HIGH SCHOOL EDITION
Journal of Student Research

Volume 13 Issue 3 (2024)

Mechanisms in Crossing the Blood-Brain Barrier Using PLGA Nanoparticles

Pranav Kavandal[1], Kristina Lilova[#], and Jothsna Kethar[#]

[1]Vista Del Lago High School
[#]Advisor

ABSTRACT

Poly(lactic-co-glycolic acid) (PLGA) is a unique nanomaterial and polymer that stands out due to its unique properties. With crossing the BBB for drug delivery, PLGA shows promise. This literature review aims to examine the current research on the promising use of PLGA nanoparticles and their use of crossing the BBB. Crossing the BBB has remained a roadblock in drug delivery for a long time, and crossing this barrier would revolutionize drug delivery and would significantly benefit the medical community. This review will dive deep into the properties of PLGA nanoparticles, methods of drug delivery through the use of PLGA nanoparticles, and how these PLGA nanoparticles can efficiently and safely cross the BBB. Additionally, this review will highlight many studies showing the potential and effectiveness of PLGA nanoparticles in drug-delivery systems in the brain. Effectively crossing the BBB for drug delivery in the brain can change how doctors treat many neurological diseases and change the field of medicine and a future run by nanotechnology and nanomedicine. By utilizing current strategies in using PLGA nanoparticles in crossing the BBB, this review also aims to make PLGA one of the more critical nanoparticles for research in the future of nanomedicine.

Introduction

Medical technology is the future of medicine. In the past years, nanotechnology has been at the forefront of this future since nanotechnology is at a nanoscale, which opens a whole new world for its unique properties that can be applied to drug delivery. Although there are many nanomaterials, poly(lactic-co-glycolic acid) (PLGA) has shown much promise in drug delivery due to its biocompatible and biodegradable properties. PLGA is also an FDA-approved polymer, making it safe for many therapeutic applications. In the context of crossing the blood-brain barrier (BBB), PLGA has shown much promise, especially in the drug delivery challenge for many brain disorders. The BBB is a protective barrier of endothelial cells that aligns with the blood vessels. The primary function of the BBB is to protect the brain from toxic substances and tightly regulate what goes in and out between the blood and the brain. This allows for essential nutrients and chemicals to enter the brain while harmful toxins are prevented from entering the brain. Although the function of BBB is crucial in protecting the brain, it creates a problem in regards to drug delivery. The BBB limits many therapeutics in crossing towards the brain, disallowing many treatment options for many neurological diseases such as Alzheimer's, Parkinson's, and brain tumors. PLGA nanoparticles offer a potential solution to this problem. Due to their small size and ability to allow for a wide range of surface modifications PLGA nanoparticles offer a promising solution to crossing the BBB. Additionally, PLGA nanoparticles are biocompatible and biodegradable. Thus, this can help with the aspect of potential toxicity when delivering drugs to brain tissue.

Methodology

The focus of the study was to analyze and find the potential in the application of PLGA nanoparticles in crossing the blood-brain barrier. This is a literature review and not an actual in the field study so all secondary sources were used. Using the data gathered and the articles we have researched, we conclude that PLGA nanoparticles show much promise in nanomedicine, specifically in crossing the blood-brain barrier. While writing the literature review, we did not use any physical tools in this study and purely used online sources to conduct this literature review, so we can disregard ethical considerations when reading this paper.

Blood-Brain-Barrier(BBB)

Structure/Function of the BBB

The BBB is a semi-permeable membrane that permits the entry of foreign substances from the bloodstream into the central nervous system. First off, understanding the structure and physiological aspects of the BBB is crucial to understanding the blood-brain barrier as a whole. The BBB core comprises endothelial cells, and substances can usually diffuse through this membrane. However, tight junctions exist between these endothelial cells. These tight junctions are really what regulate what substances travel from the bloodstream to the central nervous system as these tight junctions do not allow anything to pass through towards the central nervous system (Wu et al., 2023). The next aspect of the structure of the BBB that is vital in involving its function is the existence of pericytes. These pericytes are enclosed in the basement membrane and lie around the endothelial cells. These pericytes essentially sense what is in the bloodstream. They can contract and relax, and by doing this, they can regulate what comes in from the bloodstream (Serlin et al., 2015). Astrocytes are the next aspect of the structure of the BBB. Astrocytes lie around the blood-brain barrier, and what makes them specifically valuable for the function of the BBB is that the end feet of these astrocytes are polarized. This means they essentially control fluids and ions' movement from the BBB toward the central nervous system (Serlin et al., 2015). Due to the unique properties and function of the BBB, only small and lipid-soluble molecules can cross the BBB. Lots of macromolecules cannot get through the BBB successfully. Thus, 95 percent of the molecules in drug delivery are stopped by the BBB (Dong, 2018).

