Review:

The ins and outs of nanoparticle technology in neurodegenerative diseases and cancer

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Abstract

As we enter the twenty-first century, several therapies based on using nanoparticles (NPs) ranging in size 1 – 1000 nm have been successfully brought to the clinic to treat cancer, pain and infectious diseases. These therapies bring together the ability of NPs to target the delivery of drugs more precisely, to improve solubility, to prevent degradation, to improve their therapeutic index and to reduce the immune response. NPs come in all shapes and sizes, designed specifically for biomedical applications such as solid lipid polymers, liposomes, dendrimers, nanogels, and quantum dots. These NPs offer many attractive characteristics such as biological stability and biocompatibility can incorporate different biological or drug molecules. Among the major challenges in human disease therapy in neurological diseases through to cancer is the development of nanomaterials that are able to be effective against the disease. In the case of neurodegeneration, one of the most difficult areas to penetrate for drug discovery in the body is the central nervous system, protected by the blood-brain-barrier. Whilst in the case of cancer, the biggest problem is how to specifically target the tumor with sufficient drug without causing side effects or inducing resistance. A new generation of intelligent NPs are emerging in the treatment of human disease such as neurological disorders and cancer. The use of natural alternative therapy is an encouraging idea in drug discovery. To this end as we gain more knowledge into the biological function of exosomes, this will allow us to harness their potential as natural NPs in future therapeutics.

Introduction

Nanoparticle (NP) research is a field of growing scientific interest due to the different potential uses within the field of biomedicine [1, 2]. From the first discoveries back in 1965 until the last 25 years has seen a massive exponential growth in the nanotechnology field (Figure 1). NPs in nanotechnology are termed as small units that can act by themselves facilitating both their transport and properties. The entity of NPs with therapeutic potential are usually comprised of small-molecule drugs, proteins and nucleic acids assembled with for example, lipids and polymers (Fig. 2). At the present state of the art, numerous scientists throughout the world are involved in the study of vesicles and other colloidal structures as carriers for drug delivery. Improvements to pharmacokinetics and pharmacodynamics, these particles are designed to have enhanced anticancer effects allowing more specific targeting to tumor tissues. The efficacy of these NPs depends upon integral properties such as surface properties and size. Here, in this review, we discuss the recent published investigations on the important role that NPs both natural and synthetic play in physiological and pathological pathways such as in neurodegeneration to cancer through communication and microenvironment control. In addition, we discuss how these NPs have been utilised in human therapy and their future relevance in diagnosis and therapeutics.

Nanoparticles

NPs can range in size from 100 to 2500 nm, smallest particles between 1 and 100 nm. NPs that have a therapeutic value are used as a vehicle carrying small molecules such as drugs, peptides, proteins, and nucleic acids. There are several types of particles (nanofibres, nanotubes, NPs, nanogels) that can be assembled with a therapeutic entity (Fig. 2A). The NPs physiochemical properties such as shape, surface charge, size, hydrophobicity etc. can all affect the absoprtion and/or correct targeting to certain areas in the body such as certain

tissues and these properties are necessary to consider in the NP design (Fig. 2B) [3]. The properties of NPs may or may not depend upon the size of the fine particles. Thus, individual molecules could be the size of ultrafine particles are not classed as NPs. We will discuss herein the vehicles used for drug delivery that have been developed from solid lipid NPs to nanogels.

Types of nanoparticles-vesicles

Solid lipid nanoparticles (SLNs) are colloidal carriers, spherical in structure (50 to 1000 nm) containing physiologically tolerated lipid components such as fatty acids, waxes, or glycerides, with emulsifiers such as polyoxyethylene ethers, polyvinyl alcohol, phospholipids, bile salts, or Tween®, with solid shape at room temperature (Fig. 2, Table 1) [4]. The major advantages of SLNs are that the surface is hydrophilic-coated, thus reducing toxicity of these NPs as they are not readily absorbed by cells of the reticuloendothelial system (RES) cells and therefore can bypass the liver and spleen [5]; are easily manufactured since they have a high and improved drug content, good biodegradation [6], increased bioavailability of encapsulated compounds, and offer very high long-term stability [7]. In addition, SLNs have contributed to major advances in the development of oral bioavailability of poor soluble drugs [8] such as curcumin for treating a number of human diseases including neurodegenerative diseases (ND), cancer and liver disease [9-11].

Liposomes/micelles are spherical micro- or nano-structures consisting of one or more bilayer with liquid in the core and between the lipid bilayers (Fig. 2). Since the first discovery of liposomes by Alex Bangham in 1965, liposomes have been of upmost interest to the pharmaceutical industry and extensively studied as drug carriers [12, 13]. In the last 30 years

they have had many clinical uses and have been used to change pharmacokinetics, biodistribution, and cellular trafficking of drugs, nucleic acids, and proteins. There are different types of liposomes that vary in size and composition. **Multilamellar vesicles**: with a size range of 500 to 5,000 nm and have multiple bilayers. **Large unilamellar vesicles**: with a size range of 200 to 800 nm. **Small unilamellar vesicles**: approximately 100 nm in size and consist of a single bilayer. **Long-circulating liposomes**: these are modified liposomes (can have specific polymers at their surface) increasing their lifetime in the blood circulation than conventional non-modified liposomes. **Immunoliposomes**: these have antibodies grafted to their surface facilitating them to accumulate in the area within a region of the body where the attached antibody can be recognized.

Polymeric NPs

Polymeric NPs (PNPs) are defined as solid, colloidal particles between 10 – 1000 nm in size [14] (Fig. 2). The PNPs is a general name used for any polymer nanoparticle, normally for nanocapsules and nanospheres. Nanocapsules have a solid shell, are spherical in shape and have a liquid cavity. Nanospheres are spherical solid particles and the surface is used to adsorb molecules at the surface or captured within the particle. PNPs are used mainly as drug carriers offering controlled delivery and good biodegradation [15].

Gold NPs

Colloidal gold has origins from ancient times as a method for glass staining. In 1857, Michael Faraday first reported the optical properties and the quantum size effect of gold nanoparticles (AuNPs) [16]. Since these first works, AuNPs have generated immense interest and in the last decades many applications of using these particles in nanotechnology have been developed [17, 18]. AuNPs are a suspension of gold particles with a size between 3 to 200 nm (Fig. 2).

AuNPs have great optical and electronic properties, highly stabile, biologically compatible, and reproducible morphology [19].

Quantum dots

Quantum dots (QDs) were first investigated in 1983 by Rossetti and colleagues [20]. QDs are the smallest of the NPs between 3 to 30 nm, composed of semiconductor materials synthesized as core–shell or alloy nanocrystalline colloids from II-VI groups (for example, CdSe) or III-V (for example, InP) of the periodic table [21] (Fig. 2). The size of the QD correlates with electronic excitation energy i.e. smaller QDs have a higher energy than larger ones. As a result, the oscillator strength is confined to a few transitions providing finely tuned phototonic and electronic quantum properties [22]. QDs are fairly flexible particles offering great biological function and are highly stable [23]. They have a multiple roles in nanotechnology not only in bioimaging and biosensing but also in the diagnosis and therapy of disease [24].

Carbon nanotubes

In the early 1990s, after the discovery of a third allotropic form of carbon fullerene, Sumio Iijima found a new cylindrical structure of carbon fullerene and hence named these carbon nanotubes (CNTs) [25]. CNTs are composed of rolled graphene sheets as open cylinder or capped ends with a size in diameter by 0.4-3 nm and length by 20-1000 nm [26] (Fig. 2). There are two types of CNTs depending upon the number of sheets rolled in the cylinders, the single-walled or multi-walled CNTs. Single-walled CNTs are made up of a one graphene layer held together by Van der Waals forces giving more flexibility whilst the multiwalled CNTs have several layers of coaxial cylinders around a single graphene sheet giving less flexibility and resulting in structural defects. For a number of years CNTs have been used in

various commercial products such as rechargeable batteries to water filters [27]. Research using CNTs as carriers of biomolecules such as DNA and proteins is ongoing and are still not available as clinical medicines. However, CNT nanotechnology is attracting attention in the industry as ideal candidates for drug delivery. Several groups have developed potential applications using CNTs to specifically target tumor drug delivery, for example in the treatment of leukemia [28], liver [29], and bladder cancer [30]. The potential toxicity elicited using CNTs remains a problem and has been attributed to their physical properties but can be avoided by surface-grafting biomacromolecules or polymers [31].

Dendrimers

Dendrimers are macromolecules comprising of symmetrically arranged branches ascending from a multi-functional core with a size range between 2 to 10 nm (Fig. 2). Tomalia and colleagues described the first synthesis of dendrimers back in 1985 which were referred to as "starburst polymers." Dendrimers can encapsulate active drugs and deliver these active drugs to target tissues. Also, dendrimers can present targeting ligands at their surface aiding correct delivery to the target tissue. [32]. Polyamidoamine dendrimers are the most well-characterized dendrimer family composed of a diamine core reacted with methyl acrylate and ethylenediamine to give consistent radial concentric layers.

Iron oxide NPs

Iron oxide nanoparticles (IONPs) comprise of magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3) in the range size of 5 to 200 nm (Fig. 2). IONPs represent an important class of NPs that are backing the current revolution in nanotechnology and nanomedicine [33-35]. Owing to their exclusive physical properties such as their high surface area to volume ratio and super magnetism (whilst Cobalt and Nickel are highly magnetic, the disadvantages are they are

fairly toxic and easily oxidized), are useful features for medical approaches such as magnetic resonance imaging (MRI), tissue engineering, bioseparation, and drug/gene delivery [35]. Subsequently, to reduce toxicity the hydrophobic IONPs can be coated with different inorganic materials such as phospholipids, amphiphilic polymers or silica, thus improving their solubility and biocompatibility in vivo.

