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Review

The role of peptides in blood-brain barrier nanotechnology‡

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Abstract: The blood-brain barrier (BBB) regulates the passage of molecules between the bloodstream and the brain. Overcoming the difficulty of delivery drugs to specific areas of the brain is a major challenge. The BBB exerts a neuroprotective function as it hinders the delivery of diagnostic and therapeutic agents to the brain. Here, we provide an overview of the way in which peptides and nanotechnology are being exploited in tandem to address this problem. Peptides can be used as specialised coatings able to transport nanoparticles with specific properties, such as targeting. The nanoparticle can also carry a peptide drug. Furthermore, peptides can be used in less conventional approaches such as all-peptide nanoparticles. In summary, the combined use of peptides and nanotechnology offers tremendous hope in the treatment of brain disorders. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: peptides; blood-brain barrier; nanoparticle; nanoplatform; nanotechnology

INTRODUCTION

The adage that 'good fences make good neighbors' is perhaps nowhere better illustrated than in the human body, which encompasses various gas- and fluid-filled sacs, (e.g. the lungs, colon, and stomach) separated by specialised tissues (uterus, brain capillaries,...) that act as barriers regulating the passage of molecules from one compartment to another [1]. The performance of these barriers is directly related to health; indeed, myriad disease states are associated with loss of barrier function. This holds especially true for the blood-brain barrier (BBB), due to the central role of the brain. The BBB regulates the passage of molecules between the bloodstream and the brain, preventing the entry of harmful substances into the brain while actively regulating the uptake and efflux of ions, nutrients, and metabolites [2]. Physiological barriers are thus vital to homeostasis at the tissue, organ, and organism levels [1].

Circumventing these physiological barriers can be of strategic importance in drug delivery and medical diagnostics. Herein, we provide an overview of how peptides and nanotechnology are being exploited in tandem for these applications.

Nanoparticles, which are gradually being developed as drug delivery systems, can be envisaged to carry peptide cargo [3]. Furthermore, peptides can be used in less conventional approaches such as *all-peptide* nanoparticles or as specialised coatings able to impart nanoparticles with specific properties, such as targeting.

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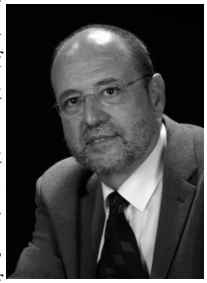
In the present article we have focussed our attention on the BBB, summarising literature reports on the use of peptides and nanotechnology for the treatment and diagnosis of brain disorders, and comparing these approaches to other methods.

THE BLOOD-BRAIN BARRIER

The BBB is the most important barrier involved in the regulation of molecules accessing the brain. The brain is highly vascularised, containing a very intricate network of capillaries (nearly every brain cell is located within 20 μm of a capillary [4]. The endothelial cells that form the brain capillaries are sealed together by tight junctions, and have no fenestrations and very low pinocytosis. This combination of features creates the BBB [5], which is both a physical and enzymatic barrier (Figure 1).

The BBB can become damaged in certain situations, as described by Couvreur [6]. In neurodegenerative diseases such as Alzheimer's disease, although $A\beta$ crosses the BBB by the RAGE receptor [7], no damage of the BBB has been described. In cerebrovascular diseases the integrity of the BBB is variable; it depends on the hypertension, severity and duration of the cerebral ischemia [8]. In the case of inflammatory diseases caused by an infection, the BBB opens as an indirect consequence of the immune response, which leads to the release of cytokines, chemokines, cellular adhesion molecules, and matrix metalloproteases at the infection site. These molecules have been shown to alter the structure and function of the BBB [9]. The opening of the tight junctions in brain tumours is one of the most important abnormalities in brain cancer, and becomes more pronounced as