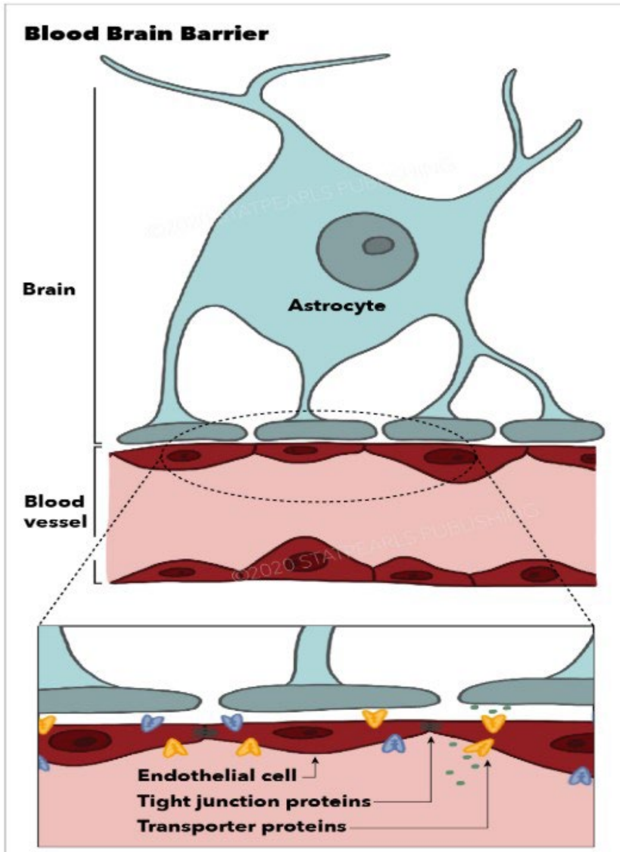

Figure 1. This shows the structure of the BBB and how it would look in the central nervous system. You can see the Astrocytes and the Endothelial cells. Illustration by Emma Gregory. From the article by (Dotiwala et al., 2023).

Properties of PLGA(poly(lactic-co-glycolic acid))

Chemical Properties of PLGA

PLGA is a biodegradable and biocompatible polymer created by polymerizing two monomers, lactic acid and glycolic acid. What makes PLGA biodegradable is that when PLGA breaks down, it breaks down into lactic acid and glycolic acid. Both of these can enter the Krebs cycle, and thus, they can become carbon dioxide and water when they break down. This overall makes the degradation of PLGA safe, non-toxic, and efficient (Lou et al., 2023). PLGA is also a biocompatible polymer; thus, when in the body, PLGA does not inflict a harmful immunological response. Furthermore, PLGA can also be formulated into microspheres and nanoparticles that also do not cause harm to the body (Lou et al., 2023). The ratio between lactic acid and glycolic acid mainly affects the chemical properties of PLGA. For example, the higher the glycolic acid, the faster the degradation rate of PLGA. The main aspects in drug release applications that affect the chemical properties of PLGA, apart from the differing ratios between lactic acid and glycolic acid, are the crystallinity, Tg, viscosity, and molecular mass of PLGA. In degradation and drug delivery systems in PLGA, all of these properties and parameters have to be carefully taken into consideration, as for specific drugs, specific degradation rates will be needed when in drug delivery (Lu et al., 2023).

PLGA Nanoparticle Formation

There are multiple ways to create PLGA nanoparticles, and based on the desired application, there are many different methods to produce these PLGA nanoparticles. We will discuss the most common techniques to create these PLGA nanoparticles.

Single Emulsion Method

This method to create PLGA nanoparticles uses the processes of emulsion, specifically oil-in-water emulsification. This method is one of the most popular ways to create PLGA nanoparticles to encapsulate hydrophobic substances, and it is used vastly in many industrial and pharmaceutical applications. Now, the way this works is that, firstly, the PLGA polymer gets dissolved in an organic solvent. This solution, made of the PLGA polymer and an organic solvent, gets mixed into water with a surfactant. Surfactant is a substance that helps mix oil with water. Over time, after the solution is mixed, the solvent evaporates using techniques such as stirring at a suitable temperature or reducing pressure. Whatever is left are the resulting PLGA nanoparticles that can now be loaded with drugs or other materials (Rocha et al., 2022).

Double Emulsion Method

The double emulsion method is similar to the single emulsion method. However, this method is more complex and is used to encapsulate hydrophilic substances. This method uses water-in-oil-in-water emulsification, where a water-based solution with the materials enclosed in the PLGA is mixed into an organic solution containing the PLGA. The solution is mixed with another water-based solution and a surfactant, just like in the single emulsion method. Now, with the evaporated organic solvent, we are left with the PLGA particles (Rocah et al., 2022).

Strategies to Cross the BBB

Properties of Nanoparticles to Help Cross the BBB

Nanoparticles are small in size, usually ranging from 1-1000 nanometers. Due to their very small size, nanoparticles offer great promise in crossing the BBB, a significant hurdle that must be crossed for drug delivery and, more importantly, helping treat deadly neurological diseases. Many studies have shown that the penetrability of nanoparticles through the BBB is better as the size of the nanoparticles decreases. However, with nanoparticles larger than 200nm, there needs to be more penetrability of the BBB. Most studies have found that drug delivery is best conducted with nanoparticles with a diameter between 10-100 nm. Now, even a specific study in 2020 found that 15nm gold nanoparticles were better and more efficient in drug delivery into a mouse brain than that compared to larger diameter nanoparticles (Ohta et al., 2020). When nanoparticles cross the BBB these nanoparticles must also diffuse through the brain's extracellular space. So overall, the best size and fit for any specific nanoparticles depends on various factors, including the type of nanoparticle, surface proteins, and coating. Ensuring PLGA nanoparticles have the perfect size and fit will be vital in successfully crossing the BBB (Hersh et al., 2022).

