Metallic nanocarriers for therapeutic peptides: Emerging solutions addressing the delivery challenges in brain ailments

Shanmuga Sharan Rathnam a, Thirumalai Deepak b, Badri Narayana Sahoo a, Tanishq Meena a,
Yogesh Singh a, Abhijeet Joshi a

a- Department of Biosciences and Biomedical Engineering, Indian Institute of Technology Indore, Simrol, Madhya Pradesh-453552, India.
b- Department of Biotechnology and Medical Engineering, National Institute of Technology Rourkela, Rourkela, Odisha-769008, India.
Running title:

Metallic nanocarriers for therapeutic peptides to the brain.

Corresponding Author Information:

Dr. Abhijeet Joshi, Ph.D.
Department of Biosciences and Biomedical Engineering
Indian Institute of Technology Indore
Khandwa Road, Simrol, Indore, Madhya Pradesh-453552, India.
Email: abhijeet.joshi@iiti.ac.in; Phone: 07316603344

Number of text pages: 58
Number of tables: 3
Number of figures: 3
Number of references: 158
Abstract word count: 227
Introduction word count: 379

Non-standard abbreviations:

Abstract

Peptides and proteins have recently emerged as efficient therapeutic alternatives to conventional therapies. Although their advent a few decades back, extensive exploration of various ailments or disorders began recently. The drawbacks of current chemotherapies and irradiation treatments, such as drug resistance and damage to healthy tissues, have enabled the rise of peptides in the quest for better prospects. The chemical tunability and smaller size make them easy to design selectively for target tissues. Other remarkable properties include antifungal, antiviral, anti-inflammatory, protection from hemorrhage stroke, and as therapeutic agents for gastric disorders, Alzheimer’s, and Parkinson’s diseases. Despite these unmatched properties, their practical applicability is often hindered due to their weak susceptibility to enzymatic digestion, serum degradation, liver metabolism, kidney clearance, and immunogenic reactions. Several methods are adapted to increase the half-life of peptides, such as chemical modifications, fusing with Fc fragment, change in amino acid composition, and carrier-based delivery. Among these, nano-carrier-mediated encapsulation not only increases the half-life of the peptides in vivo but also aids in the targeted delivery. Despite its structural complexity, they also efficiently deliver therapeutic molecules across the blood-brain barrier (BBB). Here, in this review, we tried to emphasize the possible potentiality of metallic nanoparticles to be used as an efficient peptide delivery system against brain tumors and neurodegenerative disorders.

Significance Statement

In this review, we have emphasized the various therapeutic applications of peptides/proteins, including antimicrobial, anticancer, anti-inflammatory, and neurodegenerative diseases. We also focused on these peptides' challenges under physiological conditions after administration. We
highlighted the importance and potentiality of metallic nanocarriers in the ability to cross the BBB, increasing the stability and half-life of peptides, their efficiency in targeting the delivery, and their diagnostic applications.

**Keywords:** Nanoparticles, glioblastoma cells, Alzheimer’s disease, Parkinson’s disease, targeted drug delivery, therapeutic peptides
1. Introduction

The proteins have the most complex and diverse role in the body, both structurally and functionally. They are responsible for extracellular and intracellular structural support, cell signaling cascading pathways, mediating the passages across membranes, catalysis, and many more (College 2023). The therapeutic peptides usually have a size of less than 5000 Da with a specific sequence of amino acids. The significance of therapeutic peptides/proteins in the pharmaceutical industry started after the therapeutic success of the first-ever synthesized peptide, i.e., insulin, which was synthesized through recombinant DNA technology (Sun, Xu et al. 2022). It is a 51-amino acid peptide. After that, hundreds of peptides and proteins were identified, characterized, purified, and explored for their potential therapeutic applications. Special features of therapeutic proteins/peptides make them uniquely advantageous over small molecule drugs (Leader, Baca et al. 2008). Firstly, the physiological functions of proteins are too complex and specific to be mimicked by small-molecule drugs. Also, it is unlikely that a therapeutic peptide/protein creates adverse effects by interfering with other physiological functions. Thirdly, these are less sensitive to immune responses since innumerable protein derivatives are naturally synthesized by the body for therapeutic functions. Also, these therapeutics could serve as replacement therapy for genetically altered proteins without gene therapy. Finally, it is faster to develop and obtain approval compared to conventional drug molecules clinically. The therapeutic proteins could be classified into various types depending on their molecular type. They are classified into growth factors, hormones, enzymes, antibody-based drugs, interleukins, interferons, and engineered protein scaffolds (Dimitrov 2012). However, the peptides, being smaller, in size could be synthesized according to the need and specificity. Hence, to synthesize peptides for therapeutic use, they need to be identified first. They are done by their
characteristics such as their natural bioactivity and phage display. Among these, the latter is the highly efficient method in which recombinant technology modulates target ligands on the cell surface of bacteriophages. Once the peptide is identified, they are synthesized through chemical routes using solid-phase peptide synthesis. Several chemical modifications are also adapted, such as backbone modification, side chain modification, PEGylation, and genetic code expansion, to meet the specific requirements of their functions. An overview of the roadmap of therapeutic peptides, from their synthesis to application, is depicted in Figure 1.

2. Various therapeutic roles of peptides/proteins

The therapeutic peptides were initially made in order to mimic the native functions of hormones/growth factors and were principally derived from natural sources (Wang, Wang et al. 2022). However, the advancements in molecular biology, protein chemistry, genomics, and delivery strategies have extended their therapeutic applications. This has been achieved because of the recent infrastructure advancements, which can synthesize peptides with desired biochemical properties and physiological functions. The wide range of therapeutically applicable areas include cancer, treating infections, hemorrhage stroke, gut disorders, metabolic disorders, and vaccine production. The wide range of applications of peptides is depicted in Table 1. Some of these have been discussed in successive sections.

2.1 Antimicrobial properties:

Fungi have played a tremendous role in shaping our current social lives. They have been quite beneficial for us by being the source of many antibiotics and also in food fermentation and processing from time immemorial. However, on the other side, these are also responsible for deadly human infections (Fernández de Ullivarri, Arbulu et al. 2020). Some of these include community-acquired and hospital-emerged ones. The recently emerged antifungal resistance
might be one of the main driving forces in the quest for novel therapeutic compounds to combat these infections.

Moreover, the drugs that act on common pathways to inhibit or lyse the fungal cells may also be detrimental to any human host cells because of the eukaryotic nature of fungi, unlike bacteria. In this regard, therapeutic peptides/proteins might serve as potential candidates. The resistance to antimycotic drugs is mainly due to their biofilms’ architecture and local hypoxia environment(Kowalski, Morelli et al. 2020). Cm-p5 is obtained naturally from the mollusk Cenchritis muricatus and is known to exhibit minimal toxicity in mammalian cells(Lópe-Abarrategui, McBeth et al. 2015). Dennis et al. demonstrated that the derivatives of Cm-p5 peptide successfully inhibited the biofilm formation of the yeast Candida auris and significantly led to the growth arrest of matured biofilms(Kubiczek, Raber et al. 2020). The same antimycotic ability was observed in MCh-AMP1, derived from Matricaria chamomilla. The peptide showed high toxicity against the life-threatening nosocomial candidiasis causing Candida albicans by inducing intracellular reactive oxygen species (ROS), increasing membrane permeability, and deformation(Seyedjavadi, Khani et al. 2020).