BIOGRAPHY



Ernest Giralt was born in 1948. He received his first degree in 1970 and his PhD in 1974 from the University of Barcelona. After postdoctoral work at the University of Montpellier, France, he returned to Barcelona as Assistant Professor. He was subsequently promoted to Associate Professor in 1977 and to Full Professor in 1986. He was Visiting Professor at the University of California, San Diego, and Research Associate at the Scripps Research Institute, USA, in 1991. Professor Giralt's main research interests lie in the fields of molecular recognition, peptide synthesis and structure determination, in particular using Nuclear Magnetic Resonance spectroscopy in relation with molecular-recognition processes. He has published around 350 papers, review articles and two books. He is one of the founding members of the European Peptide Society. He is editor of The Journal of Peptide Science and is on the editorial board of several other journals. He received the Narcis Monturiol prize in 1992, the Leonidas Zervas award in 1994, the Distinction Research Chair, Generalitat de Catalunya in 2001, the NMR prize, GRMN of the Spanish Royal Chemical Society in 2002 and the Research National Prize and Medal, Spanish Royal Chemical Society in 2003.

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the malignancy increases. However, hyperpermeability occurs mainly in new vessels, whereas barrier function is retained in the growing margins of the tumour [10,11].

An invasive biopsy is currently the preferred method to confirm cancer diagnosis, and can provide extra information about histological type, classification, grade, and best treatment. However, it is clear that early detection is the key issue as it correlates with a positive prognosis. The actual contrast agents used for magnetic resonance imaging (MRI) fail to cross the BBB, so a disruption of the barrier is provoked to make them able to reach the CNS. The BBB can be permeabilised using either osmotic disruption by certain hyperosmolar agents, such as mannitol, or biochemical opening by bradykinin analogues. This leads to reversible opening of the tight junctions, but is not specific enough to disallow CNS entry of toxins and unwanted molecules, thus potentially resulting in significant damage. Another option is provoking the disruption by a localised hyperthermia [12]. A contrast agent able to cross the BBB without disruption would provide valuable information and could help in MRI visualisation of early stage tumours, either before biopsy or during surgery.

The delivery of drugs and diagnostic agents to the brain is generally limited in both normal and pathological conditions. Hence, there is an urgent need for new strategies to these ends. Research in this field entails differentiation of cases in which the BBB is damaged, and those in which artificial disruption is provoked. Numerous research groups are trying to avoid solutions that imply disruption due to its many inherent risks.

There have been three major paths proposed for delivery of active molecules to the brain: intracerebral, intraventricular, and intravascular. Even in the case of the latter, which is the most promising, crossing the BBB remains a challenge. Additionally, the nasal route has been recently suggested as an alternative strategy for bypassing the BBB [13–16]. To date, intravascular delivery has been tested using lipophilic analogues of the desired active molecules, in order to improve passive diffusion, or using one of the endogenous transport systems of the BBB, (e.g. carrier- or receptor-mediated transcytosis).

Medicinal chemists have focussed on creating prodrugs, including polymeric prodrugs [17], as well as using chemical delivery systems such as those described by Bodor, based on redox trapping within the brain [18], or on use of the transferrin receptor [19]. However, lipophilic analogues can enhance plasma protein binding, and peripheral distribution and accumulation, thereby impairing CNS uptake of the active molecule.

Over the past few decades, pharmaceutical technology has lead to the emergence of different nanosystems or nanoplatforms tailored to deliver drugs to the brain, including polymeric nanoparticles, liposomes, and solid lipid nanoparticles. We have focussed here on the use of polymer matrix nanoparticles.