Electrostatic Interactions and Nanoparticles Ability to Cross the BBB

One of the central and essential properties of any nanoparticle and its ability to cross the BBB is dependent on the surface charge of the nanoparticle. This is important because the endothelial cells that primarily make up the structure of the BBB are made up of a high density of negatively charged proteoglycans. Now, this means that positively charged nanoparticles are more likely and more favorable in crossing the BBB. Compared to neutral-charged particles, these particles are 100 times less likely to cross and interact with the endothelial cells that make up the BBB than particles with a positive charge. However, positively charged nanoparticles can cause toxicity in the BBB and can cause damage to the BBB (Hersh et al., 2022). For example, a study has shown that cationic nanoparticles could be toxic, while nanoparticles with neutral and even anionic charges did not show adverse effects on the BBB (Lockman et al., 2004). Similar to another study, positively charged nanoparticles caused more neuron loss than negatively charged or anionic nanoparticles in rat brains (Knudsen et al., 2014). In the aspect of PLGA nanoparticles, making the PLGA positively charged could be helpful not only in crossing the BBB but also in crossing it efficiently (Hersh et al., 2022).

Ligands and Functional Groups for Nanoparticle Ability to Cross the BBB

To better the ability of nanoparticles crossing the BBB, the surfaces of nanoparticles can be changed with ligands such as peptides, proteins, antibodies, and surfactants. What these ligands do is better improve circulation time and enable binding to the endothelial receptors. By increasing the ligand density on the nanoparticles this improves the polyvalency and avidity and thus overall increases the probability of internalization. However, if the ligand density is too high, then this can cause steric hindrance and reduce diffusion. This means that an optimal ligand density on the nanoparticles needs to be measured so the affinity and avidity can promote internalization without impairing the BBB. An example of this is in a study that found that nanoparticles with 25 percent surface glucose showed the best permeability of the BBB than that of 10 percent of 50 percent density (Anraku et al., 2017). Ligands are also crucial in targeting specific cellular receptors as these ligands can target specific cellular receptors in the BBB. These ligands that target specific cellular receptors in the BBB can cause nanoparticles to be recognized by receptor-mediated transport systems. An example of this is the use of transferrin to target the transferring receptor located on the endothelial cells. Using specific ligands and molecules to target specific receptors this can help with crossing the BBB, as nanoparticles can be designed with specific molecules to target specific receptors and proteins in the BBB and the brain (Hersh et al., 2022).

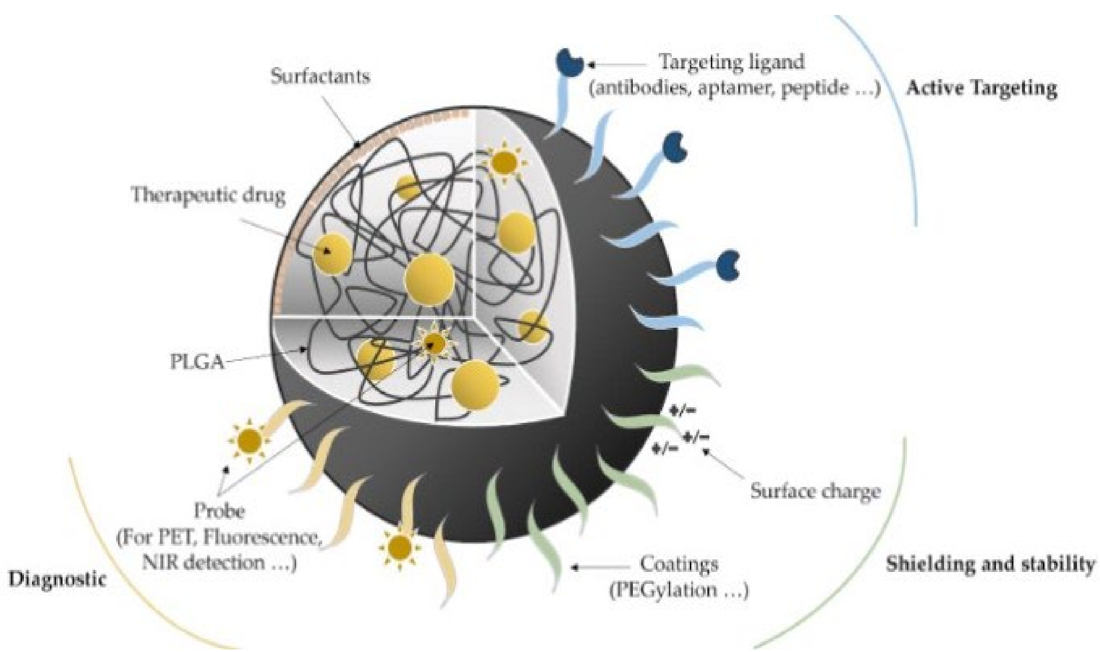

Figure 2. This image shows the representation of PLGA nanoparticles and their potential functionalization in crossing the BBB and or drug delivery (Cunha et al., 2021).