The antifungal peptides are also released into the environment as part of the competition among various species. The peptide BbAFP1, is expressed in the cell walls and released by Beauveria bassiana, inhibits the local species of Alternaria. It was shown that this peptide has glucan and chitin-binding sites and is responsible for inducing intracellular ROS and membrane damage in several phytopathogenic fungi. The transgenic tomato plant expressing this peptide also displayed fungal pathogen resistance(Tong, Li et al. 2020). Several food products are prone to fungal contamination, especially in humid environments, and cause food spoilage and exposure to fungal toxins in humans and animals(Thery, Lynch et al. 2019). Osmotin, a cationic protein, is
proven to possess antifungal properties by disrupting the membranes and affecting the membrane potential of infecting fungi (Bashir, Silvestri et al. 2020) and is used for the food preservation of several fruits and vegetables (Manghwar and Hussain 2022). Viral infections are responsible for serious health threats to animal and human welfare. The viruses are highly adaptive, and there are limited efficient therapeutics available to treat the most deadly viral infections (Agarwal and Gabrani 2021). In this context, peptides may assure better efficiencies due to their high selectivity and less toxicity to host cells. Lee et al. have developed 12 novel peptides which mimicked the amino acids of the Dengue virus envelope protein, which is crucial to bind to the receptor and fuse into the host (Lee, Anasir et al. 2023). One of the peptides has been shown to display the highest inhibitory level against all 4 Dengue serotypes. Rutger et al. showed that the CPXV012 peptide, which is derived from cowpox virus protein, hindered the infection of several enveloped viruses such as HIV, Hepatitis B, and pox viruses (Luteijn, Praest et al. 2020). This cationic peptide is responsible for interacting with the anionic regions of phosphatidyl serine of these enveloped viruses and hampering infections. Shanmugaraj et al. have proposed several monoclonal antibodies, such as CR3014, 80R, CR3022, and m396, which bind to the conformational epitope of the S1 fragment of SARS-CoV-2, and their potential to be used as antibody therapy (Shanmugaraj, Siriwattananon et al. 2020).

Combating drug-resistant bacterial infections is one of the most challenging tasks recently. In this context, antibacterial peptides might be potential candidates replacing traditional antibiotics, due to their broad spectrum. Wang et al. have newly designed a peptide Cu-GGH-AMP, which has a C-terminal KKLRLKIAFK, that is less susceptible to antibiotic resistance. It possesses an N-terminal Cu-GGH complex that could induce oxidative cleavage of bacterial DNA in the presence of ascorbic acid (Wang, Li et al. 2023). In another study, Huang et al. showed that
antimicrobial peptide NZ2114 when incorporated in hydroxypropyl cellulose-based hydrogels, promoted wound healing infected by Staphylococcus aureus. The hydrogel enabled wound closure by enhancing the production of angiogenic-based growth factors (Huang, Yang et al. 2022). Similarly, a peptide RP557 induced death of Mycobacterium abscessus. It inhibited biofilm formation by affecting peptidoglycan synthesis due to the down-regulation of nitrogen metabolism (Li, Zhang et al. 2022).

2.2. Therapeutical applications for physiological disorders

The idea of the therapeutic application for peptides started from the cure of diabetes itself. Since the approval of recombinant insulin, there have been many other therapeutic peptides to be proven as potential candidates. Out of them, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are well-studied for their glucose-regulating activities (Holst 2019, Holst and Rosenkilde 2020). Zhang et al. have demonstrated that RGD peptide hydrogel increased the stability and retention of mesenchymal stem cells-derived extracellular vesicles (EVs) in vivo. The EV-RGD hydrogel has enabled kidney cell proliferation and reduced the tubular injury and pathological damage when administered in kidney injury mice model (Zhang, Shang et al. 2020). Another exciting application of therapeutic proteins is for haemorrhagic stroke. Shao et al. have highlighted the potential of heat shock proteins, HSP20 and HSP27, in subarachnoid haemorrhage (Shao, Zhou et al. 2019). Both are analogues in their structure, but their phosphorylation function is different. The neuroprotective nature of these HSPs enables them to become potential therapeutic candidates, which were failed by most of the standard drugs. Protein-protein interactions (PPIs) are fundamental in regulating signal transduction, and the basis for several pathological phenomena (Cabri, Cantelmi et al. 2021). When these are dysregulated, it might lead to several pathological abnormalities. One such
approach could be observed in the case of autoimmune responses. Difelikefalin acetate is a pentamer peptide designed against uremic pruritus, generally observed in most dialyzed patients. It is an agonist for the K-opioid receptor, and it inhibits its entry into the central nervous system and activation of anti-opioid receptor, thereby controlling the patient's itch and discomfort (Fishbane, Mathur et al. 2020). Another such example is noted in the case of LSALT peptide, which is a 16-mer peptide designed to suppress the unwanted autoimmune response by neutrophils through the enzymatic activity of DPEP1 dipeptidase in lungs (Cabri, Cantelmi et al. 2021).

2.3 Therapeutic applications for neurodegenerative diseases

Alzheimer’s disease is characterized by intellectual disability, loss of memory, and difficulty in thinking and learning (Zhang, Zhang et al. 2022). It is a progressive neurodegenerative condition, that has no permanent cure yet. Peptide-based therapy might be a better strategy because of its high specificity. Recently, Scala et al. have designed a peptide, AmyP53, which targeted amyloid receptors, i.e., gangliosides (Di Scala, Armstrong et al. 2022). The peptide was able to reach the brain through both intranasal and circulatory routes. Mockett et al. have highlighted the prominence of soluble amyloid precursor protein alpha (saPP-α) and cationic arginine-rich peptides in neuroprotective and neurotropic perspective (Mockett and Ryan 2022). Since the practical application of saPP-α is complex due to its relatively larger size, the neuroprotective domains were identified, which might be the potential targets to look for.

Parkinson's disease is characterized by the loss of dopaminergic neurons, loss of dopamine and often affects movement (Guo, Zhao et al. 2018). Most of the drugs do not cure the progressiveness of the disease, but rather provide symptomatic relief. The peptides from natural sources might be an alternative strategy to get adapted. Ren et al. showed that an octamer peptide
derived from sea shells, Neptunea arthritica cumingii protected the dopaminergic neurons from damage and synuclein aggregation by controlling oxidative stress (Ren, Jiang et al. 2022). It was also observed from the previous studies that Parkinson’s and diabetes share common unmodulated insulin pathways. It was shown that the dual administration of GLP-1, and glucose dependent insulinotropic peptide were able to cross BBB, and improved dopaminergic synapses, protected from damage (Yang, Feng et al. 2022). In a review by Behl et al., they highlighted the significance of the neuroprotective abilities of different peptides and proteins against Parkinson’s disease (Behl, Madaan et al. 2022). These include neuropeptide Y, neurotensin, ghrelin, pituitary adenylate cyclase-activating polypeptide (PACAP), and substance P. The expression levels of these neuroprotective proteins were observed to be changed in the disease-specific areas, along with their G protein-coupled receptors. These peptides resulted in the reduction of apoptosis, inflammation, oxidative stress, and toxicity in the neurons.

2.4 Therapeutic peptides for cancer

A successful cure for the cancer is still a long-lasting vision. Conventional drug treatments through chemotherapy, or radiation treatment are not quite efficient in complete removing tumours (Sedighi, Zahedi Bialvaei et al. 2019). The resistance due to the adaptability of the tumour tissues to the available drugs, and the physiological complexity of the solid tumours make a successful treatment as a long-distant dream, in the current scenario. The focus of recent research is on providing effective strategies to combat this. Antibodies have also emerged as therapeutic molecules, and are used in conjugation with drugs to selectively deliver them (Tarantino, Carmagnani Pestana et al. 2022). However, their relatively larger size makes them practically difficult to administer. Hence, in this regard, the peptides could stand out as potential prospects, because of their unique advantages. These are smaller in size, which makes
their composition and properties easily tunable, and could be efficiently designed according to their specificity (Chiangjong, Chutipongtanate et al. 2020). The peptide fractions from the natural sources have been a common source. Zahra et al. have isolated bioactive peptides from the skin of rainbow trout fish, using flavourzyme treatments (Yaghoubzadeh, Peyravii Ghadikolaii et al. 2020). The hydrolysed protein fractions exhibited both antioxidant and anticancer properties. The fractions showed high cytotoxicity towards colorectal cancer cell lines. Similar findings were observed by Zohreh et al. They have hydrolysed the wheat germ protein with three enzymes, i.e., alcalase, pepsin, and proteinase K. The hydrolysates were found to be possessing antitumor properties against lung cancer cells, along with high antioxidant behaviours (Karami, Peighambardoust et al. 2019). Chunye et al. have highlighted the potential of several antimicrobial peptides (AMPs) displaying anticancer properties against liver cancer (Zhang, Yang et al. 2019). Modifications of AMPs such as polymerization, cyclization, fragmentation, and hybridization could achieve this. These peptides upon modification might show cytotoxicity against hepatocellular carcinoma.

