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Artificial Intelligence In Drug Transport Across Blood Brain Barrier [Bbb]

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Abstract: The blood–brain barrier (BBB) is a highly selective structural and functional interface that tightly regulates molecular transport between the bloodstream and the central nervous system, making brain drug delivery a major challenge. This review discusses the anatomy of the BBB, its cellular components—endothelial cells, astrocytes, pericytes, the basement membrane, and microglia—and the junctional complexes that restrict paracellular permeability. Conventional drug delivery approaches remain limited due to restricted permeability, active efflux mechanisms, and a mismatch between in-vitro and in-vivo models. Advances in artificial intelligence (AI) have transformed drug discovery and BBB transport research through improved target identification, molecular screening, de novo drug design, and drug repurposing. AI-driven models, including machine learning and deep learning, enhance the prediction of BBB permeability, optimize nanoparticle-based delivery systems, and integrate *In-silico*, in-vitro, and in-vivo datasets for better translational accuracy. Additionally, molecular dynamics simulations and computational nanotoxicology provide insight into nanoparticle interactions with BBB membranes, aiding the rational design of nanocarriers capable of crossing the BBB. Despite these advancements, limitations remain due to biological complexity, dataset scarcity, and reduced interpretability of AI algorithms. Overall, integrating AI with experimental BBB models offers a promising path toward more efficient CNS drug development and targeted therapeutic delivery.

Index Terms - artificial intelligence, blood–brain barrier (BBB), machine learning, de novo drug design, nanotoxicology

I. INTRODUCTION

Most medications from the blood cannot enter the brain due to the blood–brain barrier (BBB). The BBB makes it complicated to develop novel therapies for brain disorders or novel radiopharmaceuticals for brain imaging. Every biotechnology product is a huge molecule medication that doesn't pass across the blood-brain barrier. 98% of all tiny molecules are not transported across the blood-brain barrier, despite the assumption that they are. The BBB is the primary issue obstructing the development of novel treatments for brain illnesses or novel radiopharmaceuticals for brain imaging. The fundamental concepts of BBB drug transport as well as methods for reengineering medications to facilitate BBB transport will be covered in this review. The limits

of the traditional methods of delivering drugs to the brain—known as transcranial drug administration or tiny molecules—are examined. The BBB's endogenous carrier mediated transport (CMT) and RMT systems are examined, and methods for reengineering medications that enter the brain from the bloodstream through the CMT and RMT systems are evaluated. [1]. :

II. OVERVIEW OF BLOOD BRAIN BARRIER:

Only O2, CO2, water, and tiny molecules can pass through the extremely semipermeable membrane of endothelial cells that make up the BBB, a special and intricate multicellular structural barrier in the central nervous system that prevents infections and the majority of macromolecules from entering the brain.49. Pericytes, astrocytes, the basement membrane, neurons, and tight junctions (TJs) between endothelial cells make up the majority of the blood-brain barrier. [2] The blood-brain barrier (BBB) is a diffusion barrier that is necessary for the central nervous system to operate normally. The lack of fenestrations, more widespread tight junctions (TJs), and scarce pinocytic vesicular transport distinguish BBB endothelial cells from endothelial cells in the rest of the body. Tight connections between endothelial cells restrict the paracellular flow of hydrophilic substances via the blood-brain barrier. On the other hand, tiny lipophilic molecules like CO2 and O2 readily travel along their concentration gradient across plasma membranes. While bigger molecules like insulin, leptin, and iron transferrin are taken up by receptor-mediated endocytosis, nutrients like glucose and amino acids enter the brain by transporters. The capillary basement membrane (BM), astrocyte end-feet encasing the capillaries, and pericytes (PCs) embedded in the basement membrane make up the BBB in addition to endothelial cells. [3]

STRUCTURE OF BBB:

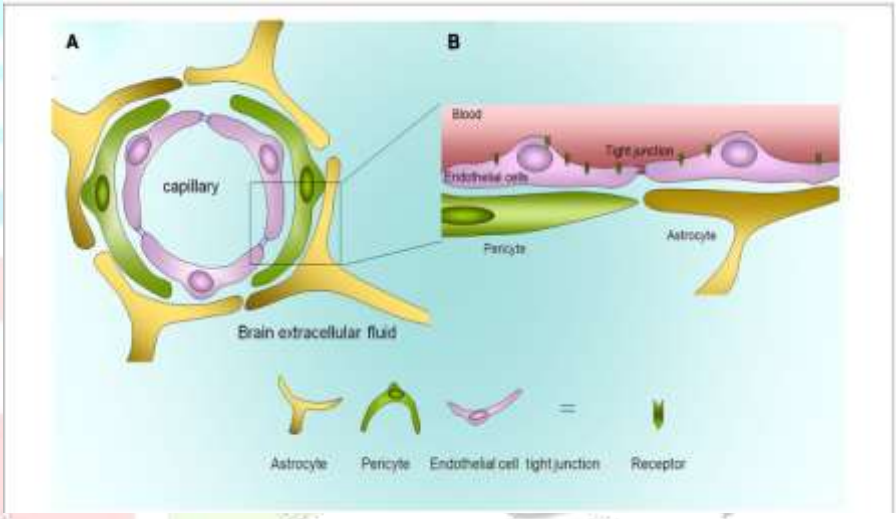

FIG:1 Anatomical structure of blood brain -barrier

NEUROVASCULAR UNIT:

Every species with a fully formed central nervous system have a blood-brain barrier (BBB), which is mainly made up of pericytes, astrocytes, and microvasculature endothelial cells. In addition to these three cells, other elements that contribute to immune activity include smooth muscles, the basement membrane, microglia, and neurons. These associated cells, which are commonly referred to as a neurovascular unit, work in tandem with endothelial cells to preserve an intact blood-brain barrier (BBB) to guarantee the proper operation of the central nervous system.