Receptor-Mediated Transcytosis(RMT): Crossing the BBB using PLGA Nanoparticles

Crossing the BBB is the main problem that PLGA nanoparticles are trying to solve, and RMT offers an auspicious way to cross the BBB with drug penetration; how the RMT works is that the endothelial cells in the BBB and the central nervous system have lots of receptors. Drug delivery systems can exploit these receptors and use this as an advantage in crossing the BBB. To exploit these receptors, drug delivery systems can be embedded with ligands that essentially target these receptors. And thus overall improving the efficiency and transport of these drugs across the BBB (Del Amo et al., 2021)

Studies on Surface-Modified PLGA Nanoparticles

Polysorbate 80(P80) and Poloxamer 188(P188) are surface modifiers that are very well known and have shown promise in drug-targeted delivery in the central nervous system. Essentially, what these agents do is they enhance the adsorption of plasma proteins into nanoparticles. This process is known as differential protein absorption, and this helps facilitate RMT and thus allows nanoparticles to travel through the endothelial cells and through the BBB (Del Amo et al., 2021). A study evaluated various PLGA nanoparticles coated on the surface with P80, P188, and chitosan. The study found that these PLGA nanoparticles coated with P80 and chitosan had better brain distribution compared to unmodified nanoparticles. P80-PLGA nanoparticles, in particular, were more efficient and effective as they absorbed the endothelial cell membranes and even crossed the BBB and went into the brain parenchyma. (Tahara et al., 2011).In another study on a P80-PLGA nanoparticle loaded with Galantamine, it was found that these nanoparticles could effectively cross the BBB. It was even found that these nanoparticles have also shown potential in drug delivery systems to treat Alzheimer's disease (Fornaguera et al., 2015). Finally, regarding surface-modified PLGA nanoparticles, there is Lactoferrin as a receptor. Now, what Lactoferrin is, it is a targeting ligand used in RMT, and the reason it is used is because it has a high affinity for the lactoferrin receptor that is abundantly found in brain endothelial cells and neurons. A study developed Lactoferrin PLGA nanoparticles loaded with Huperzine A in animal models. These nanoparticles showed improved brain delivery and increased memory (Meng et al., 2018). RMT and using PLGA nanoparticles show promise in crossing the BBB, and current and future research should still be done as this area holds promise for better drug delivery systems.

Adsorptive-Mediated Transcytosis(AMT): Crossing the BBB using PLGA Nanoparticles

AMT relies mainly on charge-based interactions, specifically with the endothelial cells, and does not really do ligand-receptor binding. Essentially what this method does in crossing the BBB is it uses positively charged nanoparticles that interact with the negatively charged surfaces of the endothelial cells located in the central nervous system and the BBB. When discussing electrostatic interactions, nanoparticles with a positive surface area are better and more suitable for cellular drug delivery due to their attraction to negatively charged endothelial cells. This effect can be created if the nanoparticles were coated with cationic or positively charged surfactants such as dioctadecyldimethylammonium bromide(DODAB) or didodecyldimethylammonium bromide(DMAB) (Del amo et al.,2021). A study prepared DMAB PLGA nanoparticles and loaded them with coumarin-6. The study showed better and improved cellular uptake and brain delivery in vitro with a nanoparticle size of 100 nm (Xu et al.,2012). Another study, instead of using cationic surfactants, used a cationic polymer chitosan(CS). It was found that (CS) modified PLGA nanoparticles increased brain concentrations, and even these nanoparticles adhered to endothelial cells due to the electrostatic interactions. This overall enhanced cellular uptake and increased efficiency in drug delivery (Tahara et al.,2011). This highlights the promise of AMT as it shows that this method can be used with nanoparticles, specifically PLGA nanoparticles.