Similarly, it was also reported that few AMPs showed minimal systemic toxicity, and are highly targeted in displaying toxicity against non-small cell lung cancer (Kunda 2020). It was reported that the anticancer properties of peptides were dependent on their secondary structure. The cationic amphiphilic peptides, with secondary structures in hydrophobic domains enabled higher cellular uptake by cancerous colorectal cells and cervical cells compared to normal fibroblast cells (Hadianamrei, Tomeh et al. 2022).

Targeting peptides, especially for the brain tumour case is in high demand due to the selective complexity of blood-brain barrier (BBB). One such approach was made by Niklas et al., where they have synthesized a dg-brevican targeting peptide (BTP7). Dg-brevican is an isoform of
brevican, and is highly expressed in the extracellular matrix of glioma cells. The BTP was successfully able to cross BBB and was internalized by glioma cells, and displayed toxicity in both *in vitro* and mice models (von Spreckelsen, Ghotmi et al. 2019). Angiopeptide-2 (angiopep-2) is a 19-residue peptide that binds to LRP-1, and the angiopep-2 based drug delivery system is one of the principal gateway systems adopted for the delivery to the central nervous system (Habib and Singh 2022). Recently, it was reported that angiopep-2-modified carboxymethyl chitosan nanogels were able to cross BBB and efficiently deliver doxorubicin (Selvarathinam, Thekkumalai et al. 2021).

### 3. Challenges of delivering therapeutic peptides

As discussed, peptides and proteins have emerged as potential biotherapeutic compounds in most healthcare and medicine fields. They have drawn significant attention of the scientific community to explore and find their way into diverse areas. Despite these potential advantages, these molecules possess some of the critical challenges, which must be addressed in order to make them ideal candidates for practical medical applications. The challenges may exist in any of the stages of therapeutic peptide development, i.e., synthesis, processing, stability or delivery (Wu, Sahoo et al. 2022). These molecules are highly subjected to enzymatic degradation, stomach hydrolysis, liver detoxification, or renal clearance, especially when administered orally or by circulatory routes. Anna *et al.* reported that the CREKA pentapeptide, which is predominantly used in the diagnosis of cancers, was engineered to replace glutamate with N-methyl glutamate to be stable against proteolytic degradation. The engineered peptide was loaded onto a conducting polymer, PEDOT, for the electrical stimulative administration (Puiggali-Jou, Del Valle et al. 2020). The oral delivery of therapeutic peptides is quite challenging, due to their exposure to gastric fluids, which makes the enteric release and
absorption inefficient. Yuting et al. have encapsulated five model peptides into complex coacervated matrices composed of succinic acid, alginate, and gelatin. The release of the peptides was not observed in water, or simulated gastric fluids, but in simulated intestinal fluid(Tang, Arbaugh et al. 2023). In another study, the therapeutic insulin was protected against enzymatic degradation from chymotrypsin by PEG-8/capric glycerides, which formed a self-emulsifying drug delivery system(Liu, Hirschberg et al. 2020). Exploiting fragment crystallizable (Fc) region for the transcytosis is famously adapted to increase the half-life and cross BBB(Kariolis, Wells et al. 2020). There are several protein therapeutics that were stabilized by increasing their half-life times. Through Fc fragment fusion, the half-life of the granulocyte colony-stimulating factor was increased from 3.5 h to more than 48 h. Similarly, the half-lives of glucagon-like peptide-1 (from 2 min to 5 days), and factor IX (from 20 h to 82 h) were also extended(Zaman, Islam et al. 2019).

4. Bringing in metallic nanoparticles as peptide/protein delivery vehicles

Considering the problems that researchers face with the traditional modes of drug delivery systems, we now discuss the potential of nanoparticles as effective delivery vehicles. The nanoparticles must be in the range of 1 to 100 nm in at least one dimension, as per the International Organization for Standardization (ISO), and American Society for Testing of Materials (ASTM). Several properties of nanomaterials aid their consideration as delivery vehicles. These properties include but are not limited to low cytotoxicity or improved biocompatibility, ease of tunability of properties (and therefore, the functionality), their penetrating ability, smaller size, higher retention inside the body, considerable in vitro stability, possible inertness, and cellular uptake(Huynh, Pham et al. 2020). Smaller size reflects a better surface area-to-volume ratio, producing more rapid and efficient drug release(Zahir, Anwar et al. 2020).
The shape and size of the nanoparticles determine several factors such as their passage through the blood vessels, their circulation time, filtration by kidneys, their uptake by the cells at the target site, and the release of the drug at the intended site. Surface properties would determine the kind and extent of interaction with the biological structures they come across. Surface charge and hydrophobicity are two significant properties that influence interactions like opsonization/phagocytosis, distribution, and circulation. A negative surface charge has been reported to be better because it reduces phagocytosis, thereby increasing circulation. Positively charged NPs, on the other hand, promote phagocytosis (Zahin, Anwar et al. 2020). Various nanostructures, including dendrimers, liposomes, nanoceramics, nanogels, quantum dots, metallic and polymeric materials have been used in targeted drug delivery. Among these, metal-based nanoparticles have gradually become interesting candidates for diagnostic and therapeutic purposes. They possess unique properties which make them advantageous over others to be used for targeted delivery of therapeutic molecules. Likewise, any other class of nanoparticles, they have characteristics that depend on their composition, shape, and the mode of their synthesis. In the following sections, we emphasize on the synthesis, properties and drug delivery applications of various metallic nanoparticles, due to their significant abilities to cross BBB.

4.1 Metal Nanoparticles

In recent times, metal nanoparticles have emerged as irreplaceable drug delivery systems. The metal nanoparticles are sustainable agents for use in the field of biomedical applications due to their tiny size, shape, bio-compatibility, and moderate or low level of cytotoxicity. Some metal nanoparticles are also known for their unique applications, such as bioimaging, photo-thermal therapy, photo-dynamic therapy, and ferroptosis against cancer cells. The metal nanoparticles can easily be functionalized with antibodies, proteins, sugar moieties, and ligands which
specifically bind with the receptor of the targeted cell type. Here, in the following sub-sections, we discuss about the properties, synthesis routes and the prominence of the most used metal nanoparticles in the field of drug delivery.