ENDOTHELIAL CELLS:

The fundamental components of the BBB endothelium are endothelial cells, which join together through incredibly tight junctions to form a thin layer. The connection between endothelial cells at the BBB is approximately 50–100 times closer than that of endothelial cells at the peripheral micro-vessel wall because of the tight junction. Consequently, even when a vascular endothelial growth factor is applied, the intercellular connections between the BBB endothelial cells lack fenestration. Furthermore, BBB endothelial cells have extremely few pinocytotic vesicles, in contrast to endothelial cells in the rest of the body. These unique characteristics allow ions or tiny molecules (such glucose or iron) to be carried across the blood-brain barrier (BBB) by an enzyme-assisted mechanism, which is commonly referred to as active transport. Compared to the diffusive transport that takes place in the endothelium of other body regions, the active transport of

nutrients from the blood to the brain demands a higher energy potential. Compared to skeletal muscle capillaries, BBB endothelial cells have five to six times as many mitochondria per capillary segment. It has been hypothesized that these extra mitochondria supply the energy needed for active transport across the BBB. Because BBB endothelial cells include proteolytic enzymes including γ-glutamyl transpeptidase, alkaline phosphatase, and aromatic acid decarboxylase, they provide an enzymatic barrier in addition to the physical one. Drugs and neuroactive bloodborne solutes can be broken down by this enzymatic barrier. [4]

ASTROCYTES:

The most common type of glial cells in the brain, astrocytes, sometimes referred to as astroglia, have complex, polarized morphologies that vary widely. They are traditionally classified into two groups: fibrous, which is found in the less vascularized white matter, and protoplasmic, which is found in the well-vascularized gray matter. By connecting a group of proteins (aquaporin IV and the dystro glycan dystrophin complex) to the proteoglycan once more, their end feet connect them to the basement membrane. They are crucial for dynamic signaling in the central nervous system (CNS), including waste removal, brain blood flow regulation, vascular function regulation, ion hemostasis, and neuroimmune response balancing. The precise function of astrocytes in the BBB is still up for debate, though. While some research suggested that astrocytes can develop barrier behaviors in cerebral, other endothelial cells, and related epithelial cells, other research suggested that the BBB appears before astrocytes. Astrocytes are a type of neural cell that surrounds blood arteries in the brain together with pericytes, acting as the interface between neurons and endothelial cells. In this sense, there is no question that the BBB exists mainly through cell cooperation. Furthermore, astrocytes are the primary elements of the barrier separating humoral fluids from the central nervous system in invertebrates without a vascularized circulatory system.

Pericytes:

Mural cells called pericytes are found sporadically along the capillary blood channel walls. They are located abluminal to the endothelial cells and are embedded in the basement membrane. Pericytes that coat the endothelium of the central nervous system are almost 100% long. It should be mentioned that pericytes are essential to the operation of the neurovascular unit. Pericytes and endothelial cells are in intimate communication with one another because to their physical proximity. One such communication is the PDGF-B signaling route, for instance.

Pericytes are drawn to blood arteries by endothelial cells' secretion of PDGF-B, which binds to PDGFRβ on pericytes. By controlling the quantity of tight connections and polarizing the end foot of astrocytes, pericytes can also release signaling molecules that impact endothelial cells. Tight connections between endothelial cells will decrease if there are fewer pericytes. Pericytes have roles in regulating cerebral blood flow, vascular growth and maintenance, and neuroinflammation in addition to controlling and maintaining the blood-brain barrier. [5]

OTHER COMPONENTS:

Basement Membrane:

Located primarily underneath endothelium and epithelial cells, the BM is a distinct type of extracellular matrix (ECM). It performs numerous crucial tasks, such as signaling transmission, cell anchoring, and structural support. There are two forms of BM in the brain: endothelial BM and parenchymal BM, which are divided by pericytes. In areas without pericytes, the two BM layers appear to be one under physiological conditions. The BM is a highly ordered protein sheet that ranges in thickness from 50 to 100 nm. Collagen IV, laminin, nidogen, and perlecan are the four main ECM proteins that make up the BM biochemically. At the BBB, BMECs, pericytes, and astrocytes are primarily responsible for the synthesis of these ECM proteins. [6]

Microglia:

The overall blood-brain barrier system, which is thought to comprise the hemato-encephalic, liquor-encephalic, and hemato-liquor barriers (HEB, LEB, and HLB, respectively), is primarily regulated by microglia. The brain is mostly cut off from immune cells in the bloodstream as a result of these barriers. In the brain, microglial cells are in charge of immunological defense. Together, they make up the internal (own) brain immune system, which serves as the central nervous system's first line of defense against a variety of pathogenic pathogens that can pass through the endothelium. [7]

III TYPES OF JUNCTIONS IN BBB:

Tight Junction: TJ and adherens junction (AJ) make up the BBB's junction complex. The outer leaflets of the neighboring endothelial cells' plasma membranes appear to fuse at the TJs' ultrastructural locations. TJs are shown as a collection of continuous, anastomosing intramembranous strands or fibrils on the P-face with a corresponding groove on the E-face in freeze fracture replica electron micrographs. Both the quantity of TJ strands and the frequency of their consequences vary. A cadherin-catenin complex and the proteins that are connected to it make up adherens junctions. The TJ is made up of several cytoplasmic auxiliary proteins, such as ZO-1, ZO-2, ZO-3, cingulin, and others, as well as three integral membrane proteins, claudin, occludin, and junction adhesion molecules. Actin, the key cytoskeleton protein for the preservation of the endothelium's structural and functional integrity, is connected to membrane proteins by cytoplasmic proteins. [8]

Adherence Junction: Adherens junction is especially important for the proper assembly of tight junction proteins and the structural integrity of the blood-brain barrier. They create physically, chemically, and geographically distinct microdomains at cellular interfaces. Adherens junctions are composed of transmembrane and cytoplasmic plaque proteins and are connected to the cytoskeleton, just as tight junctions. The primary elements of adherens junctions include vascular endothelial-cadherin, scaffolding proteins catenins, scaffolding proteins p120, and plakoprotein.Cell-to-cell adhesion is the main function of vascular endothelial-cadherin. It is a homo-dimeric transmembrane protein whose cytoplasmic domain can interact with actin filaments through scaffolding proteins, and its extracellular domain can link to other molecules of nearby endothelial cells in the paracellular gap.Two Meanwhile, catenins, p120, and plakoprotein form a bridge that interacts with actin filaments and zonula occludens-1 (tightjunction proteins) to support physics and regulate junctions. Additionally, other proteins that may be connected to the adherens junction have been discovered, including nectin, CD99, and platelet and endothelial cell adhesion molecule 1. Adherens junctions are essential for maintaining the integrity of the blood-brain barrier, and any alteration to them may cause inter-endothelial cell connections to break. [9]

Gap Junction: A direct route for electrical and metabolic signaling between neighboring cells is provided by gap junction channels, which are made up of two opposing hemichannels (connexons) in the adjacent cells. There are twenty members of the mouse connexin gene family and twenty-one in the human genome. All tissues express connexins, with the exception of mature sperm cells, differentiated skeletal muscle, and erythrocytes. Homotypic, heterotypic, and heteromeric gap junction channels can form between cells because different tissues express different types of connexins. The fundamental gating and permeability characteristics of homotypic and heterotypic gap junction channels are briefly reviewed in this article, along with current developments in the study of how transjunctional voltage, intracellular calcium, pH, and phosphorylation. [10]

IMPORTANCE OF BBB IN CNS PROTECTION:

In order to sustain homeostasis and other essential processes, the brain, a delicate and extremely sensitive neuronal organ system, requires a steady supply of fuels, gasses, and nutrients. However, the central nervous system's blood-brain barrier (BBB) serves as a physical barrier and presents a number of challenges. It prevents several medications, such as antibiotics, antineoplastic medicines, and neuropeptides, from reaching the brain through the endothelium capillaries and prevents the administration of therapeutic substances to the central nervous system. Although a number ofmedication delivery techniques and strategies have been developed for the treatment of CNS-related diseases, the majority of them have been shown to be intrusive and lack target specificity. Even more remarkably, all conventional drug delivery techniques rely on trial and error. These are always used to administer a small number of carefully chosen medications with intact structure-transport relationships and suitable structure-activity or drug-receptor interactions. [11]

IV TRANSPORT MECHANISM:

The two broad categories of drug transport through endothelial cells are paracellular and transcellular routes. Molecules are transported between cells via the intracellular space via the paracellular route. Hormones, alcohol, and gasses (CO2, O2) are examples of small lipid-soluble substances with molecular weights less than 400 Da that can passively flow through endothelial cell plasma membranes. While paracellular transport is prevalent in peripheral capillaries, it is strongly restricted in the blood-brain barrier (BBB) because of tight junctions that compel most transport to transcellular channels. For instance, one of the three transcellular pathways listed below is used to move nutrients and macromolecules over the blood-brain barrier:

1.carrier-mediated transcytosis,
2.receptor-mediated transcytosis,
3.adsorptive-mediated transcytosis.

Carrier- Mediated Transcytosis:

Transcytosis Mediated by Carriers Nutrient transporter proteins and efflux transporter proteins are transporter protein carriers found on the luminal and basolateral sides of endothelial cells, respectively. Proteins that are specialized to solutes like glucose, hormones, and amino acids are known as nutrient transporters. These solutes cause a reversible conformational change when they attach to the corresponding transporter proteins. Following a gradient of high to low solute concentration, the solutes are taken up by cells and moved to the basolateral side of the membrane. For instance, the glucose transporter GLUT1 facilitates the transit of glucose. Conversely, a variety of ATP-binding cassette transporters, often known as efflux pumps, are used to actively remove medicines and non-specific substrates from endothelial cells. P-glycoprotein (P-GP), multi-drug resistance proteins (MRP), and breast cancer resistance protein (BCRP) are examples of efflux pumps that are located on the luminal side of brain capillaries. They bind to a range of substrates, effectively preventing drug accumulation in endothelial cells and impeding drug transport to the brain.