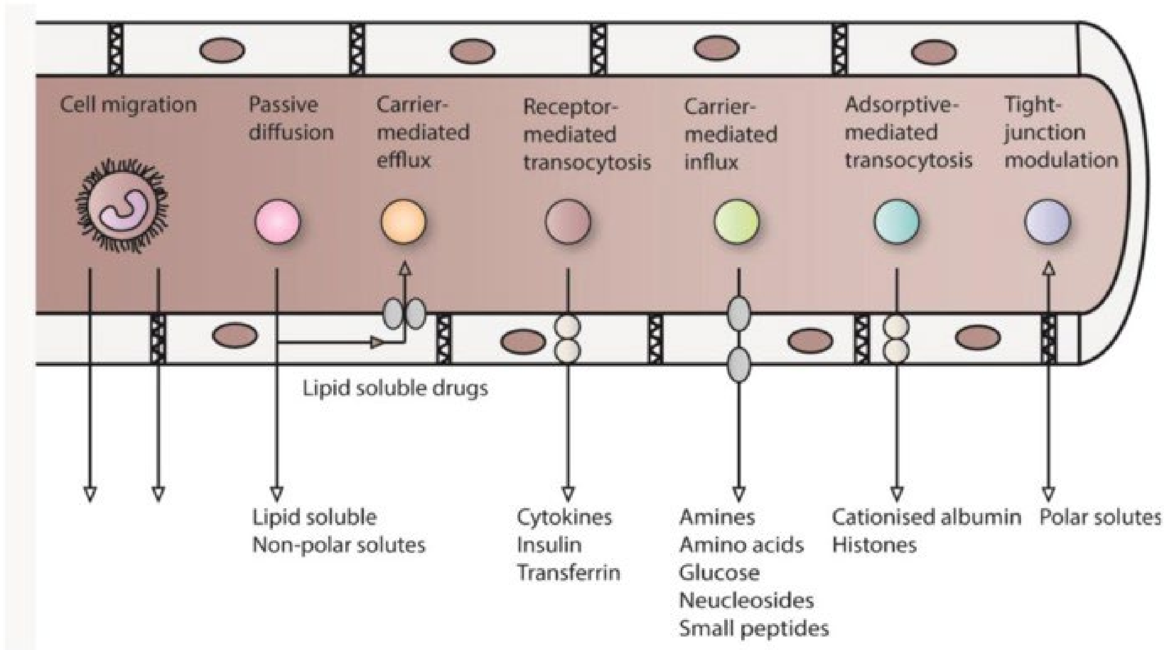

Figure 3. This image shows the potential ways to cross the blood-brain barrier (Serlin et al., 2015).

Applications of PLGA Nanoparticles in CNS Drug Delivery

Alzheimer's Disease

Alzheimer's disease(AD) is a very common neurodegenerative disorder that essentially progressively declines your cognitive function. The cause of this disease is associated with two brain lesions: extracellular amyloid-beta protein deposits and intracellular hyperphosphorylated tau protein aggregates. Now due to the accumulation of these proteins, this leads to the overall neuroinflammation and oxidative stress that causes the eventual degeneration of brain regions that are mainly used for memory and language. In the current research field for curing AD, there are no cures, and the existing treatments for AD are minimal to not very effective. PLGA nanoparticles and their unique properties offer a promising solution to help with drug delivery to the brain (Cunha et al., 2021).

Thymoquinone

Thymoquinone (TQ) is a compound that is composed of antioxidant and anti-inflammatory properties, and this compound has been researched and found to be a potential solution to counteract oxidative stress and neuroinflammation in AD (Cunha et al., 2021). An in vitro study found that PLGA nanoparticles that were embedded with TQ and coated with polysorbate 80(P-80) showed an initial release of TQ, which was followed by a sustained release. However, in vivo, P-80 TQ PLGA nanoparticles crossed the BBB through LDL receptors, and this significantly increased superoxide dismutase (SOD) in mice and, thus, overall reduced oxidative stress. Other behavioral tests showed cognitive improvement, and an examination showed a reduction in protein aggregates in the brain (Yusuf et al.,2021). This study demonstrates the potential for PLGA nanoparticles coated with TQ for AD treatment, which could be a potential solution in curing Alzheimer's disease.

Huperzine A

Huperzine A(HupA) has been shown to be a neuroprotective in its effects in treating AD, but it has poor brain selectivity and often leads to adverse side effects. A study developed PLGA nanoparticles and loaded them with HupA, which was also co-modified with Lactoferrin (Lf) and N-trimethyl chitosan(TMC), which was meant to enhance nose-to-brain drug delivery. These nanoparticles were created using the single emulsion method and had no cytotoxicity at concentrations of 12.5 to 25ug/ml. The Lf-TMC modification improved mucin adsorption, which overall resulted in a high binding efficiency of 86%. In vitro showed a release of Hupa in 4 hours followed by a sustained release over 48 hours. The cellular uptake experiments showed a higher uptake of Lf-TMC PLGA nanoparticles compared to the unmodified nanoparticles. In vivo studies showed better brain delivery, especially in the olfactory bulb and hippocampus (Meng et al.,2018). This overall indicates the potential of these PLGA nanoparticles again in treating AD as the brain delivery, especially in the CNS, is very good when using PLGA nanoparticles, and further research in these nanoparticles can really help overall in cross the BBB.

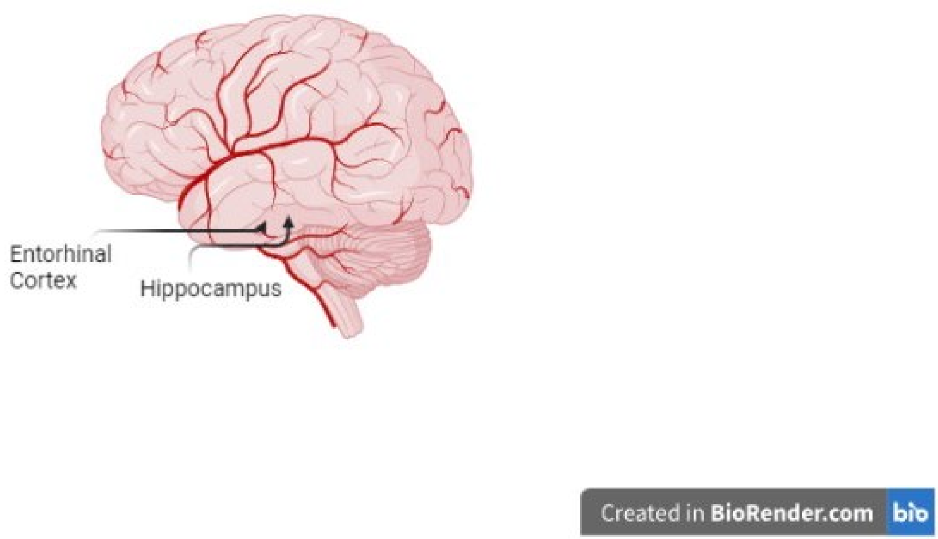

Figure 4. This image shows areas in the brain affected after getting diagnosed with Alzheimer's. Created by Pranav Kavandal.