4.1.1 Iron nanoparticles

Ferrous-based nanoparticles play a major role in various biological and non-biological perspectives. These are sustainable, effective, and could be synthesized with minimal resources and effort. They are known for biomedical applications such as potential drug carriers, magnetic hyperthermia-induced killing of cancer cells, and as contrasting agents in magnetic resonance imaging (MRI) for diagnostic purposes (Montiel Schneider, Martín et al. 2022). These are synthesized by several techniques; which broadly come under either of the top-down approach or bottom-up approaches. The former approach addresses on the physical method of synthesis which focuses on breaking bulk materials by crushing and grinding to its tiny form by mechanical actions (O’Carroll, Sleep et al. 2013). It also includes mechanical, milling, laser ablation, and sputtering, in order to obtain fine nanoparticles. In contrast the latter approach involves the synthesis based on chemical reactions of smaller molecules and their scaling up to synthesize nanoparticles. In general, chemical synthesis routes yield better in comparison with physical and biological methods. The chemical synthesis includes co-precipitation, thermal decomposition, hydrothermal process, sol-gel method and micro-emulsion techniques (Crespi, Quici et al. 2016). The co-precipitation method is the most popular among them due to its simplicity and inexpensiveness. To avoid the level of toxicity, researchers are now focused on green synthesis of iron nanoparticles. In this method of synthesis, bioreagents are used as precursor agents instead of harsh chemicals. It has been shown that several plant extracts have been used for the formation of nano-irons. The brief description is depicted in Table 2.
Ferric nanoparticles synthesized by co-precipitation generally range from 11-20 nm in size (Vargas-Ortiz, Gonzalez et al. 2022) with the spherical shape. Magnetic nanoparticles with different size ranging from 1-100 nm can show superparamagnetic properties (Rikken, Nolte et al. 2014). The magnetocaloric effect is another interesting aspect in which, when a magnetic nanomaterial is placed in between a magnetic field, it causes vibration due to the existing magnetic field, resulting in heat generation. This can be useful for eradicating cancer cells by generating magnetic hyperthermia-induced killing (Giustini, Petryk et al. 2010). Magnetic nanoparticles can be useful for several other applications like separation, sensing, drug delivery, diagnosis and therapy. The magnetic nanoparticles are used in the form of beads for the separation of several bio-molecules like DNA, RNA, and proteins (Pérez, González-Martínez et al. 2020). These particles are also used as magnetic resonance imaging (MRI) contrast agents and in labelling, identification of cells, and diagnosis (Joshi and Joshi 2022). Tracking of the transplanted cells in vivo is quite challenging, to view the real-time progress of the functions. In this context, it was recently reported that stem cells labelled with iron nanoparticles could be tracked through MRI for 21 days at the dermal matrix of burn wounds (Mehrabani, Nazempour et al. 2022). Another phenomenon of magnetite is the generation of local heat when subjected to an alternating magnetic field. This hyperthermia is used for ablation therapies, to kill cancer cells. Jabir et al. demonstrated that superparamagnetic magnetite nanoparticles exhibited alternating magnetic field induced cytotoxicity to ovarian cancer cells, resulting in apoptosis (Jabir, Nayef et al. 2019). Due to their excellent physicochemical properties and tunability, they are also helpful to load and deliver several kinds of drugs to the target sites. Ebadi et al. showed that the sorafenib-loaded iron nanoparticles coated with polyvinyl alcohol exhibited specific cytotoxicity against cancerous liver cell lines, compared to normal fibroblasts (Ebadi, Buskaran et al. 2021).
They are also known as delivery agents especially to the tissues inside brain, which is otherwise challenging, due to BBB. Likewise, from a study it was shown that the superparamagnetic nanoparticles increased the bio-availability of quercetin in the case of a malignant brain tumour. The iron particle along with quercetin was able to cross the BBB to deliver quercetin, for efficient promising cancer therapy(Enteshari Najafabadi, Kazemipour et al. 2018).

4.1.2 Gold Nanoparticles

Researchers are fascinated with the unique chemical properties of gold nanoparticles, which are subjected to broad applications like bio labelling, enhanced optical imaging and drug delivery to different parts of the bodies(Jain, Hirst et al. 2012). Here we focus more towards biological application of gold nanoparticles. The Au NPs are used vastly in biomedical applications due to their ease of synthesis, functionalization, compatibility, and superior surface plasmon resonance properties(Hu, Chen et al. 2006). It is also highly accessible for functionalization with a broad spectrum of molecules like antibodies, ligands, receptors, oligonucleotides, drugs, and growth factors for target-specific interaction(Yeh, Creran et al. 2012). The unique property of gold nanoparticles is that they accumulate inside the target cancer cells and could be display optical scattering. Several methodologies have been adapted to synthesize gold nanoparticles, which include chemical, electrochemical, thermal, and sono-chemical methods(Yu, Chang et al. 1997, Mandal 2014). Among them, the chemical methods are easiest, provide better yield, and also the particles formed are relatively more uniform and stable in aqueous systems. The chemical synthesis method includes two different steps, i.e., the first method comprises the reduction, followed by stabilization(Zhao, Li et al. 2013). The chemical reduction method involves reducing agents like formaldehyde, hydroxylamine, citric acid, oxalic acid, sugar and carbon monoxides. The stabilization method involves stabilizing agents like trisodium citrate dihydrate,
oxygen and nitrogen-based ligand compounds, polymers, and cetyltrimethylammonium bromide (CTAB) to minimize agglomeration. Turkevich method is a well-optimized reduction-based method used in the recent times. Here, the gold nanoparticles are synthesized using hydrogen tetrachloroaurate (HAuCl4) and trisodium citrate dihydrate with rapid agitation in boiling water (Kimling, Maier et al. 2006). In order to minimize the long-term toxic effects of these particles, using harsh chemicals can be avoided, using natural compounds and plant extracts as reducing agents. The natural compounds used as reducing agents also yielded nanoparticles with controlled size and shape (Table 2).

Generally, it has been reported that the gold nanoparticles synthesized by Turkevich method are more stable and sustainable. The size range of the nanoparticles formed by this method is within 20nm (Frens 1973). Gold has a vast range of applications in multiple fields. The large surface-to-volume ratio, impressive bio-compatibility and low toxicity are the main reasons to consider Au NPs as qualitative emerging tools in the field of bio nanotechnology. Apart from that the surface plasmon resonance action and the quenching ability to fluorophore enhances its susceptibility towards biomedical applications. It is also used for SERS analysis with Raman spectroscopy. They can be surface functionalized with protein, ligands, sugars or any specific moieties. There are several theranostic applications of these nanoparticles. Gold nanoparticles conjugated with a transactivator can deliver doxorubicin, which is an anticancer drug and gadolinium to the glioblastoma. Doxorubicin has shown anticancer effect and in addition to that, brain tumour imaging was possible by gadolinium for a prolonged period due to its low retention time, after the particles successfully crossed BBB (Joh, Sun et al. 2013). It has been seen that the radiotherapy accompanied with gold nanoparticles cause enhanced cellular DNA damage that is carried by ionizing radiation in the glioblastoma-derived cell line of human. It has resulted
reduced clonogenic viability and survival in a dose dependent manner (Lai, Ko et al. 2015). There are other nanostructures of gold nanomaterials, such as nanoflowers and nanorods, which are proven to have good potential to be used to target brain ailments. Daniel et al have showed that gold nanoflowers, when conjugated with the drug levodopa (L-DOPA), were able to penetrate BBB effectively. Further, these nanocarriers did not induce inflammation in brain macrophages (Gonzalez-Carter, Ong et al. 2019). The conjugation of gold nanorods with angiopep-2, enhanced their biodistribution and promoted accumulation in brain parenchyma (Velasco-Aguirre, Morales-Zavala et al. 2017).

4.1.3 Silver nanoparticles

Silver nanoparticles (Ag Nps) are also one of the metallic nanomaterials in demand for various sectors like healthcare, food, and industrial applications. These are also commonly used for nanomedicine which is applicable in the biomedical sector. The biological effectivity of the Ag Nps depends upon the shape, size, agglomeration, dissolution rate and toxicity (Iravani, Korbekandi et al. 2014). They have been extensively used as strong antimicrobial agents. Further, these nanoparticles are known for their cytotoxicity against cancer cell lines (Locatelli, Naddaka et al. 2014).

Like other metal nanoparticles, the synthesis of Ag Nps also requires three major materials, i.e., the metal salt precursor, reducing agent and the stabilizing agent. The chemical reaction occurs for the reduction of silver salts such as nitrate in the presence of reducing agents like citrate, ascorbate, borohydride. The surfactants are used as stabilizing agents such as polyvinylpyrrolidone, and polyethylene glycol (Oliveira, Ugarte et al. 2005). The conformation of these nanoparticles are highly dependent on the initial concentration of silver precursor, molar ratio of reducing agent to the precursor, and the ratio of the stabilizing agents. Ag Nps can also
be prepared using plant extract or microbes for reducing silver nitrate in a proposed manner. The phytochemicals, which are predominantly present in the plant extracts, are used for this purpose (Table 2). Apart from this, even microbes are also used for synthesis of Ag Nps (Gudikandula and Charya Maringanti 2016).

The Ag Nps that are formed by different synthesis methods vary each other by their size. Generally, the size range of the Ag Nps those are formed by chemical method ranges within 10 nm. The characteristic absorbance maxima of Ag Nps is 410 to 430 nm (Iravani, Korbekandi et al. 2014). It has been shown that Ag Nps that are formed by green synthesis technique using cannonball leaves, possess size about 25 nm and the morphologies are variable as spherical triangle, truncated triangles, and decahedral (Devaraj, Kumari et al. 2013). The Ag Nps comprise of numerous properties like antibacterial, antiviral, anti-tumour, antifungal, and anti-inflammatory. They also serve as drug carriers for target specific drug delivery to different tissues due to its controlled cytotoxicity and smaller size.