Receptor Mediated Transcytosis:

Highly specialized receptors for macromolecules including hormones, enzymes, and plasma proteins are expressed by cerebral endothelial cells. Insulin, transferrin, and low-density lipoprotein (LDL)-cholesterol are the three most researched ligands that are crucial for BBB transport. These ligands attach to insulin, transferrin (TF), and LDL receptors on endothelial cells, respectively. Ligands attach to the plasma membrane's receptors on the luminal side of the endothelium and are internalized by vesicles. After passing through the cells' cytoplasm, these vesicles release the ligands on the basolateral side. Cargo internalization by endocytosis can take place via pathways depending on caveolin or clathrin. The majority of ligands, including insulin, transferrin, and LDL-1, undergo transcytosis via the clathrin-dependent route. Nucleation (binding the cargo to the plasma membrane), cargo selection (starting the creation of a clathrin-coated pit), clathrin coat assembly, membrane scission, and clathrin coat deconstruction comprise this five-step process. The caveolin-dependent pathway, which is mediated by the caveolin protein and leads to the production of uncoated vesicles, is only carried out by a small number of substances, including folate. But in neurological disorders, this pathway is more pertinent to leaky BBB.

Adsorptive Mediated Transcytosis:

Adsorptive-mediated transcytosis is possible for positively charged drugs or substrates. It is caused by electrostatic interactions between the negatively charged heparin sulphate proteoglycans found on the endothelial cells' plasma membrane surface and the positively charged substrate surface, which is typically polycationic proteins like protamine. Compared to transport mediated by carriers or receptors, this mechanism is comparatively slower and has a reduced transport capacity. [12]

V ROLE OF AI IN DRUG DISCOVERY AND DELIVERY:

The complex process of creating, recognizing, and creating novel drugs with the goal of enhancing human health and preventing illness is known as drug discovery. Target identification, lead compound discovery, optimization, stringent preclinical testing, and careful clinical trials are all part of this extensive process, which is essential for bringing successful medications into medical practice. Drug development tactics are changing as a result of AI's role in systems pharmacology, drug-target interaction predictions, and clinical trial design. In order to improve trial efficiency and results, the technology helps optimize patient selection, monitor patient responses in real-time, and modify procedures. But integrating AI with a careful balance is essential to maintaining scientific integrity, ethical considerations, and regulatory compliance. This review explores how AI is revolutionizing the drug discovery process. It demonstrates how AI supports drug development quality

assurance, expedites the creation of innovative treatment approaches, and simplifies clinical trials. This study highlights AI's potential to accelerate the development of life-saving drugs, greatly improving global healthcare, and advocates for its responsible usage in pharmaceutical research. [13]

DRUG DELIVERY:

A crucial step in the medication development process is drug delivery. a drug's effectiveness depends on both its pharmacological properties and its capacity to enter the body at the desired location. many drug delivery methods have been developed over time to increase medication efficacy and reduce side effects. however, developing innovative medication delivery methods is a complex and time-consuming task. therapeutic delivery using ai has the potential to improve therapeutic efficacy and speed up the creation of new delivery methods. drug delivery basically means giving the right amount of medicine at the right time to the right place.

DRUG DISCOVERY:

- Target Identification: AI systems can find possible therapeutic targets by analyzing a variety of data sources, such as genomic, proteomic, and clinical data. AI helps create drugs that can control biological processes by identifying disease-related targets and molecular pathways.
- Virtual Screening: AI enables the effective screening of large chemical libraries to find therapeutic candidates that have a high likelihood of binding to a particular target. AI helps researchers prioritize and choose compounds for experimental testing by modeling chemical interactions and predicting binding affinities, which saves time and money.
- Structural Activity Relationship Modelling: AI is able to identify relationships between a compound's chemical makeup and its biological effectiveness. By designing compounds with desired properties, such as increased potency, selectivity, and favorable pharmacokinetic profiles, researchers can improve the potential of therapeutic candidates.
- DE - Novo Drug Design: AI algorithms can suggest new chemical compounds that resemble pharmaceuticals by using generative models and reinforcement learning. AI broadens the range of chemical possibilities by integrating data from chemical libraries and experiments, which makes it easier to develop novel drug candidates.
- Optimizing Drug Candidate: AI algorithms can analyze and improve drug candidates by taking into account a number of aspects, including pharmacokinetics, safety, and efficacy. By using this computational method, researchers can optimize therapeutic compounds to increase their efficacy and reduce the possibility of adverse consequences.
- Drug Repurposing: Analyzing vast amounts of biomedical data to find authorized medications with therapeutic promise for various ailments is a noteworthy use of AI in drug development. AI speeds up the medication discovery process and lowers costs through this repurposing process. [14]

VI AI MODELS FOR PREDICTING BBB PERMEABILITY:

In-silico models: In-silico models of BBB penetration are growing in popularity due to the growing demand for high-throughput drug discovery techniques. However, in-vivo log BB values have served as the foundation for the majority of in-silico models. BB is a volume of distribution that represents the brain to blood ratio at a specific point in time. BBB permeability plays a significantly less role than the cytoplasmic binding of medicines in the brain. Since the amount of free drug in the brain is dictated by the total drug concentration in plasma, the PS product, and the proportion of drug in plasma that is available for transport into the brain, the BBB PS product is a better indicator of BBB permeability. However, the majority of in-silico models rely on log BB value calculations, and the absence of log PS data has hindered the creation and verification of models that forecast BBB permeability. [15] With a prevalence of about 28% across all patient age groups, neurological disorders rank among the most common medical conditions. The number of neurological disease-related mortality has risen to 39% during the past thirty years, despite a decline in communicable neurological disorders. This significant rise in the total number of patients suggests that, given the current shifting global demographics, there are few treatments available to prevent and treat neurological disorders. [16]

VII MATERIALS AND METHODS:

Formulation and Test Compounds for BBB Research Amersham provided radiolabelled sucrose, mannitol, vincristine, dexamethasone, hydrocortisone, do pamine, phenytoin, antipyrine, propranolol, diazepam, and nicotine. NEN-Dupont supplied radiolabelled urea, pirenzepine, caffeine, and lidocaine. According to HPLC analysis, all labelled substances had radiochemical purity of more than 97%. Solutions containing equivalent unlabelled compounds were mixed with aliquots of radiolabelled isotopes. Unlabelled morphine, codeine, pindolol, acetylsalicylic acid, and terbutaline were acquired from Sigma (Stockholm, Sweden) and the University of Uppsala, respectively. The test chemicals' final concentrations ranged from 0.01 to 0.1 mm. HEPES buffered Ringer's solution (NaCl 150 mM, Kcl 5.2 mm, CaCl2 2.2 mm, MgCl2 0.2 mM, NaHCO3 6 mM, Glucose 2.8 mM, HEPES 5 mM, water for injection) was used to dissolve all test chemicals. Sigma provided all of the reagents. [17]

In-vitro models:

Any in-vitro BBB model has the following benefits: it requires fewer compounds, can be assayed directly in physiological buffer, has a higher throughput than in-vivo models, can evaluate transport mechanisms, can detect early indicators of cell toxicity, and is generally less expensive. However, an in-vitro model needs to have certain fundamental features in order to accurately replicate the BBB in vivo. Table 1 provides a summary of these. The selected in vitro model should include as many of these features as possible while still being workable and practicable for screening at moderate to high throughput.

Characteristics	Specific requirements for an acceptable in-vitro BBB model
Restricted paracellular pathway	TEER>2kΩcm^{2a} Low paracellular permeability ($P_{sucrose}$~3-12 10^{-8}cm s^{-1}
Brain capillary endothelial cell characteristics	Morphology, endothelial cell and BBB markers (gamma-glutamyl transpeptidase, alkaline phosphate, enzyme expression (monoamine oxidase, angiotensin converting enzyme)
Functional expression of BBB-specific transport mechanisms	Nutrient transfer (glucose transporter, L-amino acid transporter) efflux pumps (P-gp, OATP, BRCP), receptors (transferrin, insulin), low leucocyte adherence
In-vivo modulation	Permeability altered by bradykinin, interleukins, glial factors, dexamethasone
Practicality	Availability, convenience, predictability, and reproducibility

Table 1: Basic characteristics and requirements of in-vitro blood–brain barrier (BBB) models

It has been consistently shown that a minimal trans-endothelial electrical resistance (TEER) of more than 150-200Ωcm2 is sufficient for evaluating drug permeability over the BBB in vitro, even though a TEER of 2kΩcm2 is thought to be optimum. P-gp, p-glycoprotein, OATP, organic anion transporting polypeptide, BCRP (breast cancer resistance protein), and the permeability coefficient of sucrose across the blood-brain barrier.

Bovine brain endothelial cell culture: Audus & Borchardt (1986) created the first bovine brain endothelial cell (BBEC) culture model, which has been widely utilized and described. In order to produce BBECs, the grey matter of two bovine brains is separated and processed mechanically or enzymatically to produce about 100 million viable cells. The cells don't need to be isolated every time an experiment is conducted because they can be kept at -80°C for up to two months after being isolated. To increase plating efficiency, the BBECs must first be treated with rat tail collagen before growing as primary cultures on conventional plates or inserts (Trans-well). Following cell confluence, general mechanisms of transport can be clarified and the permeability of compounds can be evaluated in both the apical-to-basolateral and basolateral-to-apical directions. This method works effectively for high throughput compound screening because of the quantity of cells available. Co-culturing endothelial cells with primary astrocytes obtained from neonatal rats is the most popular method for enhancing the barrier qualities of BBEC cultures. This approach has been shown to maintain the characteristics of the BBB without the use of stimulants, in addition to up-regulating P-gp function and significantly increasing trans endothelial electrical resistance (TEER) values (416Ω cm2 for BBECs compared with 661Ω cm2 for BBECs co-cultured with astrocytes). Since astrocytes produce several characteristics of the BBB in vivo, astrocytic foot processes invest over 99% of the cerebral vasculature in vivo. It is not surprising that co-culturing BBECs with astrocytes produces a more restrictive in-vitro BBB

model.The main drawback of this co-culturing method is that rat astrocytes must be grown and cultured in addition to BBECs. Instead of isolating and culturing rat astrocytes, C6 glioma cells can be co-cultured with BBECs, which has been demonstrated to increase the TEER by 75% and decrease sucrose permeability by 50% (Raub1996). The main drawback of using C6 glioma cells (or other cancer cells) is that they may produce a tumor-like BBB instead of a healthy BBB (de Boeretal 1999), which could result in a poor association with brain uptake in a healthy person.

