencephalic barrier) was proposed by Lina Stern in 1921. It was not until the introduction of the scanning electron microscope to the

medical research fields in the 1960s that the actual membrane could be demonstrated.

PATHOPHYSIOLOGY

The blood-brain barrier acts very effectively to protect the brain from many common bacterial infections. Thus, infections of the brain are very rare. However, since antibodies are too large to cross the blood-brain barrier, infections of the brain that do occur are often very serious and difficult to treat. The blood brain barrier becomes more permeable during inflammation however, meaning some antibiotics can get across. Viruses easily bypass the blood-brain barrier by attaching themselves to circulating immune cells.

An exception to the bacterial exclusion are the diseases caused by spirochetes, such as Borrelia, which causes Lyme disease, and treponema pallidum, which causes syphilis. The bacteria seem to breach the barrier by physically tunneling through the blood vessel walls [11, 12].

DRUGS TARGETING THE BRAIN

Overcoming the difficulty of delivering therapeutic agents to specific regions of the brain presents a major challenge to treatment of most brain disorders. In its neuroprotective role, the blood-brain barrier functions to hinder the delivery of many potentially important diagnostic and therapeutic agents to the brain. Therapeutic molecules and genes that might otherwise be effective in diagnosis and therapy do not cross the BBB in adequate amounts.

Mechanisms for drug targeting in the brain involve going either "through" or "behind" the BBB. Modalities for drug delivery through the BBB entail its disruption by osmotic means, biochemically by the use of vasoactive substances such as bradykinin, or even by localized exposure to high-intensity focused ultrasound (HIFU). Other strategies to go through the BBB may entail the use of endogenous transport systems, including carrier-mediated transporters such as glucose and amino acid carriers, receptor-mediated transcytosis for insulin or transferrin, and blocking of active efflux transporters such as p-glycoprotein. Strategies for drug delivery behind the BBB include intracerebral implantation and convection-enhanced distribution [13, 14 - 15].

NANOPARTICLES

Nanotechnology may also help in the transfer of drugs across the BBB. Recently, researchers have been trying to build liposomes loaded with nanoparticles to gain access through the BBB. Delivering drugs across the blood brain barrier is one of the most promising applications of nanotechnology in clinical neuroscience. Nanoparticles could potentially carry out multiple tasks in a predefined sequence, which is very important in the delivery of drugs across the blood brain barrier.

Nanoparticles were first developed around 1970. They were initially devised as carriers for vaccines & anticancer drugs. Simultaneously the use of nanoparticles for ophthalmic and oral delivery was investigated.

A significant amount of research in this area has been spent exploring methods of nanoparticle – mediated delivery of antineoplastic drugs to tumors in the central nervous system. For example, radio-labeled polyethylene glycol coated hexadecylcyano acrylate nanospheres targeted and accumulated in a rat gliosarcoma. This method is not yet ready for clinical trials due to the accumulation of the nanospheres in surrounding healthy tissue [16, 17, and 18].

It should be noted that vascular endothelial cells and associated parasites are often abnormal in tumors and that the blood-brain barrier may not always be intact in brain tumors. Also, the basement membrane is sometimes incomplete. Other factors, such as astrocytes, may contribute to the resistance of brain tumors to therapy.

In nanotechnology, a particle is defined as a small object that behaves as a whole unit in terms of its transport and properties. It is further classified according

to size: in terms of diameter, fine particles cover a range between 100 and 2500 nanometers, while ultrafine particles, on the other hand, are sized between 1 and 100 nanometers. Similar to ultrafine particles, nanoparticles are sized between 1 and 100 nanometers. Nanoparticles may or may not exhibit size-related properties that differ significantly from those observed in fine particles or bulk materials. Although the size of most molecules would fit into the above outline, individual molecules are usually not referred to as nanoparticles.

Nanoclusters have at least one dimension between 1 and 10 nanometers and a narrow size distribution. Nanopowders are agglomerates of ultrafine particles, nanoparticles, or nanoclusters. Nanometer-sized single crystals, or single-domain ultrafine particles, are often referred to as nanocrystals. Nanoparticle research is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields [19, 20 - 21].

Properties

Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. A bulk material should have constant physical properties regardless of its size, but at the nano-scale this is often not the case where size-dependent properties are often observed. Thus, the properties of materials change as their size approaches the nanoscale and as the percentage of atoms at the surface of a material becomes significant. For bulk materials larger than one micrometer (or micron), the percentage of atoms at the surface is insignificant in relation to the number of atoms in the bulk of the material. The interesting and sometimes unexpected properties of nanoparticles are therefore largely due to the large surface area of the material, which dominates the contributions made by the small bulk of the material.

Synthesis

There are several methods for creating nanoparticles, including both attrition and pyrolysis. In attrition, macro or micro scale particles are ground in a ball mill, a planetary ball mill, or other size reducing mechanism. The resulting particles are air classified to recover nanoparticles. In pyrolysis, a vaporous precursor (liquid or gas) is forced through an orifice at high pressure and burned. The resulting solid (a version of soot) is air classified to recover oxide particles from by-product gases. Pyrolysis often results in aggregates and agglomerates rather than singleton primary particles.

Thermal plasma can also deliver the energy necessary to cause evaporation of small micrometer size particles. The thermal plasma temperatures are in the order of 10,000 K, so that solid powder easily evaporates. Nanoparticles are formed upon cooling while exiting the plasma region. The main types of the thermal plasma torches used to produce nanoparticles are dc plasma jet, dc arc plasma and radio frequency (RF) induction plasmas. In the arc plasma reactors, the energy necessary for evaporation and reaction is provided by an electric arc, which is formed between the anode and the cathode. For example, silica sand can be vaporized with arc plasma at atmospheric pressure. The resulting mixture of plasma gas and silica vapor can be rapidly cooled by quenching with oxygen, thus ensuring the quality of the fumed silica produced. In RF induction plasma torches, energy coupling to the plasma is accomplished through the electromagnetic field generated by the induction coil. The plasma gas does not come in contact with electrodes, thus eliminating possible sources of contamination and allowing the operation of such plasma torches with a wide range of gases including inert, reducing, oxidizing and other corrosive atmospheres [22, 23 - 24].