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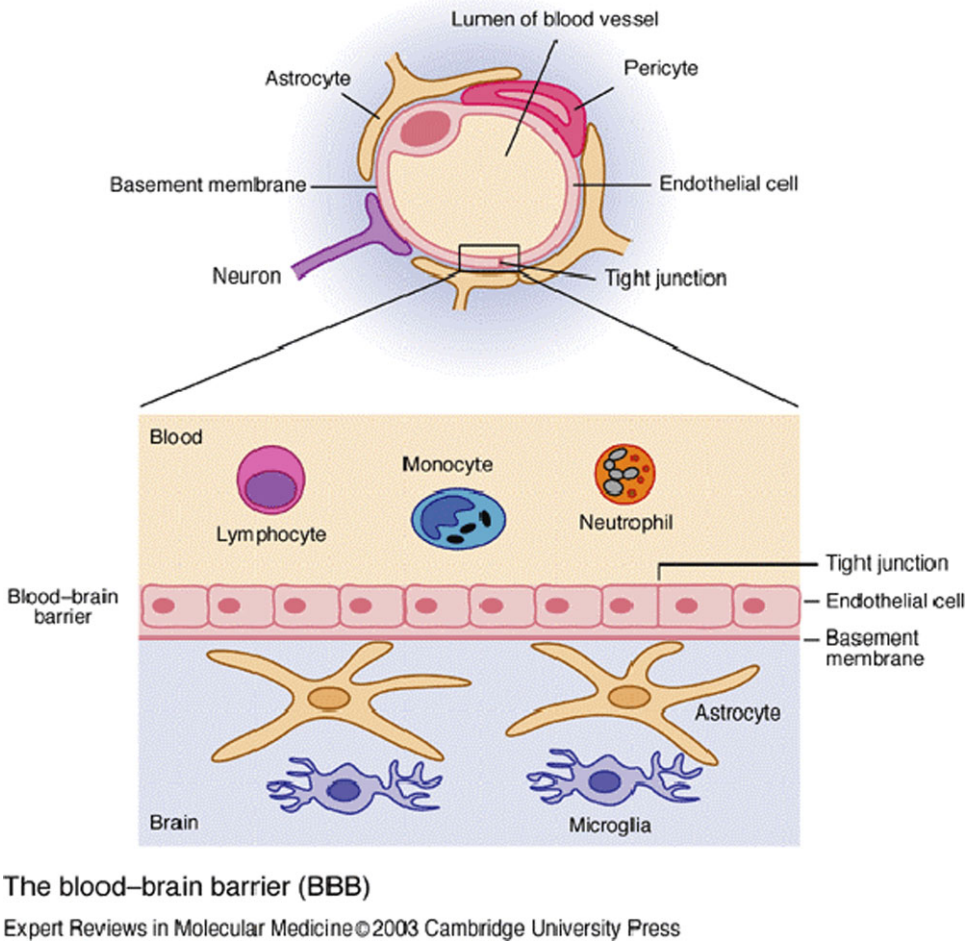

Figure 1 The blood-brain barrier is created by the tight junctions between the endothelial cells of the blood vessels in the brain, protecting it from harmful substances. Reproduced with permission of the publisher, full acknowledgment to the authors and Cambridge University Press (K. Francis et al., Innate immunity and brain inflammation: the key role of complement, Expert Reviews in Molecular Medicine (2003) Vol. 5: 119, Cambridge University Press, DOI: 10.1017/S1462399403006252). This figure is available in colour online at www.interscience.wiley.com/journal/jpepsci.

TARGETING THE BRAIN WITH POLYMER MATRIX NANOPARTICLES

Although the first review of nanoparticles appeared only three decades ago [20], they are hardly new; silver and gold nanoparticles have been used to colour ceramic glazes and stained glass since the tenth century, and their use may even date to the fourth century [21]. There are also natural sources of nanoparticles, such as fires and volcanic eruptions. Life can be seen as depending on nanoscale objects, including proteins, enzymes, and DNA. A perfect example of nano-entrapment is the ability of ferritin to store excess iron in our bodies. Nanoparticles have been exploited for a broad array of applications. Among the most promising of these is the transport of drugs across the BBB.

As previously mentioned, we have focussed here on polymer matrix nanoparticles. These are solid colloidal particles, made of polymeric materials ranging in size between 1 and 100 nm according to the usual definition, although there are examples of nanoparticles of several hundreds of nm. These particles can carry therapeutic drugs or diagnostic agents complexed by adsorption, entrapment, or covalent attachment. The nanoparticle may improve the transport properties and stability of the transported agent. Once the nanoparticle reaches the desired target, release can be achieved by one or more mechanisms such as desorption, diffusion, and nanoparticle erosion.