Cerium oxide NPs

Cerium oxide nanoparticles (CONPs) are composed of a cerium core with an external oxygen lattice. These NPs have been used frequently for many biological and engineering applications, for example as solar cells [36], in high-temperature oxidation protection materials [37], in solid-oxide fuel cells [38], and catalytic materials [39]. CONPs are of particular interest in nanomedicine as they have shown the capacity for a number of approaches [40]. The cell or tissue conditions are believed to be important in the role of determining redox activity, since CONPs have additionally been observed to present a number of antioxidant reactivity such as catalase mimetric activity [41], superoxide dismutase (SOD) activity [42], hydroxyl radical scavenging [43], and nitric oxide radical scavenging [44]. In addition, CONPs have been shown to possess oxidant behavior [45] and these activities (oxidant or antioxidant) are believed to be driven by the pH levels [46, 47].

Mesoporous silica NPs (MSNPs)

MSNPs were originally discovered in 1992 by the Mobile Oil Corporation. In recent years, they are emerging as a promising candidate for drug delivery in the nanotechnology field. The MSNPs have a size of 50 to 200 nm and are surface-grafted with polyethyleneimine (PEI) or PEI- polyethylene glycol (PEG) or polyamidoamine. Modifying the surface of MSNPs offers both the benefits of loading small interfering RNA (siRNA) and improved uptake and

delivery to cells. The most common types are Mobil Composition of Matter-41 and Santa Barbara Amorphous-15 also known as MCM-41 and SBA-15 respectively [48]. The physical properties of these NPs are ideal for the purpose of drug loading, controlled drug release and delivery, and multi-functionalization [49, 50]. MSNPs could be used for the delivery of drugs but also for a number of other applications including intracellular controlled catalysis and as a biosensor in tumor imaging.

Nano-hydroxyapatite (n-HA)

Hydroxyapatite (HA) is a bio mineral composed of calcium and phosphate, represented by the chemical formula ($Ca_{10}(PO_4)_6(OH)$). It is the main organic component of human hard tissues such as tooth and bone. In the past years, HA has been of particular interest to the medical field due its application in prosthetics. The proportion of the aging population is increasing and as an effect the number of people presenting with bone illnesses such as fractures due to osteoporosis and patients bedridden due to apoplexy are on the increase [51]. HA can be manufactured in different forms from a composite ceramic to powder form depending upon its application. nHA with the size of >100nm has been developed over the past years. Several groups have shown that nHA NPs have minimal effects on healthy cells whilst at the same time inhibit specifically the cellular proliferation of cancer cells, such as liver and glioma cells [52-54]. nHA NPs coated with a fluorescent dye are great carriers for applications such as imaging or for photodynamic therapy. The development of future technologies offers many interesting avenues of new research including the development of ceramic particles incorporating drugs such as doxorubicin [55] that are required to be slow-released over several days or months. The time of release could be controlled by the CaP composite having varying times of degradation.

Nanogels

Nanogels are nanosized particles with a size range between 20 to 200 nm, are formed by physical or chemical cross-linked polymer networks that are able to swell upon contact with solvent. The use of "nanogel" (NanoGel™) was first described to explain the cross-linked bifunctional networks of a polymer (polyion and a non-ionic polymer) in the delivery of polynucleotides (cross-linked PEG-cl-PEI or PEI and PEG) [56]. Nanogels have pores that can be filled with small molecule compounds and biomacromolecules [57]. Their physical properties can be controlled, such as swelling, degradation, and chemical functionality which all are important in delivering drugs in a controlled, sustained and targetable way.

Administration routes for therapy with NPs

Given their modularity and the possibility to build/customize NPs for the drug delivery, NPs are very promising for therapeutics especially owing to that they permit control of drug release and to increase their bioavailability with a low immunogenicity. Furthermore, by dedicated engineering it is possible to target specifically the lesion site limiting the side effects of highly toxic compounds. Besides, an important concern to take account is how to bring these drug carriers into the organism to facilitate the targeting of the affected organ, maximizing its bioavailability and limiting the drug side effects. As an example, the intranasal delivery is studied for antibiotherapy against tuberculosis with streptomycin, to limit its ototoxicity and nephrotoxicity and increase its bioavailability [58]. By consequence, the choice of the administration route is crucial [59]. Numerous routes were described in the literature concerning NP administration (Fig. 3):

- parenteral (intravenous, intramuscular or subcutaneous) [60];
- oral [61]

- rectal [62]

- transdermal [63]

- pulmonary [64, 65]

- nasal [66, 67]

- ocular [68]….

Amongst these multiple choices, the first point to consider is the anatomical localization and particularities linked to the targeted defect choosing between a local or a systemic delivery. In this way, the nasal route is of great interest for drug delivery directly to the brain, bypassing the BBB (blood-brain-barrier) [69]. The oral route is the most convenient but between the mouth and the intestinal mucosa, the difficult point for the NPs is to pass the gastro-intestinal tract due to its microenvironment (low pH, presence of digestive enzymes) [61]. These characteristics contribute to the elaboration of different systems such as the pH-sensitive NPs made with Eudragit® S100 enhancing intestinal absorption of the entrapped compound [70]. In the context of inflammatory bowel disease, ligands were grafted on poly(lactic-co-glycolic) acid-block-polyethyleneglycol (PLGA-PEG) to actively target the affected tissue [71]. PLGA-PEG were also studied for the oral delivery of insulin avoiding thus parenteral administration [72] which may represent a quality-life improvement for patients. This is particularly crucial for the delivery of peptides. An alternative, especially in the case of cancer treatment is to use the lymphatic drainage to administer nanoparticulate drugs, the lymphatic system can be reached by parenteral (subcutaneous) route, but also through the oral mucosa or the respiratory tract. Local NP administration is thus an interesting alternative. Moreover, some organs are more easily accessible by another way than a systemic route. This is the case in the eyes where NP-loaded drugs or gene-delivery systems can be delivered by instillation into the conjunctival sac, intravitreous or subtenon injections of NPs that were set up for the treatment of eye diseases such as retinal neurodegeneration [73], glaucoma [74, 75], virus-infection [76]

or retinoblastoma [77]. Another example is the rectal administration that is used for colorectal cancer therapy purpose [78] but also for delivery of anti-vomiting drugs as an complement of chemotherapy [79]. The advantage of local administration is the limited area of action for highly active drugs. Thus, ocular administration of polymethylmetacrylate NP containing carboplatin, a chemotherapy drug with adverse side effects, allow a sustained-release of the drug in the vitreous and the retina without systemic detection of the drug for retinoblastoma treatment after a single subtenon injection [77]. Amongst local targets can be cited the inner ear [80] and hair follicles [81]. Following from that, whatever the administration route, nature, composition and building of the NP should be highly studied. The majority of the targeted ways (eyes, nose, lungs, gastrointestinal tract, cervico-vaginal) involves the crossing of a mucus barrier before internalisation of the NPs by the cells [82, 83]. In the case of local administration into the nose or the eyes, for example, the mucoadhesive properties of the NP must be high enough to enhance the retention time in the aim to extend the time of contact with the biological membranes to improve the drug absorption [84]. That is obtained playing mainly on the NP coating with surfactant molecules such as PEG, chitosan, lecithin which influences the pharmacological and chemical parameters characterizing the NPs [85-87]. In this way, Holden and colleagues have tested, using an *ex vivo* corneal model, PEGylated-polyamidoamine dendrimers *in situ*-forming hydrogel for the delivery of timolol maleate and brimonidine for glaucoma treatment [88]. Then, it is noteworthy that the timing for drug action (short or long term action) will influence the choice as well as the stability of the NP encapsulated molecule. Hence, systemic administration through the nasal route is preferred when a rapid onset of the drug action is essential [67]. A meta-analysis of literature data concerning the clinical pharmacokinetics of three kinds of drugs dedicated to target the brain (opioids, benzodiazepines and anti-migraine) highlights differences between the formulations with a quicker effect when drugs are administered intravenously [89]. In the case of epilepsy-

related convulsions, the rectal administration of diazepam leads to an effect within 15 to 20 minutes after administering. In a rabbit model, the effect is visible as soon as 5 minutes after delivery of a submicron emulsion [90]. Finally, some administration pathways are preferentially studied for certain populations of patients such as young children or patients with neurological disorders. Thus, the rectal route is easier for paediatric patients whilst the nasal delivery is of interest for non-compliant patients suffering from cognitive disorders [59, 90].

For a systemic intervention, three routes are favored : parenteral, oral or nasal delivery [91]. In most cases, to increase the NP bioavailability and its stability, a coating is necessary; PEG or hydrophilic molecules with close properties are mainly used. In the case of a parenteral administration, the goal is to increase the life-time of the substance in the blood, to limit interaction with phagocytic cells or with proteins such as opsonins [92]. The mode of administration (i.e. intramuscular, intradermal) influences also the efficacy of the drug. A study shows the differential organ/cellular uptake of pluronic-stabilized poly(propilene sulfide) NPs in an *in vivo* murine model with a better efficacy for the intradermal injection upon an intramuscular one to reach lymph node, blood and spleen and immature and cross - presenting antigen-presenting cells [93]. The development of stimuli-responsive NP is a promising way in therapeutics. Indeed, NP systemically delivered can be guided to the target site using physical properties conferred by surface functionalization of drug nanocarriers [92], limiting thus a dispersion through the body. Stimuli can be exogenously applied and can vary when a magnetic field, light or temperature is increased. Moreover, NP can be created in such a manner that they could be responsive to endogenous stimuli such as pH variation and activated by enzymes. The progress of technology allow even the building of multi-stimuli-responsiveness drug delivery systems [92]. In this way, such a method was used *in vivo* by the way of MRI-guided focus ultrasound technology for enhancing brain delivery of

intravenously administered AuNPs. This method was then applied to visualize the tumor margin within xenografted animals by Raman spectroscopy [94, 95].