Inert-gas condensation is frequently used to make nanoparticles from metals with low melting points. The metal is vaporized in a vacuum chamber and then super-cooled with an inert gas stream. The super-cooled metal vapor condenses in to nanometer-sized

particles, which can be entrained in the inert gas stream and deposited on a substrate or studied in situ.

Characterization

Nanoparticle characterization is necessary to establish understanding and control of nanoparticle synthesis and applications. Characterization is done by using a variety of different techniques, mainly drawn from materials science. Common techniques are electron microscopy (TEM, SEM), atomic force microscopy (AFM), dynamic light scattering (DLS), x-ray photoelectron spectroscopy (XPS), powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), matrix-assisted laser desorption / ionization time-of-flight mass spectrometry (MALDI-TOF), U.V Spectroscopy, dual polarization interferometry and nuclear magnetic resonance (NMR).

Whilst the theory has been known for over a century (see Robert Brown), the technology for Nanoparticle tracking analysis (NTA) allows direct tracking of the Brownian motion and this method therefore allows the sizing of individual nanoparticles in solution [25,26].

Safety

Nanoparticles present possible dangers, both medically and environmentally. Most of these are due to the high surface to volume ratio, which can make the particles very reactive or catalytic. They are also able to pass through cell membranes in organisms, and their interactions with biological systems are relatively unknown. However, free nanoparticles in the environment quickly tend to agglomerate and thus leave the nano-regime, and nature itself presents many nanoparticles to which organisms on earth may have evolved immunity.

Applications

An Important area of application of nanotechnology includes novel drug delivery techniques, which are quicker and less risky, compared to the cost of developing new drugs. A large number of drugs taken through oral route are destroyed by the gastric fluids and in the liver, then distributed throughout the body, despite the fact that optimal effects would arise from focusing the drug to target organ. Targeted drug delivery by the nanoparticles has the potential to overcome some of these problems, and render treatment more effective, ensuring cost & safety benefits.

The reason why nanoparticles are attractive for such purposes is based on their importance and unique features, some of these features are mentioned below:

1. Their surface to mass ratio is much larger than other particles which promotes catalytic activity.
2. Due to their small size nanoparticles penetrates small capillaries and are taken up by the cells, which allows for efficient accumulation of drug at target organ.
3. The use of biodegradable materials for nanoparticle preparation allows for sustained drug release at the target organ over a period of days or even weeks after injected.

LIPOSOMES

A liposome is a tiny bubble (vesicle), made out of the same material as a cell membrane. Liposomes can be filled with drugs, and used to deliver drugs for cancer and other diseases.

Membranes are usually made of phospholipids, which are molecules that have a head group and a tail group. The head is attracted to water, and the tail, which is made of a long hydrocarbon chain, is repelled by water.

In nature, phospholipids are found in stable membranes composed of two layers (a bilayer). In the presence of water, the heads are attracted to water and line up to form a surface facing the water. The tails are repelled by water, and line up to form a surface away from the water. In a cell, one layer of heads faces outside of the cell,

attracted to the water in the environment. Another layer of heads faces inside the cell, attracted by the water inside the cell. The hydrocarbon tails of one layer face the hydrocarbon tails of the other layer, and the combined structure forms a bilayer.

The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. A liposome can be formed at a variety of sizes as uni-lamellar or multi-lamellar construction, and its name relates to its structural building blocks, phospholipids, and not to its size. In contrast, the term Nanosome does relate to size and was coined in the early 1990s to denote special liposomes in the low nanometer range; liposome and Nanosome are not synonyms. A liposome does not necessarily have lipophobic contents, such as water, although it usually does [27, 28].

Discovery

Liposomes were first described by British haematologist Dr Alec D Bangham FRS in 1961 (published 1964), at the Babraham Institute, in Cambridge. They were discovered when Bangham and R. W. Horne were testing the institute's new electron microscope by adding negative stain to dry phospholipids. The resemblance to the plasmalemma was obvious, and the microscope pictures served as the first real evidence for the cell membrane being a bilayer lipid structure.

Manufacturing

The correct choice of liposome preparation method depends on the following parameters:

1. The physicochemical characteristics of the material to be entrapped and those of the liposomal ingredients;
2. The nature of the medium in which the lipid vesicles are dispersed;
3. The effective concentration of the entrapped substance and its potential toxicity;
4. Additional processes involved during application/delivery of the vesicles;
5. Optimum size, polydispersity and shelf-life of the vesicles for the intended application; and,
6. Batch-to-batch reproducibility and possibility of large-scale production of safe and efficient liposomal products

It should be noted that formation of liposomes and nanoliposomes is not a spontaneous process. Lipid vesicles are formed when phospholipids such as lecithin are placed in water and consequently form one bilayer or a series of bilayers, each separated by water molecules, once enough energy is supplied. Liposomes can be created by sonicating phospholipids in water. Low shear rates create multilamellar liposomes, which have many layers like an onion. Continued high-shear sonication tends to form smaller unilamellar liposomes. In this technique, the liposome contents are the same as the contents of the aqueous phase. Sonication is generally considered a "gross" method of preparation as it can damage the structure of the drug to be encapsulated. Newer methods such as extrusion and Mozafari method are employed to produce materials for human use [29].

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