Nanoparticles are highly advantageous as delivery vehicles: the loading and release of cargo can be controlled; specific molecular-targeting factors can be attached; a hydrophilic coating can prevent undesired uptake of the nanoparticle by the reticuloendothelial system; and the matrix nature of the nanoparticle can provide protection against enzymatic and/or chemical degradation of the active agent. Additionally, the nanoparticle can prevent exclusion of the active agent by p-glycoprotein (p-gP) or multidrug resistance (MDR) protein, and may reduce the immunogenicity or other some side effects of the active agent. Finally, unlike conventional conjugate vehicles, which are generally

limited to 1 : 1 stoichiometry of vehicle to active agent, a single nanoparticle can carry up to thousands of molecules of cargo.

Compared to liposomes, nanoparticles are much simpler to prepare and to scale-up, as a low number of excipients are used [22]; they are also highly stable, both during storage and *in vivo* [23]; and their sustained release over a period of weeks is more easily achieved. PEGylated nanoparticles can even be lyophilised [24,25].

There are some concerns about the security, handling, and administration of nanoparticles [26,27], including their possible effects on iron homeostasis in the body [28]. However, once the protocols for the delivery of drugs and other molecules across the BBB using nanoparticles are safely, efficiently, and specifically established, they may prove to be one of the highest impact contributions to clinical neurosciences.

MRI, A WONDERFUL WINDOW ON BRAIN ACTIVITY

One of the most promising applications of nanoparticles is the transport of MRI contrast agents to the brain to enable studying the CNS of patients.

Iron oxides are classic superparamagnetic MRI contrast agents that directly affect the T1 and T2 water molecule relaxation times [29,30]. As iron oxides are insoluble in water, they must be delivered as modified colloids or matrix nanoparticles. This is achieved by using hydrophilic molecules such as dextrans [31].

Contrast agents such as Ferumoxtran-10 [32] and Gadolinium [33] are generally unable to cross the BBB alone; at the time of delivery, they are usually administered with a solution that provokes opening of the BBB. Hence, there is a pressing need for the development of delivery systems that do not disrupt the BBB; yet, do not imply prolonged exposure of the patient to the MRI magnetic field.

Nanoparticles are being used to study the integrity of the BBB in different situations. Dextran-coated USPIONs (ultra-small superparamagnetic iron oxide nanoparticles) are used to obtain additional information to that obtained by conventional gadolinium-enhanced MRI on BBB damage in patients with malignant brain tumours [32]. Cerebral ischemia can cause an increase in BBB permeability, due to the up-regulation of proinflammatory cytokines. This effect can be studied by a novel approach involving *in vivo* microdialysis and fluorescently labelled nanoparticles [34].

Recently, two groups have been working on the design and application of multifunctional nanoprobes that are detectable by MRI and fluorescent microscopy, and that could be used for the determination of brain tumour margins during presurgical and surgical phases [35,36]. The researchers have also worked on targeting contrast agents by attaching monoclonal antibodies that identify cells from specific tumour types [37]. Recently nanoparticles delivering markers of the fibrillar β-amyloid, such as thioflavin-T, have been shown to act as targeting moieties for Alzheimer's disease [38]. Unfortunately, all these approaches are limited by the fact that they imply disruption of the BBB.

Contrast agents that cross the BBB without disrupting it would have many applications for brain cancer diagnostics and other severe brain diseases such as Alzheimer's disease.

HOW CAN NANOPARTICLES IMPROVE DRUG DELIVERY?

Nanoplatforms comprise a nanoparticle, a specific nanoparticle coating, and a drug or diagnostic agent. An ideal nanoplatform could be intravenously or even orally administered; would be stable in blood and have a prolonged circulation time; would not provoke the activation of neutrophils; and would target the CNS while exhibiting minimal systemic effects, cross the BBB, and only release the drug upon arrival into the desired cells of the CNS. The development of nanoplatforms requires a multidisciplinary team of engineers, physicists, chemists, cell biologists, pharmacologists, and others. Several good reviews of nanoplatforms have recently appeared, which introduce the idea of a nanoplatform [39] cover the topic from different perspectives [40–46] and numerous patents on nanoplatforms have also been registered.