4.1.4 Zinc oxide (ZnO) Nanoparticles

ZnO has been classified as GRAS (Generally regarded as safe) by the US FDA (Anjum, Hashim et al. 2021). Their safe nature makes them suitable for a variety of applications. These include their recent use as additives in food products, ointments, cosmetics, lubricants, and drug carriers. They have excellent UV – adsorbing ability which is why they are in most common sunscreens. The mode of their synthesis determines the shape and size of these NPs (Technology. 2023). Various methods of preparing ZnO nanoparticles have been reported in the literature. They can be synthesized using various chemical, physical, or biological methods. These include solochemical method (Souza, Haberbeck et al. 2019), sol-gel method (Hasnidawani, Azlina et al. 2016), and hydrothermal method among others (Gudkov, Burmistrov et al. 2021). Similar to other
metallic nanoparticles, ZnO are also synthesized through green synthetic routes. ZnO nanostructures occur as a white powder which is water insoluble. They are also known to be excellent antimicrobial agents. Proposed mechanisms of their antimicrobial activity include disruption of cell structure, generation of reactive oxygen species, altering the activity of DNA and proteins, and regulating gene expression. Sundraraman et al. loaded taxifolin in ZnO NPs and tested the system for targeted drug delivery in MCF-7 cell line (Sundraraman and Jayakumari 2020). Compounds like curcumin, quercetin, and naringenin have been loaded in ZnO NPs and tested against cancer cell lines (Anjum, Hashim et al. 2021). Zn nanostructures have reportedly been used in treating brain tumours. ZnO NPs are found to reach the brain either by crossing the BBB or by transport via neurons (Mukhtar, Bilal et al. 2020). A study reported the potential of ZnO NPs in countering neurodegenerative disorders (Ashraf, Ansari et al. 2018). Hawwary et al. reported the anti-Alzheimer potential of green synthesized ZnO NPs (from Sabal blackburniana) by evaluating their acetylcholinesterase activity. The data showed a positive result revealing the anti-Alzheimer potential of the mentioned NPs (El-Hawwary, Abd Almaksoud et al. 2021). Mousavi et al. investigated the effect of ZnO NPs on neuroblastoma cells and found that these bind to Tau P proteins and alter their structure. Alteration of Tau P structure leads to them losing their microtubule-binding ability. This reduces the viability of neuroblastoma cells (Mousavi, Fard et al. 2021).

4.2 Challenges in translating metal nanocarriers in healthcare

Despite promising biomedical applications, the metal nanocarriers also display few unexpected threats once administered to the body. Physicochemical characteristics of NPs especially the size, shape, surface chemistry, dispersibility in aqueous fluids, and agglomeration, determine their toxicity (Zhang, Xiong et al. 2022). These properties significantly affect the biological
performance of nanocarriers such as inducing ROS production, oxidative stress, apoptosis, genotoxicity, and loss of membrane integrity. The persistence of the metallic nanocarriers inside the body post medical action, is one of the major contributing factors in preventing translational use (Cassano, Pocovi-Martínez et al. 2017). For this purpose, there are several methods deployed to mitigate the unwanted outcomes post administration. Some of them include surface coating with biocompatible polymers (Patsula, Tulinska et al. 2019), usage of naturally derived membranes (Rao, Xu et al. 2016), modifying charge density and hydrophobicity of the carriers. These strategies are aimed to improve biocompatibility and bioavailability, surpassing immune rejection and enable renal clearance.

5. Metal nanocarrier-mediated delivery of peptides/proteins for the treatment of brain disorders

5.1 Complexity of brain anatomy

BBB is the primary unit of the neurovascular system and contains two parallel barriers such as vascular BBB and blood-cerebrospinal fluid (CSF) (Teleanu, Preda et al. 2022). Vascular BBB is composed of a capillary bed and lined by an endothelial cell which is derived from the squamous epithelial cells (Daneman and Prat 2015). The different types of secretary molecules like laminin, heparan sulfate proteoglycan, and type IV collagen are present in the vascular BBB and anchor many signaling molecules. On the other hand, blood CSF is present inside the vascular BBB and is produced by the choroid plexus through ependymal cells which are present in the brain ventricles and function is to make a cerebrospinal blood barrier (Solár, Zamani et al. 2020). The primary role of BBB is to control the transfer of chemicals and ionic components in cells between the brain and the blood and brain homeostasis (Solár, Zamani et al. 2020). It protects the
brain from toxins and pathogens, which may otherwise hinder brain functions. It is highly selective in allowing the passage of molecules through it. The ions and solutes pass across the BBB by passive diffusion process and depend on physiochemical properties like charge, molecular weight, size, and solubility (Ramirez and Tolmasky 2010). Loss of these qualities can lead to dysregulation of ions, molecules, blockage of signal homeostasis, and may lead to neural dysfunction. Besides its selective permeability nature, other methods could be exploited to target the brain tissue such as carrier-mediated transport, adsorptive transcytosis, and receptor-mediated endocytosis (Figure 2A). The first case happens with the aid of transport proteins such as glucose transporter (GLUT-1), which is present on the luminal side of the endothelial cells. Nanoparticles could be tagged with the glucose derivatives to enhance their permeation through BBB via GLUT-1 (Zhang, Mehta et al. 2021). Adsorptive transcytosis occurs due to the interactions between positively charged molecules/particles and the negatively charged luminal membrane (Lombardo, Schneider et al. 2020).

Further, specific receptors are present on the endothelial cells which are involved for the transportation of macromolecules such as insulin and transferrin (Grabrucker, Ruozi et al. 2016). Hence these receptors aid in the movement of nanoparticles in the case of receptor mediated endocytosis. Liu et al demonstrated that magnetic nanoparticles modified with transferrin efficiently delivered small interference RNA against the polo-like kinase I (siPLK1) into the murine model and displayed anti glioblastoma activity (Liu, Cheng et al. 2018). The cell-penetrating peptides (CPP), also known as trojan peptides are specific class of molecules that have the special ability to cross the cell membranes. They possess membrane translocating sequences with the amino acid residues <30 (ref1). These are rich in amphiphilic positively charged residues such as arginine and lysine (Silva, Almeida et al. 2019). The CPP delivers the
molecules into the cell membrane through phagocytosis and clathrin-mediated endocytosis (Ghorai, Deep et al. 2023). Gold nanoparticles when conjugated with CPP, could effectively deliver doxorubicin to brain (Gessner and Neundorf 2020). On the other hand, the administration of drug loaded nanoparticles through intranasal route is one of the non-invasive ways to reach the target site in the brain. In the nasal region, the olfactory mucosa contains olfactory neurons which are present in the upper part of the septum that responsible for sensory smell (Zhang, Li et al. 2016). These olfactory nerves along with trigeminal nerves serve as routes through which the metal nanomaterials move, which bypasses the BBB (Lee and Minko 2021). This allows direct access to specific areas, and could target specific neural pathways.

It has been reported that there are different mechanisms of interactions between nanoparticles and therapeutic peptides (Liu, Fang et al. 2021). One of the approaches involves direct encapsulation of peptides into the nanoparticles by in situ synthesis. Additionally, covalent conjugation, thiolate bonding and electrostatic interactions are also possible among them (Figure 2B). We have focused on the therapeutic molecule delivery by metal nanocarriers against brain tumor, and neurodegenerative diseases as a function of their ability to cross BBB (Table 3).

5.2 Brain cancer

The most common tumor in the brain is gliomas tumor, which resembles the BBB activity due to the rapid proliferation of cells and movement (Teleanu, Preda et al. 2022). Once the gliomas cross the BBB, they will form a blood-brain tumor barrier (BBTB) which leads to new blood vessels formation in the brain (Knox, Aburto et al. 2022). The BBB and BBTB act as two different barriers which prevent the delivery of therapeutic drugs to the target site. During pathological conditions, pericyte reduction or degeneration occurs, and astrocytes swell and detach from the vascular bed (Ramirez and Tolmasky 2010, Knox, Aburto et al. 2022).
result, the BBB will lose integrity and compromise the cellular transport ion channels like efflux transport nutrient transporters. In general, brain cancer is caused by either gliomas or glioblastomas originating from the neuroglial cells. Gliomas are malignant tumors characterized by poor prognosis and substantial invasion, and 50% of brain tumors are typical central nervous system (CNS) tumors that affect adults (Guo, Khattak et al. 2023). Cellular atypia and increased cell density are common features of brain cancer. Vessel co-option, angiogenesis, and replicative immortality are commonly characterized for brain tumor (Figure 3).