Diseases and therapy advances

Nanoparticles and neurodegenerative diseases

ND are commonly late onset, progressive and fatal disorders of the central nervous system (CNS, the brain and spinal cord) leading to a neuronal loss in a specific brain area depending on the nature of the pathology associated with a neuroinflammation (microglial activation). This results in a gradual overcoming of cognitive and/or motor disabilities corresponding to the disease relative symptoms (phenotype). ND are currently affecting approximately 50 million people worldwide. Alzheimer's disease (AD) and Parkinson's disease (PD), the most common ND with respective prevalence estimated to be more than 44.4 million in 2013 (source: AD international) and 6 million people around the world [96-98]. Whatever the disease origin, i.e. familial or sporadic, ND are generally associated with aggregate-prone proteins accumulating in an insoluble form as cytoplasmic (tau, α-synuclein), extracellular (β-amyloid peptide), or nuclear (ataxin) inclusions in or close to degenerating cells. Most of the resulting aggregates are highly ordered because they are enriched in crossed β-sheet structures (i.e. amyloid-like polymers) can be stained with dyes such as Congo red or Thioflavin T [99-101]. It has been suggested that oligomeric species are the more toxic forms compared to larger aggregates [102]. Moreover, growing evidence is indicating that the spread of pathology is similar to prion-like form with an initial oligomer seeding event followed by the spread of the aggregates and a cell-to-cell communication of the pathology [100, 103, 104].

Ageing is the common shared and essential risk factor associated to the appearance of such a kind of pathology [105]. Pathological proteins linked to genetically transmissible ND are natively mal-conformed constituting a constant risk throughout life [106]. In such a case,

the aggregation induction is a process linked to the time and to the risk factors relative to the concerned disease. For sporadic forms of ND, the involved proteins acquire their aggregate-prone features mainly through covalent post-translational modifications such as oxidation, phosphorylation, nitration, or by proteolytic cleavage (for examples, see [107, 108]. Misfolding can induce either toxic gain-of-function or loss-of-function which can promote the oligomerization of pathological proteins. In all cases, the presence of aggregate-prone protein associated is challenging the proteostasis network (PN). Protein homeostasis (also called proteostasis, [109] depends on a network involving the cellular mechanisms dealing with protein from their synthesis to their elimination as well as nutritional status detection systems and quality control pathways [109-113]. Consequently, the proteostasis network (PN) corresponds to an integrated complex of sub-networks with different physiological roles linked by central master protein factors allowing coupled stress responses [114]. Mechanisms involved in the synthesis, quality control and protein degradation, the pathways linked to the energy sensing, metabolism regulation, stress response detection and response systems are counted among the PN.

Consequently, strategies to treat ND are targeting either the pathological protein downregulating their expression using gene therapy (Huntington disease (HD), Amyotrophic Lateral Sclerosis (ALS), familial PD) (reviewed in or activating pathways belonging to the proteostasis network [109, 115, 116] such as antioxidant machinery and activation of catabolic processes.

Challenges for nanomedicine in the context of ND

Diagnosis and therapeutic strategies

Except a post-mortem examination of the patient brain, in most of sporadic forms of ND, it is difficult to set up a definitive diagnosis. Nevertheless, using imagery techniques (positron

emission tomography (PET)or MRI) associated with the use of the appropriate tracer (such as in the case of AD or in PD context for example) allow the clinician to establish a probable diagnosis correlating neuroanatomy criterions with cognitive or motor deficiencies. For example, in the context of dementia of Alzheimer's, the diagnosis criteria are based upon recommendation of the NINCDS-ADRDA (*National Institute of Neurological and Communicative Disorders and Stroke - AD and Related Disorders association*) [117] associating biological and clinical evidences for the establishment of a possible, a probable or definite AD diagnosis [97]. This evidence is brought by neurologic examination associated to morphologic (MRI), structural, functional or PET imaging and biochemical analysis by detection of biomarkers (Aβ peptide and total and/or phosphorylated tau) in the cerebrospinal fluid (CSF) but often, taken alone, this information is not sufficient for diagnosis. These techniques are useful and are more efficient in the assessment of the pathology at advanced stages of the disease. Indeed, ND are often characterized with a lengthy prodromal phase for the duration of which irreversible neuronal lesions are set up precedes cognitive and/or motor decline symptoms, this is the case for AD, PD, or ALS [118-120]. In the later, the first clinical signs appears when the motor neurons loss is over 50% [121]. Clinical trials for neurodegenerative targeting drugs are made with cohorts of symptomatic patients. Given that the existence of a long prodromal stage during which a compensation mechanism masks the neuronal degeneration that already took place, (as it is discussed in [103, 122]), it would be relevant to include patients at earlier phases of the disease. However, beyond the problem due to an extension of the trial duration - for example the AD prodromal phase was estimated to be an average of 9 years before the first cognitive symptoms detection [118] - is the problem of the cohort recruitment owing to while a huge progress in imaging techniques for ND diagnosis, it is still difficult to certainly asses the presence of the pathology and to name it. Furthermore, as the clinical trials in patients represent an important effort, there is no

satisfactory treatment as yet available to cope with NDs, only some drugs can alleviate the symptoms.

The blood-brain barrier: a shield too strong?

Another major challenge is the particular status of the CNS due to the existence of the BBB, a protective neurovascular unit restricting the entry of the blood content into the brain and regulating nutriment and ion delivery to the brain tissue conferring it a privileged immunologic status (reviewed in [123, 124]). The BBB is a dynamic structure composed of brain microvasculature endothelial cells. They are characterized by specific tight junctions restricting paracellular diffusion and polarizing the structure, lacking fenestration and a minimal pinocytic activity. Whilst the endothelium is surrounded by pericytes, all embedded in a basal lamina and its extracellular matrix and all ensheathed by astrocytic end-feet [125-127]. The BBB corresponds to a physical, metabolic and transport barrier segregating the brain blood flow from the rest of the organism.

Bypassing this complex and very selective shield is a critical point for the engineering of nanocarrier-based therapeutic strategies [128]. Only few small lipophilic molecules (< 500Da) can diffuse freely through the BBB. Nutrients are transported across the BBB by specific transporters excluding or effluxing potentially neurotoxic molecules. The uptake of larger molecules (protein) such as iron transferrin or insulin is regulated *via* the highly specific receptor-mediated endocytosis. An adsorptive-mediated transcytosis, less specific is also described. The strategies for drug delivery to the brain should consider BBB permeability [127, 128]. Indeed, a systemic administration of a drug will not signify that it will enter the brain and while administration of higher doses which is related with more side effects (Fig. 3).

Consequently, the putative pharmacological approaches should consider the existence of this protective shield representing the main challenge in the treatment of brain affected

diseases. Two approaches are opposed for CNS drug delivery: invasive by breakage of the BBB (transcranial injection, ultrasound activated microbubbles, osmotic modulation of BBB permeability) or non-invasive delivery using small molecules, lipidation (prodrugs) or use of carrier-mediated transport [128]. Amongst the breakage methods, some, such as the use of ultrasound activated microbubbles are transient and reversible [95]. Consequently, the engineering of NP bypassing the BBB are promising tools for diagnosis and treatment, neuroprotection against ND as the image of the works of Kreuter and co-workers in 1995 who put in evidence the importance of use of surfactant coating (polysorbate 80 in this study) of drug-loaded NP to allow the passage of the BBB [129]. Finally, the drug driven to the brain through the BBB should not be rejected back by exchange channel at this level such as the efflux pump P-glycoprotein [130]. The last point to consider is the way of administration. Indeed, the ideal treatment should be easily administered to non-compliant patients who, due to the nature of their pathology i.e. a degenerative disease affecting the CNS, can present cognitive or psychiatric troubles. Hence, three major routes are privileged: systemic administration by oral or parenteral route on one hand and the intranasal route on the other hand (Fig. 3).

Aims and strategies in the ND context

Diagnosis, theranostics and drug delivery: targeting the brain with NPs

As presented above, the first challenge to consider in the ND context is to establish the diagnosis as earlier as possible. Some dyes are available to stain amyloid or amyloid-like structures such as a thioflavin T derivative, can cross the BBB, and the Pittsburgh compound B, used in PET-scan imaging [131]. However, for example for the case of AD, the amyloid burden is not well correlated with the cognitive decline but the cognitive decline is associated

with a decrease in the cortical thickness [103] and many efforts are made with the goal to develop diagnostic tools [132, 133]. It was also demonstrated that some healthy old people can have an extensive brain accumulation of Aβ peptide [134]. Moreover, a given aggregate-prone protein is not exclusive for a given ND. Indeed, PD patients can present an Aβ accumulation. In addition, neurofibrillary tangles, constituted by aggregation of hyperphosphorylated tau, are not only an AD hallmark, but are characteristic pathological lesions in a group of diseases named tauopathies amongst them the fronto-temporal dementia or Pick disease [133, 135]. Consequently, ND diagnosis relies upon a body of evidence. Consequently, efforts are made in the aim to enhance the potency of diagnostic tools and to search for efficient biomarkers [136, 137].

NPs are of a particular interest for the detection and imaging of amyloid-like protein species (imaging and radio-tracers) and in the assessment of the pathology presence. The NP will convey either antibody or dye recognizing the incriminated protein species or other significant target(s) [138]. Such NP can be ameliorated to present a therapeutic property, the term theranostic was coined to define such a technology. For example, the cyclophosphamide - a drug known to alleviate inflammation in cerebral amyloid angiopathy, which corresponds to the deposition of Aβ in the brain and leptomeninges small-to medium caliber blood vessel - was encapsulated into Magnevist ® - conjugated chitosan NPs that were also conjugated to an immunoglobulin F(Ab')2 fragment against Aβ. These NPs play a dual role as a MRI contrast agent and at the same time as a nanocarrier for a drug [139].