Huntington's Disease and PLGA Nanoparticles

Huntington's Disease(HD) is a hereditary disorder that is caused by a mutation in the IT15 gene. What this mutation does is it leads to the abnormal repetition of the CAG trinucleotide, and an effect of this is the production of the mutant Huntington protein (mHtt). Symptoms that typically occur between the ages of 30-50 which include involuntary movements, muscle rigidity, and psychiatric disorders such as dementia. HD is signified by the death of neurons in brain regions, which leads to cognitive and motor dysfunctions. The only approved medication for HD is tetrabenazine (Cunha et al.,2021). This shows how much the need for new drug delivery methods is needed to cure this disease.

Peptide-Loaded PLGA Nanoparticles

A study developed peptide-loaded PLGA nanoparticles, which were also coated with polysorbate 80. Peptides QBP1, NT17, and PGQ9P2 were found to have the highest potential in preventing mHtt aggregation. The nanoparticles were prepared using nanoprecipitation and had diameters between 158 and 180nm. In vitro using

MDCK cells it was found that the coated PLGA nanoparticles have higher brain uptake than that of the uncoated ones. In vivo studies in mice showed higher brain concentrations of the coated PLGA nanoparticles. Using a Drosphila model of HD, the peptide-loaded nanoparticles showed better motor performance. This study shows the drug delivery potential using PLGA nanoparticles to better deliver drugs to cure HD (Joshi et al., 2019).

Cholesterol-Loaded PLGA Nanoparticles

Cholesterol is very important for cellular functions, and usually, HD patients show altered cholesterol metabolism, which, in the end, leads to cognitive impairments. A study wanted to investigate using PLGA nanoparticles to deliver cholesterol. The nanoparticles loaded with cholesterol with sizes 200-300nm were prepared. In vivo studies using R6/2 mice showed that these nanoparticles could cross the BBB and repair synaptic transmission in neurons. There were some problems, though, as the cholesterol supplementation did not majorly improve behavioral symptoms, but it did, however, slow disease progression, which shows potential in not only this method but also in using PLGA nanoparticles in drug delivery (Valenza et al., 2015).

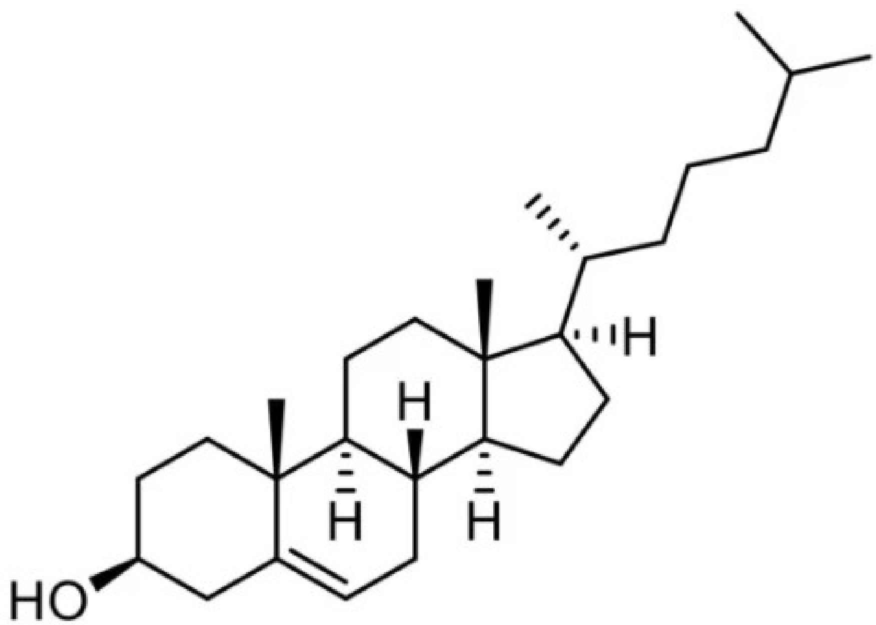

Figure 5. This image shows the chemical structure of the cholesterol encapsulated in the PLGA nanoparticles (Cunha et al., 2021).

Multiple Sclerosis and PLGA Nanoparticles in Drug Delivery

Multiple sclerosis(MS) is an autoimmune disease that affects the central nervous system (CNS). It is a disease defined by the immune system that attacks the myelin sheath, which protects nerve fibers, and eventually, due to the attacking of the myelin sheath, this disrupts nerve signals. Some symptoms include limb numbness, vision problems, and movement difficulties. There are many therapies and approved treatments, but many of these therapies can be improved by using PLGA nanoparticles (Cunha et al., 2021).