The treatment of glioblastomas occurs through therapeutic molecules such as Nimotuzumab, Panitumumab or TMZ, and radiotherapy, by applying a high radiation dose. The rapid proliferation of cancer cells is associated with drug resistance to the targeted therapeutics (Lundy, Nguyễn et al. 2021, Tang, Feng et al. 2021) The problem related to the treatment of glioblastoma includes the passage of drug to the delivery sites by BBB, high cell infiltration, which hinders conventional chemotherapy, and lack of successful treatment models. There are several challenges to attain successful treatment for the brain tumor, considering the complexity of BBB, the stability, and the bioavailability of the therapeutic compounds. In this regard, a nanocarrier-mediated approach might find its place in addressing these challenges. In a report by Grillone et al., it was showed that the magnetic solid lipid nanoparticles had the potency to permeate BBB when loaded with Nutlin-3a, and induced proapoptotic activity in glioblastoma cells (Grillone, Battaglini et al. 2019).

Apart from acting as drug carriers, the metallic nanoparticles could also serve as self-cytotoxic agents for glioblastoma cells. One such potential candidate is ZnO, which exhibits size-dependent and surface charge-dependent cytotoxicity by inducing oxidative stress to glioblastoma (Kim, Kim et al. 2014, Hamidian, Sarani et al. 2022). The metal nanocarriers are
also successful in the delivery of therapeutic proteins and peptides to the brain tumors. In one of the recent studies, Rizzuto et al. developed ferritin-based nanoparticles to deliver monoclonal antibodies to the brain (Rizzuto, Dal Magro et al. 2021). Glioblastoma multiforme is characterized by high expression profiles of epidermal growth factor receptor (EGFR). The recombinant nano vector was internalized by cerebral vascular endothelial cells of BBB. The system promptly delivered the therapeutic proteins for the antibody-dependent cell mediated cytotoxicity against EGFR and HER2, which are overexpressed in glioblastoma. In an another study, Naletova et al. have developed novel angiogenin peptide fragment-gold nanoparticle hybrids as anti-angiogenic systems against neuroblastoma (Naletova, Cucci et al. 2019). Here, the peptide segment from the cell membrane binding domain of the protein is used for functionalization with gold nanoparticles. The hybrid particles of around 10 nm size showed anti-angiogenic properties and were able to affect the proliferation, cytoskeletal structure, and the release of vascular endothelial growth factor from neuroblastoma cells. Mansur et al. have developed a hybrid metal nanocarrier system comprising of gold and super magnetic iron oxide nanoparticles coated with carboxy methyl cellulose as shell (Mansur, Carvalho et al. 2022). The nano system was functionalized with integrin binding peptide. The polymer-peptide conjugate induced oxidative stress and magnetic hyperthermia, when exposed to an alternating magnetic field.

Few in vivo studies have also reported on the usage of metal nanoparticles for the delivery of therapeutic proteins. The efficiency in animal models would reflect their translational potential. In a report, it was shown that gold nanoparticles used as a single platform, displayed both therapeutic molecule carrier agent, and as radiosensitizer. In this case, glioblastoma targeting antibody was loaded onto the nanocarrier, which was further coated with insulin for the
The nano formulation, when injected intravenously got accumulated at the tumor site. When combined with radiotherapy, the nano formulation has significantly inhibited tumor growth and reduced its vascularization and proliferation (Gal, Betzer et al. 2022). In another study, the Ag Nps were used in combination with chlorotoxin, a peptide that specifically binds to the overexpressed matrix metallopeptidase (MMP) 2 in glioblastoma (Locatelli, Naddaka et al. 2014). The composite displayed in vitro cytotoxicity and in vivo tumor reduction in mice.

Additionally, the metallic nanocarriers possess unique property to diagnose disease condition. Hence, they can potentially serve as diagnostic and therapeutic peptide delivery agents on a single platform. It was reported that gold particles act as an excellent contrast agent and provide a detailed high-resolution 3D X-ray of a mouse xenograft glioblastoma cells and angiogenic microvasculature (Lai, Ko et al. 2015).

5.3 Neuro-degenerative diseases

As per the Alzheimer's disease (AD) international report, approximately 50 million people worldwide were affected by dementia, and which estimated that this number will triple by 2050, with two-thirds of them would be from the countries with lower GDP per capita (International 2018). Moreover, above the age of 80 years, females are more prone to Alzheimer’s disease than men. Neurons in AD patients are characterized by neurofibrillary tangles of tau (hyperphosphorylated microtubule-associated protein), and by aggregation of amyloid beta (Aβ) (Figure 3). The FDA-approved drugs for the Alzheimer's disease are cholinesterase inhibitors (Donepezil, Galantamine, and Rivastigmine) and N-methyl-D-aspartate (NMDA) receptor antagonists (Memantine). These drugs are generally administered orally & work differently in the brain and are approved for different stages of the disease. Donepezil is approved for all
stages, while galantamine and rivastigmine are approved for mild to moderate stages (Hadavi and Poot 2016). Many of the investigated compounds have been deemed ineffective due to their low bioavailability, which is often a result of being restricted by the BBB.

Moreover, there has been a transitional gap between the animal models used, potentially leading to further complications in testing efficacy in humans (Prins, Visser et al. 2010). It has been observed that several animal models used in such studies make a "transitional gap" between the findings of animal studies and their applicability to humans. Developing effective treatment options presents a significant challenge, primarily due to the difficulty of designing a system that can penetrate the BBB.

One promising approach involves using nanoparticle-based systems, which can help target the drug to its specific site. The therapeutic agents can permeate through BBB by utilizing these nano-systems, thereby improving their bioavailability, pharmacokinetics, and pharmacodynamics. This can significantly increase the impact of any treatment strategy. In a report, it was shown that ZnO nanoparticles were found to be displaying anti-Alzheimer potential by acetylcholinesterase and butyryl cholinesterase activity (Al-Radadi, Faisal et al. 2022).

Osthole is a bioactive compound recently explored in Alzheimer’s treatment. In a recent study, it was found that when this compound was conjugated with ZnO, it could reduce Alzheimer-caused cell apoptosis, and increase overall cell viability (Alharthi, Aldakheel et al. 2022). Another group of researchers found that ZnO NPs could alter gene expression of Zn transporters in cells where down-regulation was observed. Low Zn levels are associated with neurodegenerative disorders (caused by 6-hydroxydopamine (6-OHDA) in neuroblastoma and Alzheimer’s). These NPs could elevate Zn levels in cells effectively reducing 6-OHDA-induced cell death (Pan, Lin et al. 2020).

A recent report showed silver composite with short peptide amphiphile nanostructures were
shown to balance the microbiota of Alzheimer’s condition (Kumar Tripathi, Kesharwani et al. 2023). The microbiota-gut brain axis highly influences the development of neurodegenerative disorders. Here, the nanostructures displayed better cell compatibility, and were used for addressing the issues of local infections of gut microbiota. Magnetic nanoparticles were studied for their ability to transport therapeutic proteins in animal model. The NU-4 antibody, which is specifically designed to target soluble beta fibril oligomers was conjugated with iron oxide nanoparticles for its delivery (Viola, Sbarboro et al. 2015). After the intranasal administration of the formulation, it was able to effectively bind to the oligomers in the animal Alzheimer’s models. Similarly, iron oxide nanoparticles were used as diagnostic agents in detecting tau proteins of Alzheimer’s when conjugated with anti-tau antibodies (Chiu, Chen et al. 2014).