Nanoparticles are rapidly removed from the bloodstream after injection, accumulating predominantly in the liver and the spleen [47,48]. However, desired localisation of the nanoparticles could be achieved through nanoplatform design.

The first step in removal of nanoparticles from the bloodstream is opsonisation. Opsonins cover nanoparticles, and then undergo phagocytosis via specific membrane receptors on monocytes and tissue macrophages. Opsonisation can be minimised by coating the nanoparticle surface with hydrophilic PEG [49] leading to longer circulation times of the nanoparticles in the blood.

Therapeutic agents that do cross BBB must still overcome another obstacle: local efflux transporters. P-glycoprotein, MDR protein, and others work as detoxification and defence mechanisms for the brain, thereby complicating delivery of compounds to the CNS. Indeed, drugs such as paclitaxel [50] are substrates of these efflux systems. The effect of the efflux system is not only restricted to anticancer drugs, but also some promising anticonvulsant agents such as MRZ 2/276 are actively transported out of the brain [51].

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Current efforts at making new chemotherapeutic agents that could reach the CNS include use of nanoparticles that circumvent the p-gP efflux system [52].

Although non-selective inhibition of efflux transporters has been suggested as an approach to facilitate nanoplatform entry, it implies a risk of CNS or peripheral toxicity [53]. Entrapment of the drug inside a nanoparticle could obviate coadministration of p-gP inhibitors. Nanoplatforms can decrease the side effects and toxicity related to the administration of anticancer drugs such as doxorubicin in organs that do not need to be treated, (e.g. the heart) [54,55]. Moreover, delivery of a compound by nanoparticles can help prevent its degradation and photobleaching [56].

PEPTIDES AS DRUGS FOR TREATMENT OF CNS RELATED DISORDERS

One of the most promising, yet challenging, applications of nanoparticles is the delivery of therapeutic peptides and proteins to the brain. The main concern during the loading of nanoparticles with peptides or proteins is the chemical stability of the peptide or protein [57] to the elevated temperatures, shear force, surfactants, free radicals, and UV radiation implied. Moreover, the function of proteins depends on their structure. Hence, the preparation needs to be optimised for each nanoparticle formulation [58–65]. Stability has been studied in depth by Van de Weert [59], Schwendeman [58] and Cleland [66].

The typical peptidic cargo delivered by nanoparticles are anaesthetic agents such as dalargin [67], kyotorphin [67], and the neuromuscular blocking agent tubocurarine [68]. The interest of using these peptides is mainly academic, as they are anaesthetic agents that only exhibit therapeutic effects when administered directly in the brain, (i.e. not after peripheral administration).

Other peptides which have been transported to the CNS by nanoparticles are Z-DEVD-FMK, a specific caspase inhibitor [69], and *NC*-1900, a vasopressin fragment analogue [70]. The nanoplatform formed by peptide Z-DEVD-FMK entrapped in PEG-overcoated chitosan nanoparticles and fuctionalised with OX26 monoclonal antibody against transferrin receptor [71] using the avidin-biotin system has shown therapeutic promise for brain ischemia [69].

PEPTIDIC NANOPARTICLES

Nanoparticles have been used for pharmaceutical and medical applications for over 30 years. Albumin nanoparticles [72] first appeared in the mid-1970s. The first albumin nanoparticles with a magnetic core were prepared shortly thereafter, but the method is very aggressive, leading to denaturation of the protein [73]. Around the same time, the first biodegradable acrylic nanoparticles made of poly(methylcyanoacrylate) and poly(ethylcyanoacrylate) [74] were developed. Similar compounds were later made with poly(*n*-butyl, isobutyl, *n*-hexyl and isohexyl-cyanoacrylates).

Poly(lactic acid) and its copolymer poly (lactic-co-glycolic) acid were first developed for transplantation purposes. It was not until 1981 that the first nanoparticles of poly (lactic-co-glycolic) acid were described by Gurny [75]. These nanoparticles were especially challenging to prepare due to their poor solubility in organic solvents, and the fact that they cannot be directly polymerised.

Albeit nanoparticles for diagnostic applications have been on the market for over ten years, it was not until 2005 that the first nanoparticle product containing a drug became commercially available (Abraxane).