For drug delivery as highlighted here and in many reviews about brain drug delivery, the main thing to point out is that one must consider engineering NPs with the capacity to cross the BBB. This can be done by coupling the NP to a ligand such as transferrin, insulin, or an amino acid which have a high transport efficacy; agonist antibodies specific of receptors (OX26 that targets transferrin receptor); transporters (LAT-1 GLUT1 for amino acids)

involved in the regulation of molecule passage at this level [140-143]; or changing their lipophilicity or their surface potential by lipidization, or by surfactant coating [144]. For example the polysorbate 80 adsorbs ApoB and E lipoprotein in the blood which allow them to pass the brain capillary endothelial cells [142].

Gene therapy

Gene therapy strategies could be envisaged for the familial forms of ND. For example, familial forms of PD can be caused by mutation in the gene *SNCA* coding for the neuronal protein α-synuclein. Huntington disease (HD) is due to the repeat of a CAG motif in the gene coding for huntingtin. The strategy would be to silence such genes. In the case of PD [145], this therapeutic method can also be used to silence molecular targets thought to be involved in the pathogenic process. Thus BACE-1, a β-secretase participating in the maturation of amyloid peptide precursor (APP) into the Aβ peptide in the AD context, was down-regulated using QDs technology in the SK-N-SH cell line model [146].

Therapeutic strategies using nanoparticles in ND

Alzheimer's disease

AD is the most common adult onset ND, affecting more than 35 million people worldwide. AD, widely characterized by two pathological hallmarks: formation of extracellular deposits of Aβ, senile plaques (SP) and intraneuronal accumulation of hyperphosphorylated tau as neurofibrillary tangles (NFT) or paired helical filaments (PHF) [147, 148]. Aβ results from the sequential cleavage of APP by the β- and γ-secretases. There are numerous drugs in the pipeline that are specific for AD but could also be used for other types of ND (Table 2). The current therapeutic strategies target aggregate formation to aid in the understanding of their involvement in AD pathogenesis. It is in the case of transferrin-PEG-QDs cross-linked to a

recombinant Aβ peptide in the goal to follow their oligomerization and evaluate the kinetics of fibril formation *in vivo [149]*. A similar approach tested in vitro used an Aβ monoclonal antibody coupled to near infrared fluorescent IONP as a potential theranostic technology [150]. Quantum dots coupled to anti-Aβ antibody were designed as lab-on-chip systems to allow the screening of potential anti-aggregating compounds in a micro-scale volume of body fluid [151]. Lab-on-chip devices using cadmium/selenide/zinc sulfide QDs in order to detect the plasma levels of ApoE *ex vivo* as a diagnosis biomarker [152]. Some other NP based systems were described for biomarker detection such as tau protein [153]. Superparamagnetic IONPs are widely tested for producing potent and specific contrast agents in MRI [154]. SPION coupled NPs were tested by *in vitro* imaging of Aβ-related aggregates [155] and *in vivo* with a transgenic AD mouse model [156-158]. This type of approach by coupling Aβ-targeting antibodies to NPs give rise to the question of a double function of such nanocarriers which could have positive side-effect capturing toxic protein species, and activating elimination pathways adding a possible therapeutic action [159-161].

Disruption of amyloid aggregates using destabilizating drugs such as green-tea also known as polyphenol epigallocatechin gallate (EGCG) is extremely encouraging [162, 163] not only in AD but also in other ND such as PD. However, the bioavailability of EGCG and access to the brain is quite poor [99]. The integration of such a compound into NPs to enhance its delivery to the brain is logically studied [78] and appears to inhibit amyloid aggregates *in vitro* when incorporated to Tet-1 peptide (a neuron-targeting molecule allowing the bypass of the BBB via a retrograde axonal transport) linked selenium NPs [164]. The same strategy was chosen using sphingomyelin and cholesterol liposomes functionalized with amyloid-binding acidic lipids such as ganglioside GM-1, phosphatidic acid or cardiolipin *in vitro* and was shown to alleviate tau pathology acting on the balance between kinases and phosphatases while transposition to an animal model should take in account that GM-1 can cause

neuropathies [165]. Curcumin, which can bind amyloid aggregates, as EGCG (the both are actually in clinical trials for a possible AD therapeutic action) is also subjected to NP encapsulation in the aim to enhance its action as a proved PN enhancer compound since it presents a poor bioavailability *in vivo [99, 166-169]* not only in AD context but also in most of the degenerative diseases linked to protein aggregation such as type 2 diabetes [170]. Several kinds of NP were elaborated and tested in either *in vitro* or *in vivo* transgenic models; nanoliposomes [171-173], PEG-PLGA-NP [174, 175]. In the same way, *in vitro* experiments were conducted with PEG-PLGA NP containing selegiline [176].

Polyphenols exert an antioxidant action presenting beneficial effects for neurodegeneration [177]. Thus, antioxidant drugs such as idebenone, an analogue of the co-enzyme Q10 are in consideration for building anti-AD NPs [178]. Oxidative stress is a common feature of proteinopathies. Consequently, similar approaches consisting of the delivery of anti-oxidant molecules loaded NPs are elaborated in other ND context [78]. For example, pomegranate seed oil nanoemulsion was shown to delay the onset of prion pathology in a model of genetic Creutzfeld-Jakob disease [179].

A deficit in the production of acetylcholine is related to AD [180]. Therapeutic strategies are targeting their efforts towards the inhibition of acetylcholinesterase or an agonist of cholinergic receptors [181] using molecules such as rivastigmine incorporated into liposomes [182, 183], or polymer [184] or galantamine [185]. This acetylcholine decrease was used as an *in vivo* biosensor system using carboxylated multi-walled CNTs and zirconium oxide NP onto which were co-immobilized acetylcholinesterase and choline oxidase and deposited on glassy carbon electrodes [186].

D-penicillamine, a copper chelating molecule, was shown to have an interest for AD-targeted therapy [187, 188] since metal ions such as iron or copper are involved in the formation AD pathogenesis due to the binding of APP, their accumulation in the AD brain

[177, 189, 190]. This compound, already used in therapy of other pathologies such as the Wilson's disease affecting the liver, rheumatoidis and lead poisoning was tested as a nanoformulation where it was covalently linked to NPs *in vitro* and was successfully shown to disaggregate Aβ peptide [191].

Parkinson's disease, Amyotrophic lateral sclerosis and other neurodegenerative diseases

PD, the second most common ND after AD. This pathology is characterized by α-synuclein aggregation, the physiological function of this protein still remains to be elucidated, and the specific degeneration of the dopaminergic neurons in *substantia nigra,* striatum and in other brainstem regions leading to the appearance of motor and cognitive clinical symptoms: bradykinesia, resting tremor, rigidity and other troubles.

As for AD, most of the PD cases (90 to 95%) are from unknown etiology, however around 5-10% of cases are familial forms due genetic mutations. There are 6 autosomal genes that are involved including *SNCA* encodes α-synuclein, *LRKK2 (*leucine-rich repeat kinase 2)*, PINK1 or PARKIN* were described [192-194]. Whatever the etiology of PD, modifications of α-synuclein playing a major role in pathogenesis, the several NP kinds were elaborated prion-like behaviour of this protein was assessed [100].

Besides therapeutic strategies based on agent destabilizing protein aggregates, playing on the enhancement of the PN or targeting oxidative stress with the reformulation of the same molecules than used in AD therapy research, such as EGCG and curcumin [162, 195], the elaboration of PD specific therapeutic solutions consist in the enhancement of existing solutions which alleviates temporally the clinical symptoms - amongst them the oral administration of levodopa presents the highest efficiency on motor symptoms - and the gene-based therapy [196]. Thus, new L-dopa formulation using NPs has been proposed: chitosan-

coated NPs [197] and tested *in vitro* for toxicity [198]. An intrathecal implanted system allowing a site-specific controlled release of cellulose acetate phthalate NPs loaded with dopamine during a period of time was designed and evaluated in a rat model for the establishment of PD chronic drug delivery system [199]. Similarly, encapsulation of bromocriptin in NPs was also tested [200], but the finality of such approaches has especially an interest in the elaboration of effectiveness and non-invasive drug delivery systems targeting brain pathologies. Consequently, interest stays with the ability for nanosystems to deliver nucleic acid to lesioned cells for gene-based therapy [201], particularly regarding the advantages presented by NPs compared to viral vectors to date giving less immunogenicity, the possibility to repeat the treatment and most importantly their ability to cross the BBB. Nanogels composed of PEG and PEI covalent cross-linked were designed for oligonucleotide delivery. PEI derivatives are routinely used in mammalian cell culture transfection. The functionalization of such nanogels with insulin and transferrin allow them to reach the brain crossing the BBB [202]. They present the advantage of spontaneously encapsulating negatively charged oligonucleotides. Gene therapy can also involve the delivery of specific microRNA. In addition, some of these small oligoribonucleotides playing a role in the regulation of gene expression and can be deregulated in most of the neurodegenerative diseases [203, 204]. By this way, it is possible to silence defective genes in neurons [205] or to overexpress genes coding for proteins with a rehabilitating function i.e. therapeutic genes. Neurotrophins such as Glial cell line-derived neurotrophic factor (GDNF) or brain derived neurotrophic factor (BDNF) expression are in exploration for AD and PD, and was tested in the ALS context as a therapy for the repair of synaptic failure [206]. In vitro exogenous expression by vascular endothelial growth factor (VEGF) or GDNF overexpressing mesenchymal stem cells of GDNF had positive effects in an ALS rat model [207], GDNF expression has also a protective effect in a murine transgenic model for AD [208]. In a rat

model, it is possible using compact DNA NPs (naked DNA), directly injected into the brain to express a gene for a period of 8 weeks with minimal inflammation [209]. Promising results were obtained after transfection of cultured dopaminergic neurons using RTX-polyplex (PEI derivative) NPs as an *in vitro* model for PD [210].