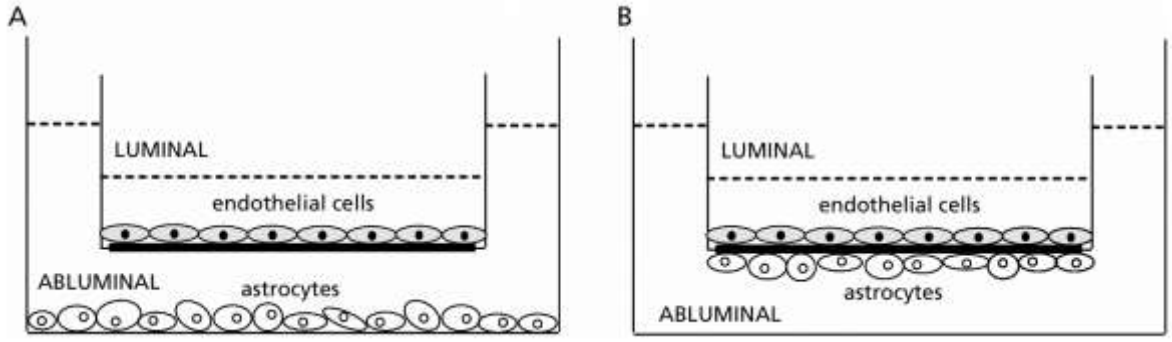

FIG:2 Schematic representation of bovine brain endothelial cells co-cultured with astrocytes using the no-contact method where the astrocytes are grown on the bottom of the multi-well plate (A), and the contact method where the astrocytes are grown on the underside of the Trans-well filter (B)

In-vivo models: Although in-vitro models are helpful for moderate-to high-throughput screening and have several benefits, in-vitro methods should not be the sole ones used in BBB research. Instead, in-vitro BBB model observations must be correlated with in-vivo research. The brain efflux index, intracerebral microdialysis, intravenous injection, single carotid injection, and in-situ perfusion are some of the in-vivo techniques that have been used to measure drug uptake into the brain. The BBB permeability–surface area (PS) product or logBB, where BB is the brain to blood ratio at a specific time, can be calculated using any of these methods. The BBB PS product, which measures unidirectional passage from blood to brain across the BBB and has units of mL min−1 g−1, is the best indicator of BBB permeability. Numerous imaging methods (such as quantitative autoradiography, magnetic resonance imaging, positron emission tomography, and single photon emission computed tomography) can be used to evaluate the transport characteristics of the BBB and are more helpful in the diagnosis of various CNS diseases, even though they are not appropriate for high throughput compound screening.

In-situ perfusion technique: An extension of the carotid artery single injection approach, the in-situ perfusion technique entails a longer experimental time with carotid artery perfusion of the brain, followed by drug level measurement within the brain. This technique involves anesthetizing the animal (often a rat) and inserting the perfusion catheter in the external carotid artery just distal to the common carotid artery's bifurcation. The perfusion fluid is fed retrogradely down the external carotid artery and up the internal carotid artery toward the brain after the ipsilateral pterygopalatine, superior thyroid, and occipital arteries are severed and ligated. The common carotid artery is cut just before the perfusion starts in order to stop the perfusion fluid from combining with systemic blood at the carotid bifurcation. The animal is beheaded after the perfusion, and the chemical concentration is measured to compute a BBB PS product. For the assessment of brain plasma volume, a reference compound, such as radiolabelled sucrose or inulin, should be added in addition to the component of interest. One of the problems with this method (and all in-vivo methods, for that matter) is that a substance that isn't in the brain's microvasculature might be there. parenchyma (transcytosis), attached to the lining of the endothelium, or found inside the endothelial cells (endocytosis). A capillary depletion approach has been devised that uses a dextran density centrifugation stage to deplete the brain homogenate of vasculature in order to distinguish endothelial binding/endothelial endocytosis from true transcytosis (uptake into brain parenchyma).This process can only be used, though, if the drug binds to the microvasculature with high affinity. If the compound is not particularly attached, it may separate from the vasculature during the homogenization process and show up in the post-vascular compartment. Therefore, when the test chemical is not linked to the brain microvascular endothelium through a high affinity procedure, the capillary depletion approach should not be employed. To separate bound and transcytosed chemicals for such compounds, a post-perfusion wash is recommended.This can be accomplished by briefly (10–30 seconds) perfusing the vasculature with physiological buffer at a rate of 4 mLmin−1. With the exception of metabolism that takes place within the brain microcirculation, the in-situ perfusion approach has the unique advantage of avoiding systemic exposure of the molecule. [18]

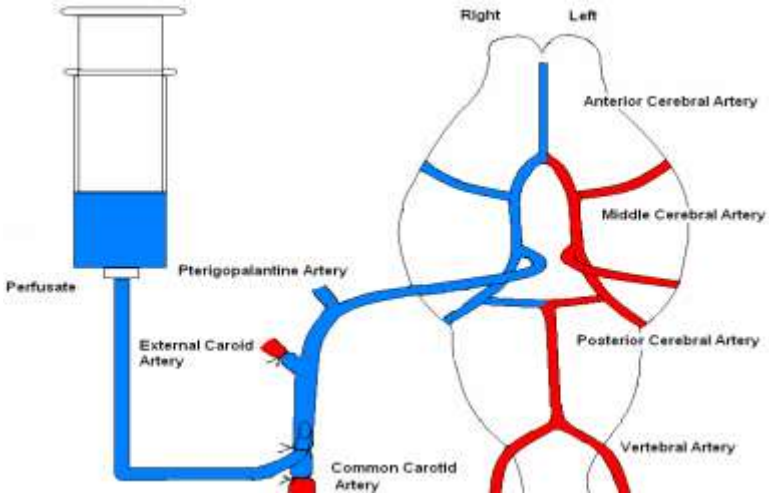

FIG:3 DIAGRAM REPRESENTING IN SITU BRAIN PERFUSION FOR PERFUSING RIGHT CEREBRAL HEMISPHERE OF RAT.