Parkinson's disease (PD) is another degenerative neurological condition that primarily affects the elderly and is marked by motor-related impairment. The primary symptoms of PD include a significant reduction in dopamine-producing neurons (Figure 3) in the substantia nigra pars compacta and subsequent synaptic plasticity defects in the basal ganglia and striatal networks (Karthivashan, Ganesan et al. 2020). It is rare among people below the age of 50 and becomes more prevalent with age, with its highest occurrence between the ages of 85 and 89. PD has higher probability among men, with a ratio of 1.4 males to every female (Dorsey, Elbaz et al. 2018). The primary approach for treating PD involves targeting the motor symptoms by either increasing the dopamine levels within the central nervous system (CNS) or stimulating dopamine receptors (Baskin, Jeon et al. 2021). The dopamine replacement therapy has not been successful as dopamine is a hydrophilic molecule making it unable to pass through the BBB and also it has a very short lifespan inside body.
Dopamine agonists are used independently or with Levodopa (L-dopa) to trigger dopamine production for PD treatment. Levodopa, which is the precursor of dopamine, has been one of the most trusted drugs since its discovery as it can pass through the BBB via L-amino acid transporters, further converting it into the active form of dopamine after decarboxylation. Various other neuroprotective agents, mitochondrial function modulators, anti-inflammatory compounds, iron chelators are still under trials to treat PD disease (Krishnan 2021). But all these drugs show some similar kind of adverse side effects including drowsiness, nausea, vomiting, leg swelling, dyskinesias, chest pain, insomnia, headache, dry mouth, heartburn & even in some cases hallucinations (Jankovic and Tan 2020). Being a very complex neurodegenerative disorder, PD requires multiple drugs at a single time to cure but low solubility, poor bioavailability, shorter lifespan, less stability become the major issues in this direction along with not crossing the BBB effectively as only 1% of the total administered drug reaches the brain milieu (Manning 2018). To cope with these poor pharmacological properties of the drugs, nanoparticle-mediated drug delivery is one such option which could enhance self-time, and sustainability by minimizing non-specific binding and side-effects. It has been shown that L-Dopa which is a potential drug for Parkinson’s disease, crossed the BBB by encapsulated with nano-flowers of gold nanoparticles via large neutral amino acid transporter. It has also successfully internalized by macrophage without any inflammation (Cheng, Dai et al. 2014). In a study, magnetic nanoparticles modified with fluorescent carboxyl magnetic nile red, efficiently crossed BBB, and successfully delivered osmotin, a potential drug for Parkinson’s disease (Amin, Hoshiar et al. 2017). Cerium oxide is one of the rarely explored metallic nanoparticles in the biomedical application point of view. However, they possess interesting antioxidant properties. In a report it was shown that CeO₂ nanoparticles greatly inhibited cytotoxicity induced by α-synuclein (α-


syn), which is the primary pathological hallmark of PD (Ruotolo, De Giorgio et al. 2020). α-syn was strongly adsorbed on the surface of these nanoparticles, preventing their foci accumulation.

6. Summary

Despite possessing potential therapeutic abilities against various disorders, the peptides/proteins face several challenges, that are needed to be addressed prior to their practical applications. Nanocarrier mediated encapsulation and delivery of peptides enhance biodistribution, bioavailability, increased half-life, stability, and targeted delivery especially across BBB. The metallic nanocarriers are unique in displaying excellent carrying capacities and in diagnosis of a disease. A few challenges exist in translating metal nanocarriers for the healthcare applications such as toxicity and persistence in the body. A few strategies such as surface functionalization and modification in charge density are necessary to make a balance between the theranostic applications and clearance issues. Therapeutic peptide delivery through nanocarriers is still in its infancy, especially against neurodegenerative diseases. However, the outstanding potential of metal nanocarriers in efficiently passing the BBB, high loading capabilities, sustained release of entrapped therapeutic molecules, magnetothermal, and bioimaging increase their scope for becoming futuristic ideal candidates for the successful therapy to brain ailments.

7. Data Availability

This article contains no datasets generated or analyzed during the current study.

8. Author Contributions
Wrote or contributed to the writing of the manuscript: Rathnam S, Deepak T, Sahoo B, Meena T, Singh Y, and Joshi A.

9. References


efficient delivery platform for enhanced malignant glioma therapy and imaging." Small 10(24): 5137-5150.


chronic inflammations as well as autoimmune pathologies and unveils a new potential


Holst, J. J. (2019). "From the incretin concept and the discovery of GLP-1 to today's diabetes

Holst, J. J. and M. M. Rosenkilde (2020). "GIP as a therapeutic target in diabetes and obesity:
insight from incretin co-agonists." *The Journal of Clinical Endocrinology & Metabolism*
**105**(8): e2710-e2716.

nanostructures: engineering their plasmonic properties for biomedical applications."

Huang, Y., N. Yang, D. Teng, R. Mao, Y. Hao, X. Ma, L. Wei and J. Wang (2022). "Antibacterial
peptide NZ2114-loaded hydrogel accelerates Staphylococcus aureus-infected wound

"Synthesis, properties, and biological applications of metallic alloy nanoparticles."


nanoparticles: chemical, physical and biological methods." *Research in pharmaceutical

Fe3O4-PEG nanoparticles combined with NIR laser and alternating magnetic field as
potent anti-cancer agent against human ovarian cancer cells." *Materials Research Express*
**6**(11): 115412.


human Wharton’s jelly stem cells seeded onto acellular dermal matrix labeled with superparamagnetic iron oxide nanoparticles in burn wounds." *Burns & Trauma* **10**.


Pan, C.-Y., F.-Y. Lin, L.-S. Kao, C.-C. Huang and P.-S. Liu (2020). "Zinc oxide nanoparticles modulate the gene expression of ZnT1 and ZIP8 to manipulate zinc homeostasis and


**Footnotes**

a. This work received no external funding.

b. No author has an actual or perceived conflict of interest with the contents of this article.

**Figure Legends:**

**Figure 1:** An overview of the therapeutic flow of peptides, starting from their identification, synthesis, and to their applications. A) Identification of potential peptides through phage library. B) Labelled peptides used for the diagnosis of tumor, such as CT imaging. C) Peptide-conjugated with nanomaterials for selected tumor therapy. D) Targeted peptide vaccines for immunotherapy.
Figure 2: A) Schematic representation of different possible transport mechanisms of metallic nanoparticles for the delivery of therapeutic peptides into the diseased brain tissue through blood brain barrier. B) Various types of interactions between metal nanoparticles and therapeutic peptides i.e., a-direct encapsulation, b-electrostatic interactions, c-covalent conjugation, and d-thiolate bonding.

Figure 3: Pathophysiology and characteristics of the most common brain ailments, i.e., brain tumor, neurodegenerative Alzheimer’s and Parkinson’s diseases.

Table 1: Examples of various therapeutic applications of peptides, with their sources