ALS, (Motor Neuron Disease, Charcot's disease, Lou Gerhing's disease) is a devastating ND affecting motor neurons in the brain cortex, the spinal cord and the brainstem. It results in a progressive impairment of the motor functions (paralysis) until the death of the patient within 3 to 5 years generally by a respiratory failure as result of paralysis of the respiratory muscles. The pathology onset is generally around 60 years old and most of the cases are sporadic (etiology unknown). Approximately, 10% of ALS cases are of familial origin among them 20% due to a mutation in the gene encoding the Cu/Zn superoxide dismutase 1 (SOD1) [211, 212] and around 34% are due to a hexanucleotide expansion in *C9ORF72* gene [213, 214]. *C9ORF72* is also involved in the development of another ND, the fronto-temporal dementia which can be associated with ALS [215, 216]. Therapy using antisense oligonucleotides that target repeats containing related-RNA is a rational approach [217]. Besides a method for the oral deliver antisense oligoribonucleotides adsorbed onto a cationic core-shell NP has been described in a mouse model of the X-linked recessive Duchenne muscular dystrophy [218].

Huntington disease (HD) is a hereditary neurodegenerative disorder due to the repeat of a CAG motif in the gene coding for huntingtin. HD belongs to a group of proteinopathies due to such CAG (coding for glutamine) repeat in the mutated gene sequence. In this context, the elaboration of NP system are aimed either to modelize the pathology *in vitro* or *in vivo* by expressing a peptidic sequence containing a CAG repeat of a pathologic length with a strong tendency to aggregate and to cause cell degeneration on one hand. Hence, organic modified silica (or also called ORMOSIL) nanostructures permitted Klejbor and colleagues to express

the CAG repeat in rat or mouse brains through an intraventricular injection to modelize HD brain pathology [219]. Organically modified silica NPs are also of interest in targeting axonal transport defects in neurodegenerative condition [220]. Interestingly, it has been suggested that such particles are promising since by coupling them to protein allowing them to be specifically targeted to neurons and then to associate them to specific subcellular structures such as a molecular motor for intraneuronal transport [220]. On the other hand, amphiphilic β-cyclodextrine oligosaccharide based molecules were successfully used as siRNA carriers to reduce Htt expression in rat striatum [221].

NPs are apparent tools in neurodegenerative diseases treatment but the arising technology in this domain is biological nanovesicles or exosome-mediated therapy [222, 223]. Exosomes are believed to participate in cell-to-cell communication, were shown to naturally convey protein or nucleic acid to receptor cells (discussed in further detail in extracellular vesicles section). Since these vesicles are produced by most cells, not surprisingly neurons, glial and microglial cells produce exosomes. The elegant work of Alvarez-Erviti and colleagues present a safe tissue-specific siRNA delivery (brain and muscle) by engineered exosomes from the transfection of exosome producing cells with a gene for the expression fusion endosomal protein (LAMP2b fused to a brain or muscle peptide), to their administration by intravenous injection after their electroporation-mediated loading of siRNA. The high efficiency of this system to knockdown the targeting gene is very promising especially as a siRNA against BACE-1 was used in normal and AD modelizing mice [224], discussed in [225]. However, exosomes produced in a pathological condition by themselves could be a new therapeutic target since they were suggested to participate in cell-to-cell transmission of prion-like aggregate-prone proteins and in degeneration progression in AD and PD [100, 226]. Microvesicles were shown to contribute to the microglial activation, induction of inflammation and to participate in neurodegeneration [227].

Neurodegenerative disorders as a consequence of an exposure to nanoparticles

Owing to the BBB protective function, it can be difficult to specifically target molecules to the brain parenchyma hence the multi-functionalization is necessary for brain targeting NP. However, as an intrinsic function of the BBB is to avoid the penetration of potential harmful molecules into the brain tissue. Consequently, neurotoxicity of NP needs to be considered for the same reasons than a more conventional therapeutic system. Since microglial activation is involved in the neurodegenerative pathogenic process in a majority of CNS disorders, it is one of the most important points to consider and to envisage in NP neurotoxicity. In this way, an *in vitro* study using cultured microglial cells has shown that amongst four types of inorganic NPs, TiO2, SiO2, Fe_3O_4, and hydroxyapatite, while Fe_3O_4/hydroxyapatite (also known as HAP) and TiO2 presented the ability to trigger the expression of inflammation related iNOS (inducible nitric oxide synthase) and activate NF-κB signaling pathway, all the tested NP induces to a variable extent the increase of pro-inflammatory molecule production (TNFα, IL-1β and IL6) which lead to a toxicity in PC12 cells [228]. Another study using IONPs, *in vivo* and *in vitro s*how that activation of the microglial cells and possible pathological changes as reflected by morphological changes in the olfactory bulb, striatum and hippocampus. However, while the activation of microglial cells reflected by production of reactive oxygen species and nitrogen oxide is assessed, it remains to be seen if it is in favour of neuroprotection or neurotoxicity since microglial cells represent the immune system in the brain tissue [229]. SiO_2 NPs were shown to induce PD's like pathology in Zebra fish [230]. Other studies regarding the toxicity for medical purpose of magnetic NPs in an *in vivo* rat model show nearly no toxicity [231]. Another study concerning the toxicity of aminosilane-

coated IONPs in three cultured cell models conclude on a satisfying safety regarding the concentration used while a differential charge sensitivity of primary cultured neurons when compared to astrocytes with a higher dosage [232]. Taken altogether these data are giving rise to the concern of side effects linked to the pharmaceutical use of NPs. The risk is to taken into account keeping in mind the benefits provided by NPs. The differential side effects of NPs depends upon the considered organ as well as the model used in the studies i.e. *in vivo* or *in vitro* model. Consequently, as for each active biological compounds, the use of NPs in human medicine should be carefully thought out considering the route of administration which well engages different organs, the dose, but also the benefits for the patients and the exposure time. At the same time, the risk to formulate self-evidence fact, toxicity of NPs, for human and other purpose should be carefully studied for the long term.

Cancer

The formation and the development of a tumor is a process of several steps since the cellular transformation following the passage with the acquisition of different properties from a normal to tumoral cell. Successive genetic alterations are at the origin of the cellular transformation process and at the present time we are not able to establish exactly the mutation(s) or genetic segregation necessary to explain the transformation event. It was proposed by Hanahan and Weinberg that the transformation depends upon acquisition of different intrinsic properties, the hallmarks of cancer, or by diverse attacks like metabolic or oxidizing stress allows DNA damage and genomic instability [233, 234]. The order by which cells acquire their properties seems to depend on the type of cancer and the cellular type. Furthermore, the identification of genetic variability within the same tumor or regional differences by self-production of growth factors highlights the heterogeneity of the tumor. Neoplastic lesion development implies the clearing of anatomical barriers. The cancer cells

invade the stoma through proteolytic enzymes where the presence of cancer cells can initiate several events to support tumor progression [235, 236]. The orchestration of this process with the anarchic cellular proliferation and a weak pressure in oxygen induces hypoxic stress (Fig. 5). In this microenvironment which becomes hostile for both healthy and cancer cells, the cells respond by the induction of a neo-angiogenesis [237]. The VEGF, produced by cancer cells is released into the tumoral matrix, it activates the formation of new blood and lymphatic vessels from the cells of the vascular or lymphatic endothelium respectively who express the VEGF receptor (VEGFR) [238]. The fixation of VEGF on its receptor stimulates migration and proliferation in endothelial cells and the permeability of capillaries. This enables three parameters - oxygen, nutriments and elimination of waste - are restored, leading to a supplementary delay for the tumoral cells. These changes have involved the recruitment of fibroblast, migration of immune cells, matrix remodelling and development of vascular networks. In this context we can evaluate a tumor as a complete organ as opposed to simply visualising a mass of cells. The tumor topography is continually being remodelled where the vasculature network limits the outgrowth. By consequence, the tumor seems accessible by blood vessels and systemic treatment. Unfortunately, the vascularization process is imperfect, hypoxic and anoxic areas appear, pulling a pressure of selection and the appearance of highly aggressive cells (Fig. 5). Gradually, the tumoral cells penetrate into the blood and lymphatic vessels and colonize the secondary organs via metastasis [239]. The conventional methods used in cancer therapy, chemotherapy, radiotherapy and surgery are limited with the appearance of resistant cells or by the access to the cancer cells themselves in the tissue invaded. The neoplastic lesions impairs the systemic drug delivery both by the remodelling of the vascular network and by the chemical properties of the tumor microenvironment such as acidic, inflammatory area, or in the presence of healthy cells perturbing the drug properties

and subsequently the efficiency. Taken together, the drug concentration in the tumoral site is suboptimal and induces several toxic effects in normal cells.