VII AI IN NANOCARRIERS AND DRUG TRANSPORT SYSTEM DESIGN:

Therapeutic medications can be adsorbed, entrapped, or covalently bonded to nanoparticles (NPs), which are solid colloidal particles with a size range of 1 to 1000 nm. NPs can be used as innovative medication delivery vehicles to various bodily regions. This is achieved by using the characteristics of the colloidal drug carrier to conceal the membrane barrier, limit the therapeutic drug molecule's characteristics, and maintain drug stability. The medicine may be able to pass through the previously impermeable membrane thanks to this disguise. The drug may be released by desorption, diffusion through the NP matrix or polymer wall, NP erosion, or a combination of any or all of these processes once the NP has reached the target region. As therapeutic drug transporters across the blood-brain barrier (BBB), NPs are currently attracting attention. [19]

Role of AI in enhancing NP drug delivery system:

The two main obstacles to the creation of new medications are decreased effectiveness and high cost. Drug development can be completely transformed by utilizing AI technologies in conjunction with processing capacity. Here, AI-driven drug development companies work with pharmaceutical behemoths. AI uses computers to mimic human intelligence for the benefit of society. Self-correction, knowledge gathering, approximated sketching, and other procedures are all included in AI.

It includes the subfields of machine learning (ML), deep learning (DL), and artificial neural networks (ANNs) and is divided into three categories: artificial general, narrow, and super intelligence. Artificial Intelligence (AI) is a vast subject of computer science that focuses on developing computers that can carry out tasks that normally require human intelligence, like solving problems, comprehending language, and identifying patterns. AI is a general phrase that includes many subfields, such as deep learning and machine learning. A branch of artificial intelligence called machine learning (ML) allows computers to learn from data and gradually improve their performance without needing to be specifically designed for every task. ML algorithms employ statistical techniques to find patterns in data and make judgments or predictions based on that information rather than hard-coded rules. DL, also known as deep neural networks, is a specialized branch of machine learning that focuses on multi-layered neural networks. These networks are capable of automatically identifying complex patterns in vast volumes of data. [20]

Predicting the BBB permeation of nanoparticles: The ability of various NPs, such as functionalized carbon allotropes, MOFs, QDs, CD-formulated medications, etc., to penetrate the blood-brain barrier is a crucial factor in determining and forecasting their potential for CNS nanotoxicity. For instance, bio conjugated QDs (CdSe and CdTe) may pass the blood-brain barrier, however the presence of cadmium in the QD composition raises concerns about cytotoxicity. In the absence of active transport, NP penetration through a lipid membrane is generally regarded as a major prerequisite for their bioavailability; failure to achieve this will typically result in lower bioavailability. The complex mechanism of BBB penetration depends on a number of variables, such as size, aggregation state, surface charge, zeta(f) potential, etc., that affect nanomaterials' capacity to enter the cell. Using the classical equation of motion for a set of interacting particles, where m and r are the mass and position of the ith particle and U is the position-dependent potential energy of the system's configuration, large-scale MD simulations have been found to be an essential tool to shed light on the

mechanisms of interactions among NPs and various components located inside or outside the cell [21]However, because of this mechanism's extraordinary intricacy, insilico modeling of NP penetration across the BBB faces significant challenges. These are primarily the constraints on the simulation's time scale and the size of the MD computational cell (number of atoms) [22] In order to lower the "calculation load" of MD simulations, coarse-grained MD models have been created and presented employing "pseudo atoms" for their presentations of the NP and membrane DNA. [23,24] Some very small or very hydrophobic NPs may just traverse the BBB, even if most NPs do not because of size constraints (>1nm) or excessive hydrophilicity (CDs, hydrophilic SiO2, etc.). Dissipative particle and discontinuous MD simulations were used to describe the latter scenario in silico (Fig. 2). Specifically, it was discovered that the 6 nm-sized coarse-grained hydrophobic NPs were spontaneously inserted into the interior of the lipid bilayer after 1.5 ms of MD simulation (Fig. 4[A]), as they directly entered the membrane and embedded themselves into the inner lipid hydrophobic core. [25] Additional findings came from examining the interactions between a 1,2-dipalmitoyl-sn-gly dipalmitoyl phosphatidylcholine (DPPC) bilayer and hydrophilic nanoparticles (Fig. 2[B]) with diameters ranging from 10 to 250 Å that were wrapped or embedded in the bilayer surface so they could interact with the hydrophilic heads of the lipid molecules. [26] The NP crossing mechanism through the BBB was further investigated using the steered MD method (SMD), which simulates the influence of magnetic force. The uncoated and insulin-coated gold nanoparticles (NPs) with a diameter of 2–10 nm were examined for their ability to interact with membranes that imitate the blood-brain barrier (BBB) made of palmitoyl-oleoyl phosphatidylcholine (POPC) and di-myristol phosphatidylcholine (DMPC). These simulations show that these gold nanostructures require a relatively constant, coating-dependent SMD force for the best NP permeability across the POPC and DMPC lipid bilayers [27,28]. Furthermore, using MD simulations and an internalization assay, the gold NPs were evaluated both in vitro and in silico for a transferrin-mediated endocytosis to effectively cross the blood-brain barrier for the treatment of solid brain tumors. Additionally, a two-compartment pharmacokinetic model for the deposition of sterically-stabilized liposomal NPs containing the anti-cancer chemical in brain tumors was created by Arnold and colleagues. This model explained the rate of drug clearance from plasma and distribution to the brain through the blood-brain barrier. [29]