Nanoparticles are currently made from a wide array of materials such as poly(alkylcyanoacrylates); poly(methylidene malonate); polyesters such as poly (lactic acid), poly(glycolic acid), poly(ε -caprolactone) and their copolymers; polysaccharides; and proteins. The choice of nanoparticle materials is based on biodegradability, price, intrinsic immunogenicity, and toxicity. Therefore, certain peptides with inherently low toxicity and immunogenicity are ideal materials from which it is possible to fabricate nanoparticles.

Preparation of a nanoplatform may result in inactivation of the therapeutic molecule. In this sense, the nanogels described by Vinogradov in 1999 [76] are promising alternatives for the delivery of oligonucleotides. Nanogels are flexible hydrophilic polymer gels of nano-size made of a network of cross-linked ionic polyethyleimine (PEI) and non-ionic PEG chains [77]. Peptides can be used instead of PEI-PEG, and can also form nanogels to entrap molecules that are sensitive to the preparation procedure [78]. Like Vinogradov's nanogels, peptidic nanogels can also carry high payloads of macromolecules, which is usually impossible with conventional nanoparticles.

Another parameter that needs to be taken into account is biodegradability of the nanoparticle. Poly(butylcyanoacrylate) has proven to be the most promising material *in vivo* because of its rapid degradation [79]. Biodegradability can work in favor of peptidic nanoparticles. The intestinal absorption of peptidic nanoparticles may be improved by designing them with the mucoadhesive properties of chitosan [80].

There are a few examples of peptidic nanoparticles. In the chemotherapeutic field, many attempts have been made to formulate paclitaxel. One of the most promising is Xyotax, a poly-(L)-glutamic acid-paclitaxel conjugate. It was designed to have greater aqueous solubility and passive tumour targeting than the parent drug as administered alone [81,82]. Xyotax is not believed

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to cross the BBB, but rather to get absorbed into the endothelial cell surface, where it subsequently releases the drug.

Another example of a peptidic nanoparticle is protamine, a small polycationic peptide that it is found in salmon sperm, and that can condense DNA and deliver it to the nucleus of the egg after fertilisation. Protamine nanoparticles could help stabilise DNA cargo, and when coated with apolipoprotein E, allow transcytosis. In a recent work by Kratzer [83], the only problem detected was the loss of cargo inside cells during transcytosis. Albeit protamine nanoparticles require further development, they represent a milestone in the fledgling field of peptidic nanoparticles. It would be also interesting to know if a peptidic nanoparticle could be used to deliver plasmids, as an alternative to cationic albumin-conjugated PEGylated nanoparticles (CBSA-S-(Maleimido-PEG-PLA-co-MPEG-PLA)) [84].

PEPTIDE COATED NANOPARTICLES

Different nanoparticle surface coatings are used for different purposes: hydrophilic surfactants [41,68,85–87] reduce nanoparticle absorption by reticuloendothelial system organs to alter biodistribution of the nanoparticle; poloxamers and poloxamines induce a steric repulsion effect, which minimises adhesion of nanoparticles to macrophage surfaces, consequently minimising phagocytic uptake [88]; surface PEGylation increases blood half-life of nanoparticles [49,89,90]; and polysorbate-80 improves BBB transport of nanoparticles [67,87,91,92]. Any coating used for CNS targeted nanoparticles must allow the interactions needed for BBB transport [88].

Das *et al.* [93] used an elegant double coating of polysorbate-80 and PEG to enable oral delivery of Dalargin–nanoparticle conjugates. The PEG was employed to protect the peptide-loaded nanoparticle from the pH and enzymes of the gastrointestinal tract [94,95], and to increase the circulation half-life by the disopsonic action of the long PEG chains [49,96–98].

From the initial idea of molecular trojan horses (conjugates of a drug that is not transported through the BBB to an MAb that undergoes receptor mediated transcytosis) [4], the use of monoclonal antibodies for the transport of nanoparticles appear [69,99]. Different peptides have been described that bind to the transferrin receptor and then undergo transcytosis [100]. One can thus imagine a nanoplatform coated with a peptide that binds to the transferrin receptor and crosses the BBB. An advantage of this approach is that peptides are much easier to prepare than monoclonal antibodies.