Oral or systemic drug delivery offers a different mechanism to reach the cancer cells and also at the same time reducing the toxicity effects on healthy tissue. Nevertheless, targeting the drug to the correct site and thus, avoiding the side effects or creating resistance is a great challenge to nanomedicine. During the last 30 years, the idea of using NPs as a vehicle to transport drugs seems to be a great candidate and improves the quality of life of patients. For example, Doxorubicin can be used to treat a range of cancers but it is highly toxic and induces heart problems. Whereas Doxil, the first success in nanomedicine as a drug carrier reduces the toxicity effects of free Doxorubicin in the heart [2, 12, 240-242]. Doxil is a lipid bubble (liposome) of 100 nm in diameter engineered by self-collapse around the drug. The size of this liposome offers an opportunity to access the tumoral vascular network whilst escaping the healthy vessels. To help the Doxil to evade the immune system or engulfment by liver cells, PEG was used to coat the lipid surface. The Doxil liposomes facilitate them to accumulate in the tumor, the surface of the liposome degrades, releasing the drug from its carrier and attacks nearby cells. The first step in drug discovery was established, the survival rate of patients and the quality of life were improved but like every original works, few questions still remain such as how to maintain and target specifically the cancer cells. The challenge was to find the component to carry the drugs, their size and the manner to deliver the active substances. The Doxil was the base of the nanoparticle design with a spherical form and membrane-coated. Larger particles impaired the access to the different parts of the tumor independently of their own entry in the tumor vascular fenestrae which could be a block and induce a clearance of the NPs. Furthermore, some types of cancer such as pancreatic and breast cancer are enriched in collagen which represents a physical barrier for drugs. The range of nanoparticle size is between 30 to 100 nm in diameter. The tumor angiogenesis vessels are

disorganized and leaky but NPs with more than 100 nm diameter fail to enter and deliver their material. Different companies have engineered microspheres specific to target cancer cells. The company BIND Biosciences have developed the BIND-014 a Docetaxel polymer nanocarrier directed against both primary and metastatic prostate cancer cells by binding to prostate-specific membrane antigen (PSMA) [243] (Table 3). BIND-014 has currently entered phase II clinical trials, specifically targets the PSMA which is a transmembrane glycoprotein (known also as Nacetyl- L-aspartyl-L-glutamate peptidase I and glutamate carboxypeptidase II) predominantly expressed on prostate cancer cells. Nowadays, some polymers are added at the membrane to target some biomarkers found at the cancer cell surface and to facilitate the drug delivery. Development of personalized therapy against oncogene(s) involved in proliferation and survival pathways represent a new insight in cancer therapy. Many oncogenes are abnormally activated in cancer cells. The epidermal growth factor receptor (EGFR) is the most common oncoreceptor deregulated in cancer. Two compounds designed against mutated EGFR, gefitinib and erlotinib used for the treatment of non-small lung cancer decrease tumor size and subsequently the survival rate of the patient. Unfortunately, the outcome of the patient still remains poor, apparition of new EGFR mutations impaired the drug efficiency and increased the number of resistant cells [244]. By consequence, therapeutic agents designed for one target are not really efficient against a multifactorial disease like cancer. Essentially, few compounds against multiple targets are designed, with predominantly the Single Drug Inhibitor (SDI) or Multi Drug Inhibitor (MDI) to inhibit cancer cell growth and survival. The SDIs affects multiple pathways simultaneously whereas the MDI are a mix of drugs to inhibit multiple pathways. These approaches seem better to avoid drug resistance that are observed and to minimize the side effects. Both types of SDI and MDI have pros and cons, however they both share the same problem of how to overcome delivery and accumulation at the tumor site. The combination therapies available today are limited due to

different drugs have different properties such as pharmacokinetics, biodistribution and transport which cause problems in the dosing and optimization [245-248]. These challenges have been the driving power to test out various ways to deliver multiple drugs within one single nanoparticle [249, 250]. The particle size is a major factor affecting nanoparticle toxicity and the success of MDIs. As such if the particles are too small (i.e. <10 nm), they are able to pass the BBB causing damage or if they too large (i.e. >100 nm) they could be inefficient as carriers [251] Owing to the heterogeneity nature of cancer cells within the same tumor, and multitude of possibilities for both oncogene and survival pathways abnormally activate the method by which could be optimized the MDI represent the new age of personalized treatments. The ambition of nanoparticle-based therapy is to deliver multiple drugs whilst addressing the specificity and optimization of pharmacokinetics [252, 253].

There are currently only a few nanomedicines using NPs approved currently for the use in the cancer treatment such as Abraxane® (Celgene Corporation, Inc., Berkeley Heights, USA), Doxil® (Janssen Biotech Inc., USA), Depocyt® (Pacira Pharmaceuticals Inc., San Diego, CA, USA), Myocet® (Sopherion Therapeutics Inc., USA), DaunoXome® (Galen US Inc., USA), Oncaspar® (Enzon Pharmaceuticals Inc., Bridgewater, USA) and Genexol-PM® (Samyang Biopharmaceuticals Corporation, Korea) (Table 3). There are a number of NPs that are entering early clinical trials or are in the pipeline (Table 3). Although the clinical potential of these NPs in nanomedicine, there still remains limitations with the technology regarding the difficulties experienced in the manufacturing and the hurdles faced in stimuli responsive drug delivery release. The success and development of these NPs to be used in nanomedicine will depend upon all the factors already discussed such as the physical properties of the NP, targeting to specific tissues/organ/cells, bioavailability, and survival rate of the patients. The challenge for the future will be to develop intelligent NPs personalized for each patient for routine use in the clinic practice.

Extracellular vesicles (EV)

Nanovesicles (also called EV) are small parcels that allow information to be transferred from one cell to the next. These vesicles form a communication network in the body between neighbouring cells and distal sites. They were first reported more than 30 years ago by Trams et al in 1981 to result from exfoliation of the plasma membrane from neoplastic cell lines [254]. These vesicles were shown to contain membrane bound enzymes which could be taken up by recipient cells When they observed the vesicles under electron microscopy, there was a mixed population containing vesicles ranging from the smallest at 40 nm to 1000 nm [254]. The smallest vesicles ranging in size from 40 nm to 100 nm are now known as exosomes. These small vesicles were believed to be part of the cells garbage disposal system removing obsolete membrane and cytosolic proteins such as in reticulocyte maturation [255]. In more recent years, exosomes have been shown to play key roles intercellular signalling, for example, in immune system functions such as in antigen presentation of T cells [256], in immune rejection of murine tumors [257] and in the activation of both B and T cell proliferation [258]. It is more commonly accepted that vesicle secretion occurs from most cell types if not all. There are three main populations of EVs that have been characterised which depend on their intracellular origin. The properties of EVs and the ability of the vesicles to deliver their content to a recipient are similar to liposomes that can be packed with therapeutic molecules. These vesicles could be used both as mediators of tumor resistance and as vehicles for targeted drug delivery.

Natural EVs consist of exosomes, ectosomes, microvesicles, oncosomes, and shedding bodies (apoptotic bodies) ranging in size from 30 to 1000 nm [259]. **Exosomes** are derived from the inward budding of the late endosome and hence have a similar lipid biolayer composition to the plasma membrane of the origin cell. Exosomes are maintained in the cell

within the multivesicular bodies (MVBs) and potentially have two fates in the secretory pathway, degradation by fusing of the MVB with the lysosome or release at the plasma membrane from the cell [260]. Exosomes can be identified in combination of specific protein markers for MVB such as for example, Cluster of Differentiation 63, ALG-2-interacting protein, and Tumor susceptibility gene 101, according to their size (30–100 nm), morphology (saucer shape is observed after fixation under transmission electron microscopy), and their density (1.15–1.19 g/ml in sucrose) [261]. It is now clear that it is difficult to distinguish exosomes from other subtypes using the current methods [262-264]. It remains a challenge to develop more sensitive methodologies to clearly define and separate the EV subtypes.

The natural alternative

Exosomes play a major role in many biological processes. They are vehicles that carry both genetic and proteomic information, and hence are presumed to play a role in cell-to-cell communication. The discovery that exosomes have the ability to transfer biological material such as proteins, mRNA and miRNA to other cells, opened up the prospect that exosomes could be utilised as vehicles to transport small molecule compounds in the body [265-267]. The ability to alter protein expression through RNA interference (RNAi) has grown with interest in respect with the applications to treat various diseases. However, a number of problems are encountered such as efficiently targeting specific tissues, preventing an immune response and toxicity [225]. Current research for the clinical application of RNAi focuses on three types of delivery: viruses, polymer-based NPs and liposomes. Nevertheless, there are a number of caveats that limit the use of these types of carriers. Viral particles are easily removed from the bloodstream via antibodies and can elicit an immune response by activation of complement or coagulation factors [268]. Polymer-RNA complexes can be protected from degradation in the bloodstream but can accumulate in other types of tissues including the

kidney, lung, liver, and spleen and thus perhaps limiting their access to the target tissue. Liposomes can adsorb opsonins that in turn can elicit an immune response leading to phagocytosis due their net charge and size [269]. The natural alternative to all these carriers would be to harness the body's own delivery system- 'exosomes' which would bypass the problems concerning immune response, targeting, and biodegradation of cargo molecules. In a first study of using exosomes as vehicle carriers from immature murine dendritic cells to deliver a small-molecule, anti-inflammatory drug to immune cells [267]. Proof of principal was established when Alvarez-Erviti and colleagues harvested modified exosomes from dendritic cells and expressed a fusion protein of LAMP2B and a neuron peptide [224]. The siRNA for BACE1 (a protease implicated in AD) was then electroporated into the exosomes and intravenously injected into mouse brain. The results were promising as siRNA for BACE1 delivered by exosomes could efficiently knockdown BACE1 levels in the mouse brain. However, the use of this technology in the clinical situation still has a number of drawbacks. Firstly, the techniques used for the characterization and purification of exosomes need to be addressed as some exosomal markers are observed in other types of vesicles [270]. The second is to address the methods used to load exosomes with siRNA need to be optimized to increase the efficiency and decrease the large amounts of exosomes administered during therapy. Thirdly, the targeting of exosomes to specific tissues and transport across the BBB needs to be improved. Once we discover how RNA is packaged into exosomes this will revolutionize RNAi-mediated therapy. There are several schools of thought of how RNA (miRNA and mRNA) is encapsulated into exosomes, the most obvious is that the RNA is derived from the cytoplasm due to an invagination event at the MVB into ILVs. In fact, inconsistences remain in the field, some studies have reported that exosomes have small amounts of 18S and 28S RNA; that not all exosomes contain mRNA and miRNA; and whilst others have shown that little of the mRNA and miRNA is specifically targeted and loaded into

the exosomes [265, 271-274]. These differences observed may reflect the cell type, dependent upon cell homeostasis, the stage of the cell cycle or due to stress conditions. Nevertheless, these studies indicate that miRNA and mRNA incorporated into ILVs is a regulated process. Exosomes are comparable to liposomes as they have the ability to cross the BBB, allowing them to be ideal carrier vehicles [275]. A number of studies have shown that exosome signature found in glioblastoma patients showed a high level of TGF-band tumor antigen *EGFRvIII* [275-277]. As the identity of exosomes depends on the cell type, exosomes could be primed to carry biological or chemical agents. Altogether, exosomes appear to be potential therapeutic carriers that would aid in the treatment of a number of devastating human diseases from neurodegeneration to cancer.