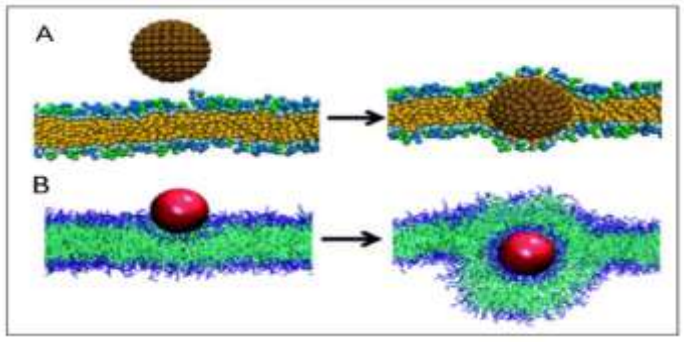

FIG:4 *In silico* modelling of NP translocation of hydrophobic (A) and hydrophilic (B) NPs in lipid bilayers as part of the BBB permeation process using dissipative particle and discontinuous MD simulations.

APPLICATIONS:

1. **Predict which small molecules cross the BBB** — ML/DL models (random forests, graph neural nets, DNNs) can classify or score compounds for BBB permeability using molecular descriptors or SMILES. These tools are already used as triage/screening steps to reduce wet-lab testing. [30]
2. **Improve accuracy with new model types and larger curated datasets** — modern methods (message-passing neural nets, transformer-based molecular encoders) show improved predictive performance versus older QSAR models when trained on carefully curated BBB datasets. [31]
3. **AI-guided design of CNS-capable molecules** — generative models and property-optimization pipelines can propose molecules with physicochemical profiles (lipophilicity, PSA, MW) that are more likely to penetrate the BBB, accelerating early drug design. Reviews of ML in drug discovery summarize these capabilities. [32]
4. **Optimize nanoparticle / carrier design for brain delivery** — ML approaches are being used to relate nanoparticle features (size, shape, coating, ligand density) to BBB uptake and biodistribution, helping prioritize nanocarriers for in-vitro / in-vivo testing. Recent experimental + review literature shows rapid progress here. [33]
5. **Integrate multi-modal data (*in silico* + in vitro)** — AI can combine computational predictions with experimental BBB models (BBB spheroids, Transwell assays, omics data) to better prioritize

candidates and suggest likely transport mechanisms. Work combining in silico prediction with experimental validation has been published. [34]

Limitations:

1. **Biological complexity of the BBB** — the BBB's active transporters, efflux pumps, tight junction dynamics, neuroinflammation and disease-specific changes mean in silico features (e.g., lipophilicity) capture only part of reality; simple predictors miss transporter-mediated or disease-altered transport. Use mechanistic/experimental follow-up. [35]
2. **Black-box models and interpretability** — many high-performing deep models lack transparent explanations. For medical decisions and mechanistic biology, interpretability / XAI methods are still needed to build trust and to suggest why a molecule is predicted permeable. [36]
3. **In vitro / in vivo mismatch** — strong performance on held-out computational test sets doesn't guarantee success in animals or humans because of ADME, immune clearance, plasma protein binding, and differences between model systems and human BBB. Experimental validation remains essential. [37]
4. **Regulatory, ethical and deployment hurdles** — clinical translation requires validation, reproducibility, explainability, and compliance with regulatory standards; slow adoption and added validation costs are realistic barriers. [38]
5. **Limited / biased datasets** — high-quality, labeled BBB permeability data (with standardized assay conditions and mechanistic labels) are comparatively scarce; models trained on heterogeneous data can overfit or fail to generalize. This is a leading practical limitation. [39]

SUMMARY AND FUTURE PERSPECTIVES:

The *Insilico* models of NP toxicity at the BBB level were covered in this review from the standpoint of computational nanotoxicology. The majority of the nanotoxicology data pertaining to the BBB level is poor, and this scientific topic is still in its infancy, despite the fact that some information is known regarding NP harmful effects in vitro and in vivo generally. However, because NPs (Au, iron oxide, QDs, CNT, etc.) as synthesized require ligands (polymer coatings in general) to ensure colloidal stability, it is still difficult to correlate the physicochemical properties of the NPs with their bioactivity.

More importantly, these NPs eventually interact with other "free" proteins and biomolecules in physiological fluids (to form "coronas") in addition to membrane proteins and membranes. With very few exceptions because of anti-fouling coats, these bio-nano-interactions may control the uptake of NPs and their nanotoxicity. They are generally an important but little-studied topic. Both hydrophilic and hydrophobic NPs can be used as drug delivery vectors to facilitate drug permeations across the blood-brain barrier (BBB) because numerous prior studies have shown that NPs can avoid and cross the BBB because of their small size and larger surface area (i.e. improved reactivity)

The later ones, which may pass through lipid bilayers but combine to cause cytotoxicity, are of special interest. The significance of the specific lipid compositions presents in the brain's microvascular endothelial cells and the significance of tight junctional proteins (ZO-1, claudin-5, occludin, VE-cadherin, etc.) and active transporters (P-gp, BCRP, and BBB-ChT) for the BBB transport should be examined in future perspectives on in silico NP modelling at the BBB.

To compute log BB, PS, and other parameters for the prediction of NPs permeation across the BBB, new in silico tools and algorithms for QSAR and ADMET need also be created. Microsecond or even millisecond time scales of MD simulations may be crucial for the study of NPs aggregation and their interactions with lipid membranes. Additionally, to increase the accuracy of such simulations, more optimal force-field parameters are needed. [40]

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