A peptidic coating could also replace the typical cationised albumin coating used to make nanoparticles cross the BBB by adsorptive-mediated transcytosis. For this case, to use D-amino acids in the peptide would increase the stability of the coating to enzymatic activity [101]. An advantage of this method is that it obviates the need for recombinant albumin.

Peptides such as TAT have been used to enable transport of fluorescently labelled silica nanoparticles *in vivo* across the BBB [102]. In addition, polylysine has been used to coat iron oxide nanoparticles for their transport across the BBB [103,104]. The polycationic nature of the polylysine coating allows conjugation of DNA cargo for gene therapy.

Constantino *et al.* have recently reported peptide-coated poly(lactic-co-glycolic) acid nanoparticles that are transported through the opioid receptor [105]. The only modification introduced into the peptide sequence is substitution of the *N*-terminal Tyr with Phe to avoid any potential opioid effects. The group has also recently described the use of dendrons to create a well-defined arrangement of the peptidic coating on nanoparticle surfaces [106]. Although the dendrons they use are based on Lin's amine, peptidic dendrimers could be another option to obtain the said arrangement [107,108].

Polysorbate-coated nanoparticles absorb apolipoproteins from blood, and then cross the BBB by the low-density lipoprotein receptor (LDL-receptor), which is overexpressed in the brain endothelium. Coating the nanoparticles with polysorbate has been shown to be superior than coating them directly with apolipoprotein. The possibility to coat the nanoparticles with a peptide that binds to the LDL-receptor to undergo transcytosis could be advantageous, as it would avoid the different protein adsorption patterns on the nanoparticle surface [109].

Binding of a peptide-coated nanoparticle to a given receptor can result in the nanoparticle being transported across a barrier by a mechanism other than that expected of the coating [105].

Nanoparticle coatings have created various controversies. A major disagreement between Kreuter and Olivier started in 1999, when the latter suggested that polysorbate 80-PBCA may compromise the integrity of the BBB [110]. Kreuter responded by publishing that there was no toxicity; only some effects due to the extremely high doses used in the assay [55,86]. This polemic led to numerous in-depth studies on surfactant effects [111,112].

TOWARDS AN ALL-PEPTIDIC NANOPLATFORM FOR BRAIN THERAPY AND DIAGNOSIS

There is an increasing interest in peptides with therapeutic potential, including peptide-based drugs for the treatment and diagnosis of CNS diseases [113,114]. Hence, transport of peptidic drugs and diagnostic agents to the CNS will be evermore required in the near

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future. Use of peptidic nanoparticles in nanoplatforms allows for mild conditions that do not affect the peptidic cargo. Finally, a peptidic coating could be used to fine-tune an all-peptidic nanoplatform by modulating its biodistribution, increasing its half-life in blood, or improving its transport across the BBB by an endogenous mechanism (Figure 2).

NANOPLATFORM CHARACTERISATION

The components of nanoplatforms are characterised by different techniques. The size and morphology of the dried nanoparticles are commonly determined by SEM (scanning electron microscopy) and AFM (atomic force microscopy), whereas the size and distribution of nanoparticle aggregates in aqueous solution are determined by light scattering. The surface charge is determined by measuring the zeta potential. X-ray photoelectron spectroscopy and Fourier Transformation Infrared Spectroscopy are used to study the surface composition of the nanoparticle, and to determine the position of the hydrophilic groups modifying the polymer.

The encapsulated amount of drug or imaging agent is determined by elemental, spectrophotometric, and chromatographic analysis. Drug release and biodistribution studies must also be performed. The potential of nanoplatforms to challenge the integrity of the BBB is evaluated by MTT assays, whereby the transendothelial resistance (TEER) or the inuline transport across a coculture of bovine brain and endothelial cells.