Conclusion

The nanotechnology approaches of drug targeting include an array of NPs that have been designed for localised or sustained-controlled release discussed within this review and elsewhere [2, 15, 278-280]. Most of these technological advances have brought products from the bench to bedside but the benefits to the patient have been slow and extend the patient's life by only a few months in some cases. In the past, the use of NPs was visualized as a competitive platform for the drug delivery. Nevertheless, nanotechnology has pros and cons (Fig. 6) which is constrained by many factors such as size, surface chemistry, shape/geometry, targeting ligands of NPs etc. The emergence of using exosomes in nanomedicine as a natural drug delivery vehicle is a promising idea. Since exosomes carry many of the desirable attributes of liposomes i.e. the attributes to transport hydrophobic drugs on the surface of the membrane and hydrophilic drugs in the liquid core. Whilst NPs might initiate an immune response, exosomes are less likely to elicit an immune attack and cellular toxicity. The major challenges will be to understand and to develop exosome-based drug delivery vehicles

without affecting the physical properties and producing reproducible exosomes that are biologically active in the treatment of human disease from neurodegeneration to cancer.

Figure legends

Figure 1 Nanotechnology evolution- the number of publications per year. Publication in the area of Nanoparticles/Nanomedicine is experiencing exponential growth over the last 15 years. These are the number of annual publications with keywords "Liposomes", "Nanoparticles" "Nanoparticles and drug metabolism", and "Liposomes and drug metabolism" between 1965 and 2014 in Pubmed.

Figure 2 A. Types of nanoparticle in drug delivery. Schematic showing the structural variation between various nanoparticles (NPs), such as solid lipid NPs, liposomes, polymeric NPs, gold NPs, carbon nanotubes, dendrimers, quantum dots, iron oxide NPs, cerium oxide NPs, and nanogels. **B. Physical properties of nanoparticles.** Schematic showing the composition, surface chemistry, targeting ligands and physical properties are all important in nanoparticle design.

Figure 3 Nanoparticle- mode of delivery. The various sites in the body used for nanoparticle delivery. In bold are indicated the site of administration, normal font corresponds to the pharmaceutical forms that can be used and italic to the targeted pathologies and/or the extent of the delivery.

Figure 4 BBB challenge for neurodegeneration. The strategies employed in optimising the engineering of nanoparticles to bypass the BBB (in italic). In bold, represented by the different BBB components (see the text).

Figure 5 Spatial relationships of O_2 levels between the blood vessel and a malignant tumor. In normoxic conditions, defined where oxygen concentrations are above 5%, present in areas less than 70 μM from the tumor blood vessels. The normoxic areas near the blood vessels are accessible by chemotherapy treatment whilst areas deeper into the tumor are inaccessible. In hypoxic conditions defined by oxygen concentration lower than 0.5%, present at a distance more than 100 μM from the tumor blood vessels. In these areas, O_2 concentrations are reduced, thus creating a hypoxic area within the tumor microenvironment. These areas are reachable by nanoparticle treatment.

Figure 6 Perspective model. Schema shows the pros and cons of nanotechnology using nanoparticles versus exosomes in the treatment of neurodegeneration and cancer.

Table 1 Types of nanoparticles and their potential uses in nanomedicine.

Table 2 Drugs approved or in the pipeline for ND.

Table 3 Nanoparticles approved or in clinical trials for cancer therapy

List of abbreviations:

Aβ, β-amyloid peptide; AD, Alzheimer's disease; ALS, Amyotrophic Lateral Sclerosis; APP, amyloid precursor protein; AuNPs, gold nanoparticles; BACE-1, beta secretase-1; BBB,

blood brain barrier; CNT, carbon nanotube; CNS, central nervous system; CONP, cerium oxide nanoparticle; EGCG, epigallocatechin gallate; EGFR, epidermal growth factor; EV, extracellular vesicle; GDNF, Glial cell line-derived neurotrophic factor; IONP, iron oxide nanoparticle; MDI, multi-drug inhibitor; MRI, magnetic resonance imaging; MVB, multivesicular body; ND, neurodegenerative diseases; NP, nanoparticle; PD, Parkinson's disease; PEG, polyethylene glycol; PEI, polyethyleneimine; PET, positron emission tomography; PLGA, poly(lactic-co-glycolic) acid; PN, proteostasis network; PNP, polymer nanoparticle; QD, quantum dot; RNAi, RNA interference; SDI, single drug inhibitor; siRNA, small interfering RNA; SLN, solid lipid nanoparticle; VEGF, vascular endothelial growth factor.

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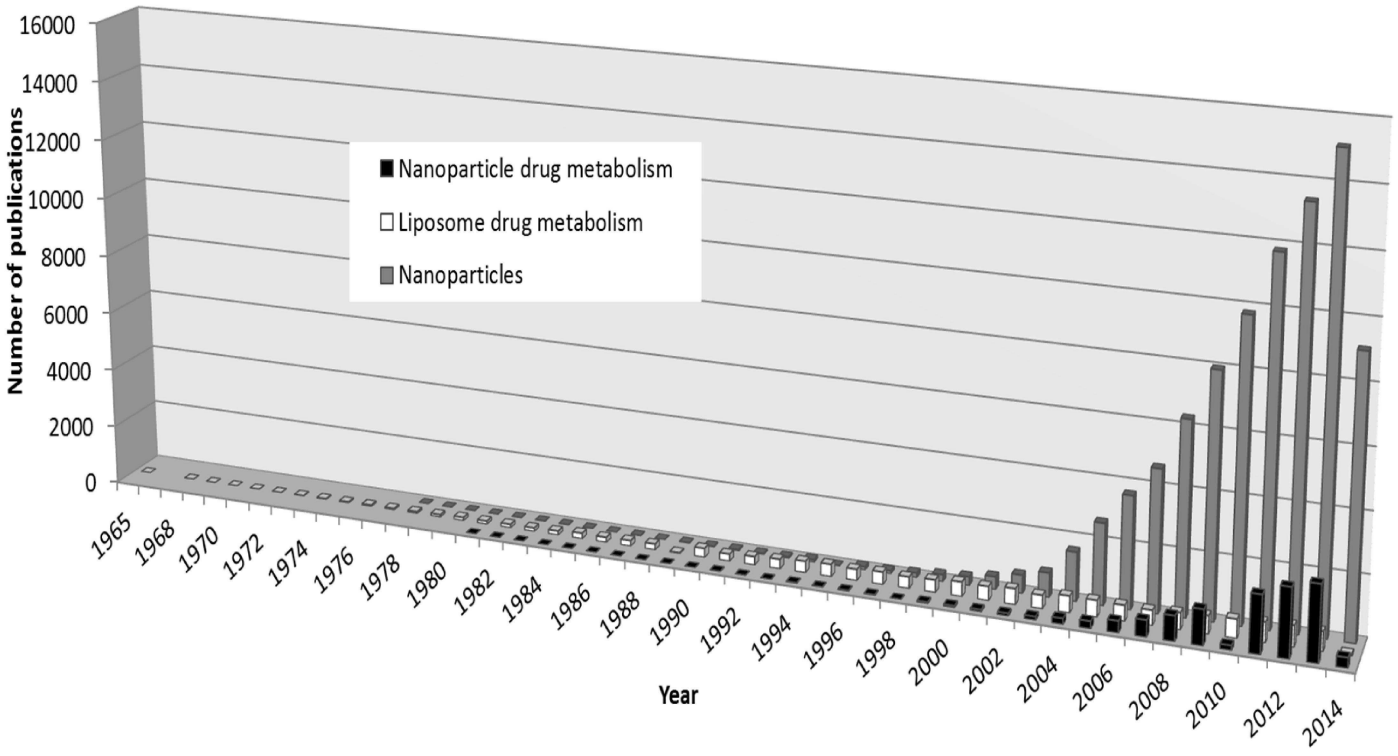
Figure 1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Number of publications
Nanoparticle drug metabolism
Liposome drug metabolism
Nanoparticles
1965
1968
1970
1972
1974
1976
1978
1980
1982
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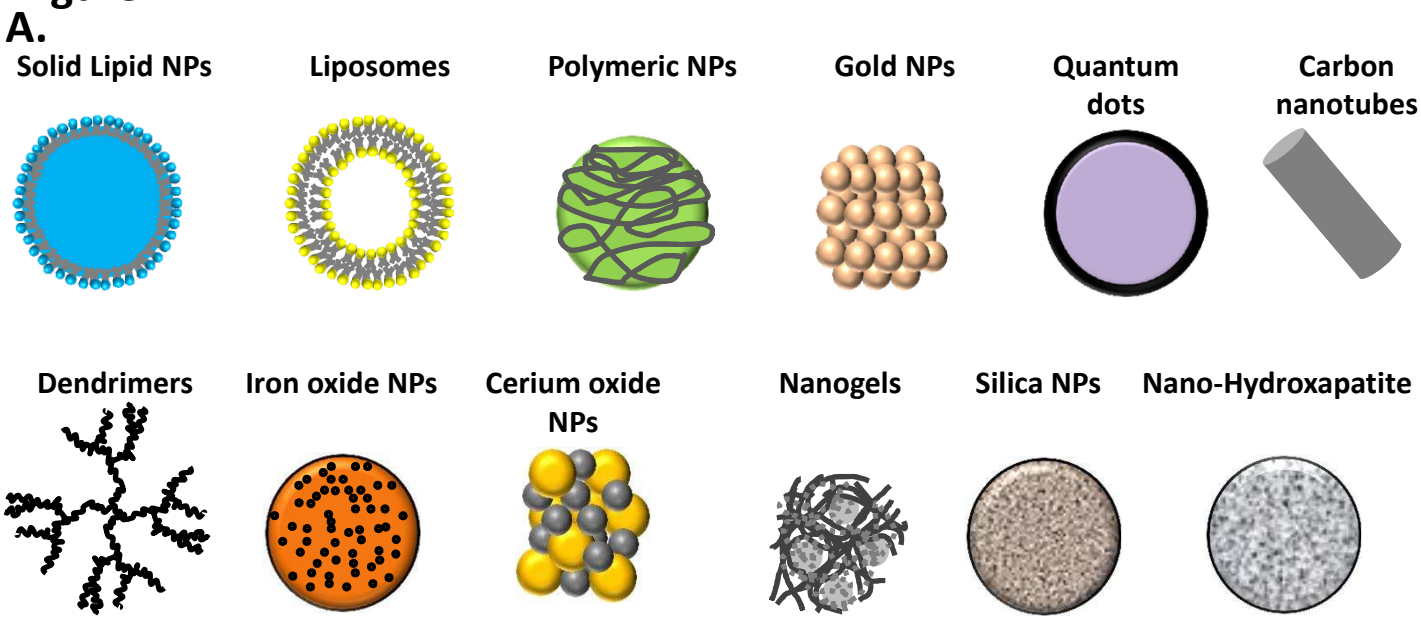
2008
2010
2012
2014
Year