<table>
<thead>
<tr>
<th>Peptide/protein</th>
<th>Source</th>
<th>Therapeutic application</th>
<th>Remarks/Findings</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>BbAFP1</td>
<td>Beauveria</td>
<td>Antifungal</td>
<td>Possess glucan and chitin-binding sites.</td>
<td>(Tong, Li et al.)</td>
</tr>
<tr>
<td></td>
<td>bassiana</td>
<td></td>
<td>Induces intracellular ROS and membrane</td>
<td></td>
</tr>
<tr>
<td>Peptide</td>
<td>Source</td>
<td>Function</td>
<td>Description</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------------</td>
<td>----------------------</td>
<td>-----------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>17BIPHE2</td>
<td>Cathelicidin LL-37</td>
<td>Antibacterial</td>
<td>Most potent antibiofilm peptide against various Gram-negative species.</td>
<td>(Wang, Narayana et al. 2019)</td>
</tr>
<tr>
<td>MPLfcinB6</td>
<td>Modification to bovine lactoferrin peptide</td>
<td>Anticancer</td>
<td>Selectively binds to the cell membrane of myeloma cells, may show cytotoxicity and not to other cancer cells such as breast.</td>
<td>(Hilchie, Hoskin et al. 2019)</td>
</tr>
<tr>
<td>Temporin-La</td>
<td>Bullfrog skin</td>
<td>Anticancer</td>
<td>Damages cell membrane of hepatocarcinoma cells, with IC₅₀ around 12 µM.</td>
<td>(Xie, Liu et al. 2020)</td>
</tr>
<tr>
<td>Kefir peptides</td>
<td>Brazilian kefir</td>
<td>Anti-Alzheimer’s</td>
<td>Peptides were shown for acetylcholinesterase inhibition and antioxidant potential, and led to better motor performance in Alzheimer’s fly model.</td>
<td>(Malta, Batista et al. 2022)</td>
</tr>
<tr>
<td>Glucagon-like peptide (GLP)-1</td>
<td>Intestinal epithelial cells</td>
<td>Anti-Parkinson’s</td>
<td>The peptide has the ability to cross BBB, and reported to protected the dopaminergic neurons from damage and increased synapses.</td>
<td>(Yang, Feng et al. 2022)</td>
</tr>
<tr>
<td>MTADV-pentamer</td>
<td>Chemically synthesized</td>
<td>Anti-inflammatory</td>
<td>The peptide inhibited the release of pro-inflammatory cytokines in intestinal bowel disease.</td>
<td>(Hemed-Shaked, Cowman et al. 2021)</td>
</tr>
<tr>
<td>Dalazatide</td>
<td>Caribbean sea anemone</td>
<td>Anti-inflammatory</td>
<td>Kv 1.3 channel blocker and used in the treatment of psoriasis.</td>
<td>(Tarcha, Olsen et al. 2017)</td>
</tr>
<tr>
<td>Nanoparticles</td>
<td>Green derivatives</td>
<td>Size range</td>
<td>Applications</td>
<td>Cell line/ Microbes</td>
</tr>
<tr>
<td>------------------------</td>
<td>--------------------------</td>
<td>------------</td>
<td>-----------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>Iron oxide nanoparticles</td>
<td>Rosemary extract</td>
<td>100 nm</td>
<td>Anti-cancer</td>
<td>C26 and 4T1</td>
</tr>
<tr>
<td></td>
<td>Satureja hortensis</td>
<td>9.3-27 nm</td>
<td>Anti-cancer and antimicrobial</td>
<td>MCF-7, K-562, Candida albicans, Escherichia coli</td>
</tr>
<tr>
<td></td>
<td>Garcinia mangostana fruit peel</td>
<td>13 nm</td>
<td>Anticancer</td>
<td>HCT116</td>
</tr>
<tr>
<td>Gold nanoparticles</td>
<td>Garcinia mangostana</td>
<td>33 nm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Ziziphus zizyphus</td>
<td>3 nm</td>
<td>Antimicrobial</td>
<td>C. Albicans, and Escherichia coli</td>
</tr>
<tr>
<td><strong>Moringa oleifera</strong></td>
<td>15-20 nm</td>
<td>Antidiabetic Antioxidant Anticancer properties</td>
<td>MCF-7</td>
<td>1. Antidiabetic property (α-Amylase reduction assay). 2. 96.2 % Scavenging activity 100 µg/ml. 3. IC50 of the nanoparticle was 130 µg/ml against cancer cell line</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Mentha longifolia</strong></td>
<td>36.4 nm</td>
<td>Anticancer effect on breast cancer</td>
<td>MCF7 Hs 578Bst Hs 319.T UACC-3133</td>
<td>Cytotoxic effect in various cancer cell lines high cell viability in normal cell upto 1000 µg/ml</td>
</tr>
<tr>
<td><strong>Cinnamon</strong></td>
<td>35 nm</td>
<td>AuNPs as a quencher</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Anabaena variabilis</strong></td>
<td>99.55 nm</td>
<td>Anti-microbial effect</td>
<td>Various fungal and bacterial strains</td>
<td>Higher doses exhibited higher antioxidant activity</td>
</tr>
<tr>
<td><strong>Couroupita guianensis</strong></td>
<td>13-61 nm</td>
<td>Anti-cancer effect</td>
<td>MCF-7</td>
<td>The Ag NP showed IC 50 of 20 µg/ml.</td>
</tr>
<tr>
<td><strong>Pedalium murex</strong></td>
<td>14 nm</td>
<td>Antibacterial effect</td>
<td>Several bacterial strains</td>
<td>High growth inhibition by Ag NPs at 15 µg/ml</td>
</tr>
<tr>
<td><strong>Daucus carota</strong></td>
<td>20 nm</td>
<td>Antimicrobial and antioxidant property</td>
<td>Staphylococcus aureus and Aspergillus niger, and U87MG cell line</td>
<td>Zones of inhibition against <em>Staphylococcus aureus</em> (18 mm), and <em>Aspergillus niger</em> (15 mm) Low cytotoxicity against U87MG cells up to 100 µg/ml</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Zinc oxide nanoparticles</strong></td>
<td><strong>Raphanus sativus var. Longipinnatus</strong></td>
<td>209 nm</td>
<td>Anticancer</td>
<td>A549</td>
</tr>
<tr>
<td><strong>Deverra tortuosa</strong></td>
<td>9.26-31 nm</td>
<td>Anticancer</td>
<td>A549 and Caco-2</td>
<td>High cytotoxicity against lung and colon cancer cells (Selim, Azb et al. 2020)</td>
</tr>
<tr>
<td>Peptide/Protein</td>
<td>Nanocarrier</td>
<td>Brain ailment</td>
<td>Mode of action</td>
<td>In vivo studies</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-------------------------</td>
<td>---------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Monoclonal antibody to EGFR</td>
<td>Iron nanoparticles</td>
<td>Glioblastoma</td>
<td>Cellular uptake by EGFR and HER2 overexpressed glioblastoma, and showed antibody-dependent cell mediated cytotoxicity</td>
<td>___</td>
</tr>
<tr>
<td>Angiogenin peptide fragment</td>
<td>Gold</td>
<td>Neuroblastoma</td>
<td>Anti-angiogenic effect on neuroblastoma cells by altering cytoskeletal structure, and proliferation</td>
<td>___</td>
</tr>
<tr>
<td>RGD tri peptide</td>
<td>Gold and iron oxide</td>
<td>Glioblastoma</td>
<td>Dual magnetothermal-chemo dynamic therapy by inducing oxidative stress</td>
<td>___</td>
</tr>
<tr>
<td>Antibody to glioblastoma cells</td>
<td>Gold</td>
<td>Glioblastoma</td>
<td>Nanoparticles were coated with insulin and cetuximab to enhance the permeation through BBB. Efficient in crossing BBB in tumour mice model and reduced tumour size and proliferation</td>
<td>___</td>
</tr>
<tr>
<td>Chlorotoxin</td>
<td>Silver</td>
<td>Glioblastoma</td>
<td>Specific binding to MMP2 overexpressed glioblastoma cells</td>
<td>Size of the tumour was observed in tumour bearing mice upon administration of chlorotoxin-Ag NPs</td>
</tr>
<tr>
<td>------------</td>
<td>--------</td>
<td>-------------</td>
<td>----------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Tyrosine based short amphiphile peptide</td>
<td>Silver</td>
<td>Alzheimer’s</td>
<td>Reported to balance microbiota-gut brain axis in Alzheimer’s condition</td>
<td>____</td>
</tr>
<tr>
<td>NU-4 antibody</td>
<td>Iron oxide</td>
<td>Alzheimer’s</td>
<td>Aβ oligomer-specific antibodies were tagged with magnetic nanoparticles for specificity</td>
<td>Successful binding to soluble fibril oligomers in mice model. Used for Alzheimer’s diagnosis of human brain tissues (Ex-vivo)</td>
</tr>
<tr>
<td>Anti-tau</td>
<td>Iron oxide</td>
<td>Alzheimer’s</td>
<td>Anti-tau antibodies are used in the detection of tau proteins through iron nanoparticles in the presence of alternating magnetic field</td>
<td>____</td>
</tr>
</tbody>
</table>
Figure 2
Figure 3

Brain tumor

1. Atypical cells and increased cell density
2. Vessel co-option
3. Angiogenesis
4. Replicative immortality

Alzheimer's Disease
- Normal dopaminergic neuron
- Healthy dopamine production
- Normal movement

Parkinson's Disease
- Degenerating dopaminergic neuron
- Compromised dopamine production
- Parkinson-related movement disorders