Interferon-Beta-1a

Interferon-beta-1a is a protein that regulates the immune system and is used to slow the progression of MS. A study developed Interferon-beta-1a loaded PEG-PLGA nanoparticles, and these nanoparticles were created using a double emulsion method. The release kinetics of the nanoparticles were slower than that of normal PLGA nanoparticles, which shows a more controlled release of the drug, which is good. Also, in vitro studies using hepatocytes had cellular uptake and no cytotoxicity. However, in vivo toxicity studies in rats showed toxicity due to the fabrication process, indicating more modified uses of the nanoparticles (Fordor-Kordos et al.,2020). Further research is needed to find a safer way to deliver the drug, but this shows promise in the use of PLGA nanoparticles and treating MS (Cunha et al., 2021).

Antigen PLP139-151

A study created PLGA nanoparticles and loaded them with the antigen PLP139-151 using again the double emulsion method. Now, the reason to deliver this is because this antigen is known to induce tolerance and have a better regulation of the immune response in MS. The encapsulation was low, with 60 percent of the antigen not encapsulated, and the drug was released in a few hours. This means that better mechanisms for making the nanoparticles are needed (Ferreira Lima et al.,2020). Although there were trials in using these nanoparticles, there still needs to be further studies to evaluate PLGA nanoparticles and how they can help with a potential treatment of MS. By improving drug encapsulation, these nanoparticles do have the potential to increase the effectiveness of current treatments and overall help with curing MS. Advancements in PLGA nanoparticles are necessary and more research and more studies on this can not only help with the curing of MS but also many other diseases (Cunha et al.,2021).

Conclusion

PLGA nanoparticles show much promise in the field of drug delivery. Due to the many challenges in crossing the BBB, their unique properties of biodegradability and biocompatibility make them efficient and safe for various biomedical applications. Not only in theory, but PLGA particles show promise in not only crossing the BBB but show promise in effectively treating neurodegenerative diseases such as Alzheimer's and Huntington's disease. PLGA nanoparticles have immense potential in drug delivery and medical treatments. That said, the future of drug delivery in nanoparticles, specifically PLGA, shows much promise. I recommend continued research on PLGA nanoparticles using the methods examined, if not more. Further exploration of this topic and improvements in drug delivery regarding PLGA nanoparticles can include better improvements in the development of these nanoparticles. These improvements, such as surface charge, size, and hydrophobicity of these PLGA nanoparticles, should be explored in the future as these small changes can change whether crossing the BBB is efficient and effective. Combined with the unique properties of PLGA nanoparticles certain mechanisms in crossing the BBB also needed to be further explored.

Limitations

The main limitation of this research paper was the use of all secondary sources rather than actually performing the studies. The main reason for this is the lack of tools to conduct these studies myself.

Acknowledgment

I would like to thank Dr. Lilova for helping me pick my research topic and Coach Jo for giving me the opportunity to write this research paper.

References

Anraku, Y., Kuwahara, H., Fukusato, Y., Mizoguchi, A., Ishii, T., Nitta, K., Matsumoto, Y., Toh, K., Miyata, K., Uchida, S., Nishina, K., Osada, K., Itaka, K., Nishiyama, N., Mizusawa, H., Yamasoba, T., Yokota, T., & Kataoka, K. (2017). Glycaemic control boosts glucosylated nanocarrier crossing the BBB into the brain. *Nature Communications*, *8*(1), 1001. https://doi.org/10.1038/s41467-017-00952-3

Cunha, A., Gaubert, A., Latxague, L., & Dehay, B. (2021). PLGA-Based Nanoparticles for Neuroprotective Drug Delivery in Neurodegenerative Diseases. *Pharmaceutics*, *13*(7), 1042. https://doi.org/10.3390/pharmaceutics13071042

Del Amo, L., Cano, A., Ettcheto, M., Souto, E. B., Espina, M., Camins, A., García, M. L., & Sánchez-López, E. (2021). Surface Functionalization of PLGA Nanoparticles to Increase Transport across the BBB for Alzheimer's Disease. *Applied Sciences*, *11*(9), 4305. https://doi.org/10.3390/app11094305

Dong, X. (2018). Current Strategies for Brain Drug Delivery. *Theranostics*, *8*(6), 1481–1493. https://doi.org/10.7150/thno.21254

Dotiwala, A. K., McCausland, C., & Samra, N. S. (2023). *Anatomy, Head and Neck: Blood Brain Barrier*. PubMed; StatPearls Publishinghttps://www.ncbi.nlm.nih.gov/books/NBK519556/#:~:text=%5B1%5D%20The%20BBB%20is%20composed

Fodor-Kardos, A., Kiss, Á. F., Monostory, K., & Feczkó, T. (2020). Sustained in vitro interferon-beta release and in vivo toxicity of PLGA and PEG-PLGA nanoparticles. *RSC Advances*, *10*(27), 15893–15900. https://doi.org/10.1039/c9ra09928j

Fornaguera, C., Feiner-Gracia, N., Calderó, G., García-Celma, M. J., & Solans, C. (2015). Galantamine-loaded PLGA nanoparticles, from nano-emulsion templating, as novel advanced drug delivery systems to treat neurodegenerative diseases. *Nanoscale*, *7*(28), 12076–12084. https://doi.org/10.1039/c5nr03474d