Nevertheless, therapeutic and diagnostic applications of nanoparticles are still limited by unresolved drawbacks. In various reports on nanoparticle delivery of CNS drugs [41,91,92], the brain is extracted and homogenated after the *in vivo* assay, and the concentration of the drug in the resulting homogenate is measured. It is frequently claimed that because the blood capillaries represent only 1% of brain volume, and the brain blood vessel endothelial cells only 0.1%, then drug concentration can be directly measured in the brain homogenate without considering that the drug could be in the brain vessel endothelial cells. The basis for this affirmation is that if the drug detected in the brain homogenate is only in the endothelial cells, then the concentration would have been absolutely toxic for the animal. To avoid this assumption, the localisation of the nanoplatform could be tracked by using a fluorophore such as 6-coumarin. Once attached, the fluorophore is not easily released from the nanoparticle [115]; hence, it can be assumed that wherever fluorescence is found, corresponds to the location of nanoparticles [116,117]. Another tracking option is to use a dye such as rhodamine, introduced during nanoparticle polymerisation [77,105]. Alternatives for localising polymeric nanoparticles include binding gold-nanoparticles, or entrapping copper chlorophyll in the nanoparticle [118]. All of these methods are preferable to the use of radioactivity, which is widely employed for labelling nanoparticles [119].

Very frequently, nanoparticle studies only include evaluation of uptake without separately exploring transport; in these studies, what happens after uptake is left to speculation [41,52,120–124]. Transport

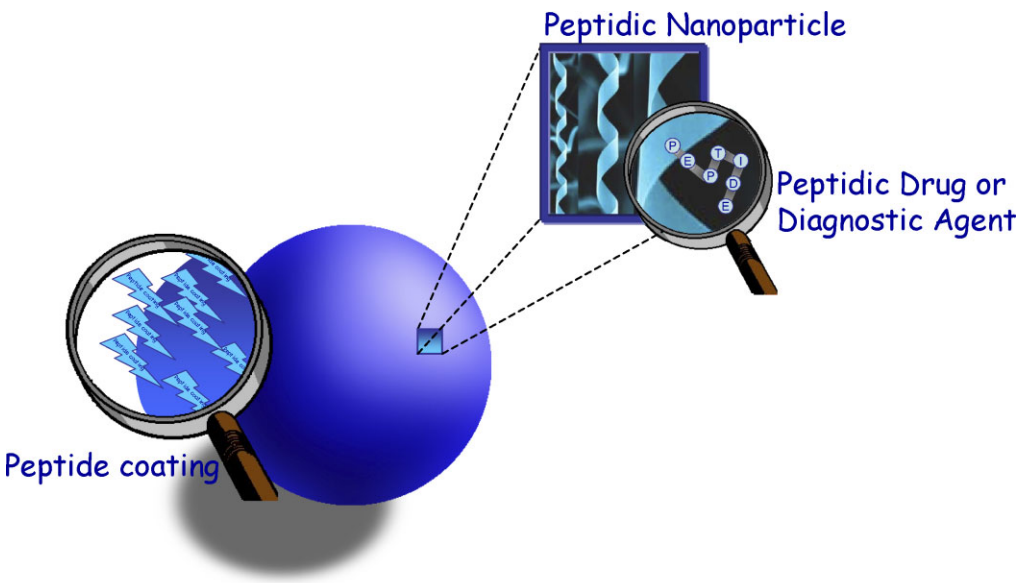

Figure 2 The idea of an all-peptidic nanoplatform would put together the advantages that a peptidic nature can bring to every piece of this nanoplatform, in terms of mild synthetic conditions, potential to modulate the nanoplatform biodistribution, to increase its half-life in blood, and to improve its transport across the BBB. This figure is available in colour online at www.interscience.wiley.com/journal/jpepsci.

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studies are now starting to be performed in different cellular models of BBB [125].

In summary, the combined use of peptides and nanotechnology offers tremendous hope to address brain disorders by crossing the BBB. Nonetheless, the mechanisms of *in vivo* nanoplatform transport remain poorly understood. Moreover, nanoparticles, coatings and coadjuvants must be further studied for their potential effects on the structural integrity and function of the BBB [86,91,126].

Acknowledgement

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