Hersh, A. M., Alomari, S., & Tyler, B. M. (2022). Crossing the Blood-Brain Barrier: Advances in Nanoparticle Technology for Drug Delivery in Neuro-Oncology. *International Journal of Molecular Sciences*, *23*(8), 4153. https://doi.org/10.3390/ijms23084153

Jin, Y., Xu, A., Yao, Li, Jin, Y., & Ying, J. (2012). A physical model for the size-dependent cellular uptake of nanoparticles modified with cationic surfactants. *International Journal of Nanomedicine*, 3547. https://doi.org/10.2147/ijn.s32188

Knudsen, K. B., Northeved, H., Ek, P. K., Permin, A., Andresen, T. L., Larsen, S., Wegener, K. M., Lam, H. R., & Lykkesfeldt, J. (2014). Differential toxicological response to positively and negatively charged nanoparticles in the rat brain. *Nanotoxicology*, *8*(7), 764–774. https://doi.org/10.3109/17435390.2013.829589

Lima, A. F., Amado, I. R., & Pires, L. R. (2020). Poly(d,l-lactide-co-glycolide) (PLGA) Nanoparticles Loaded with Proteolipid Protein (PLP)—Exploring a New Administration Route. *Polymers*, *12*(12), 3063. https://doi.org/10.3390/polym12123063

Lockman, P. R., Koziara, J. M., Mumper, R. J., & Allen, D. D. (2004). Nanoparticle Surface Charges Alter Blood–Brain Barrier Integrity and Permeability. *Journal of Drug Targeting*, *12*(9-10), 635–641. https://doi.org/10.1080/10611860400015936

Lou, J., Duan, H., Qin, Q., Teng, Z., Gan, F., Zhou, X., & Zhou, X. (2023). Advances in Oral Drug Delivery Systems: Challenges and Opportunities. *Pharmaceutics*, *15*(2), 484–484. https://doi.org/10.3390/pharmaceutics15020484

Lu, Y., Cheng, D., Niu, B., Wang, X., Wu, X., & Wang, A. (2023). Properties of Poly (Lactic-co-

Glycolic Acid) and Progress of Poly (Lactic-co-Glycolic Acid)-Based Biodegradable Materials in Biomedical Research. *Pharmaceuticals*, *16*(3), 454. https://doi.org/10.3390/ph16030454

Meng, Q., Wang, A., Hua, H., Jiang, Y., Wang, Y., Mu, H., Wu, Z., & Sun, K. (2018). Intranasal delivery of Huperzine A to the brain using lactoferrin-conjugated N-trimethylated chitosan surface-modified PLGA nanoparticles for treatment of Alzheimer's disease. *International Journal of Nanomedicine*, *Volume 13*, 705–718. https://doi.org/10.2147/ijn.s151474

Ohta, S., Kikuchi, E., Ishijima, A., Azuma, T., Sakuma, I., & Ito, T. (2020). Investigating the optimum size of nanoparticles for their delivery into the brain assisted by focused ultrasound-induced blood–brain barrier opening. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-75253-9

Rocha, C. V., Gonçalves, V., da Silva, M. C., Bañobre-López, M., & Gallo, J. (2022). PLGA-Based Composites for Various Biomedical Applications. *International Journal of Molecular Sciences*, *23*(4), 2034. https://doi.org/10.3390/ijms23042034

Serlin, Y., Shelef, I., Knyazer, B., & Friedman, A. (2015). Anatomy and physiology of the blood–brain barrier. *Seminars in Cell & Developmental Biology*, *38*, 2–6. https://doi.org/10.1016/j.semcdb.2015.01.002

Tahara, K., Miyazaki, Y., Kawashima, Y., Kreuter, J., & Yamamoto, H. (2011). Brain targeting with surface-modified poly(d,l-lactic-co-glycolic acid) nanoparticles delivered via carotid artery administration. *European Journal of Pharmaceutics and Biopharmaceutics*, *77*(1), 84–88. https://doi.org/10.1016/j.ejpb.2010.11.002

Valenza, M., Chen, J. Y., Di Paolo, E., Ruozi, B., Belletti, D., Ferrari Bardile, C., Leoni, V., Caccia, C., Brilli, E., Di Donato, S., Boido, M. M., Vercelli, A., Vandelli, M. A., Forni, F., Cepeda, C., Levine, M. S., Tosi, G., & Cattaneo, E. (2015). Cholesterol-loaded nanoparticles ameliorate synaptic and cognitive function in
Huntington's disease mice. *EMBO Molecular Medicine*, *7*(12), 1547–1564. https://doi.org/10.15252/emmm.201505413

Wu, D., Chen, Q., Chen, X., Han, F., Chen, Z., & Wang, Y. (2023). The blood–brain barrier: structure, regulation, and drug delivery. *Signal Transduction and Targeted Therapy*, *8*(1), 1–27. https://doi.org/10.1038/s41392-023-01481-w

Yusuf, M., Khan, M., Alrobaian, M. M., Alghamdi, S. A., Warsi, M. H., Sultana, S., & Khan, R. A. (2020). Brain targeted Polysorbate-80 coated PLGA thymoquinone nanoparticles for the treatment of Alzheimer's disease, with biomechanistic insights. *Journal of Drug Delivery Science and Technology*, *61*, 102214. https://doi.org/10.1016/j.jddst.2020.102214