Figure 2


A.
Solid Lipid NPs
Liposomes
Polymeric NPs
Gold NPs
Quantum dots
Carbon nanotubes
Dendrimers
Iron oxide NPs
Cerium oxide NPs
Nanogels
Silica NPs
Nano-Hydroxapatite

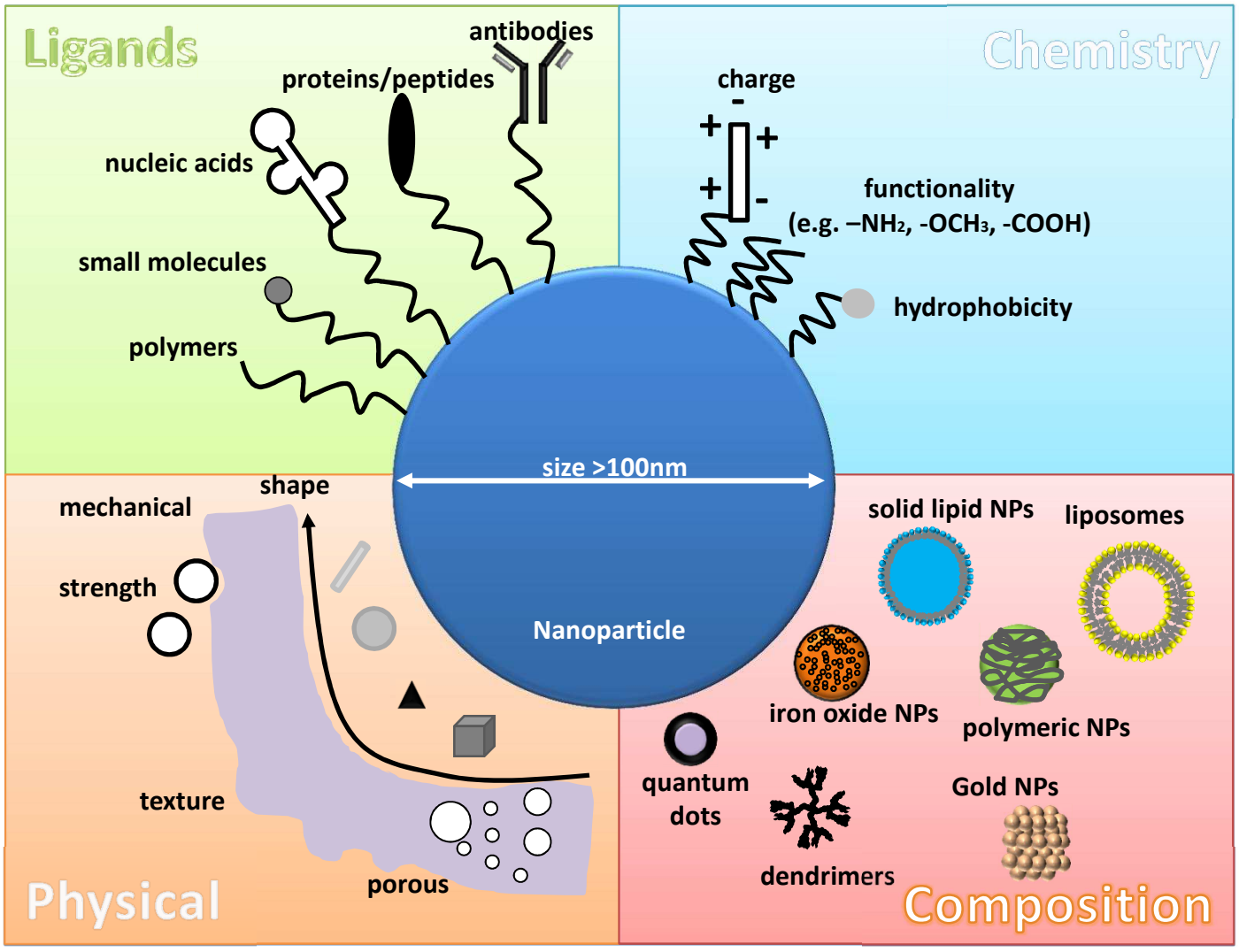

B.
Ligands
antibodies
proteins/peptides
nucleic acids
small molecules
polymers
Chemistry
charge
functionality
(e.g. –NH2, -OCH3, -COOH)
hydrophobicity
size >100nm
Nanoparticle
shape
mechanical
strength
texture
porous
Physical
solid lipid NPs
liposomes
iron oxide NPs
polymeric NPs
quantum dots
Gold NPs
dendrimers
Composition

Figure 3 Nanoparticle- mode of delivery

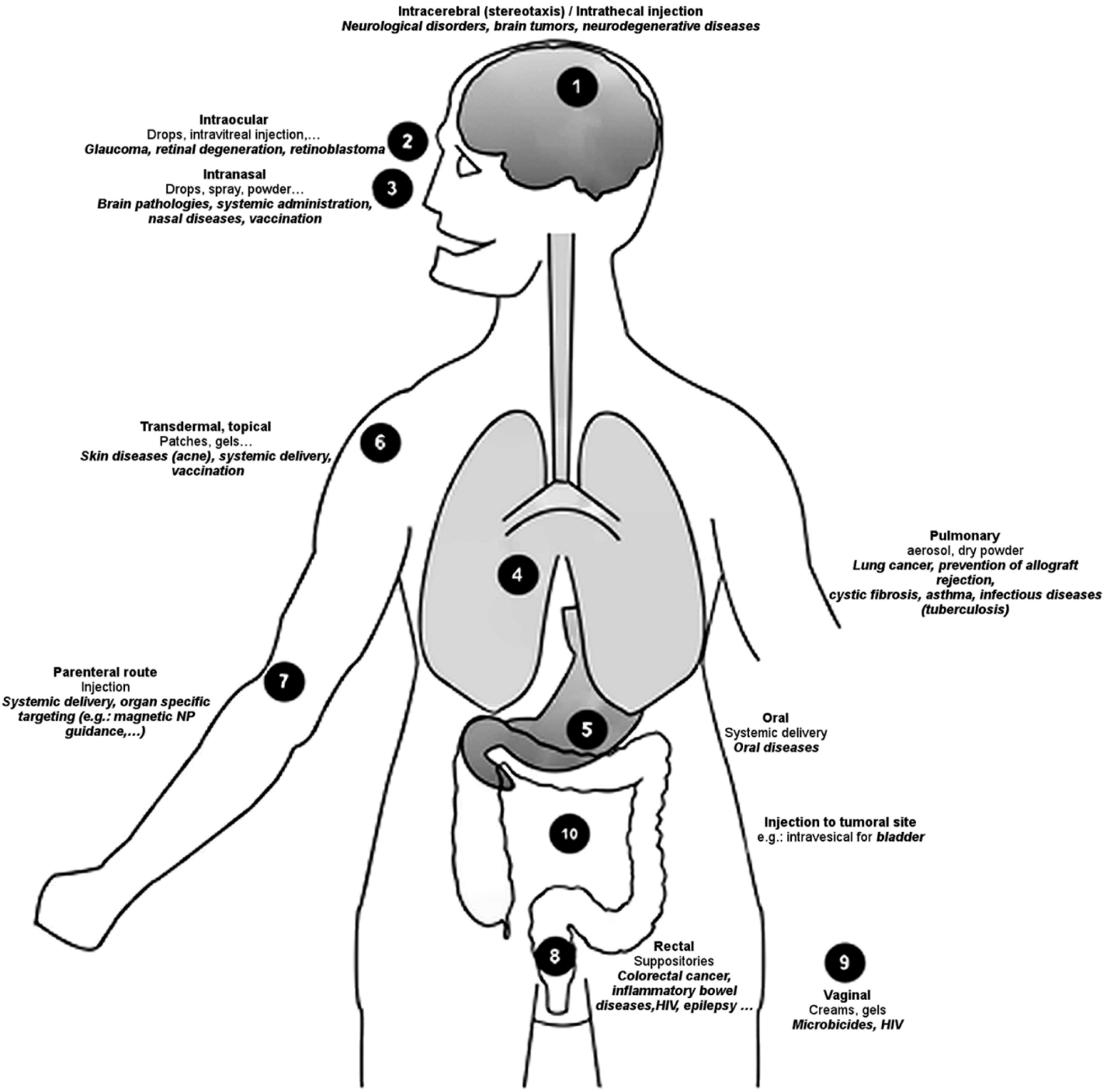


Figure 4 BBB challenge for neurodegeneration

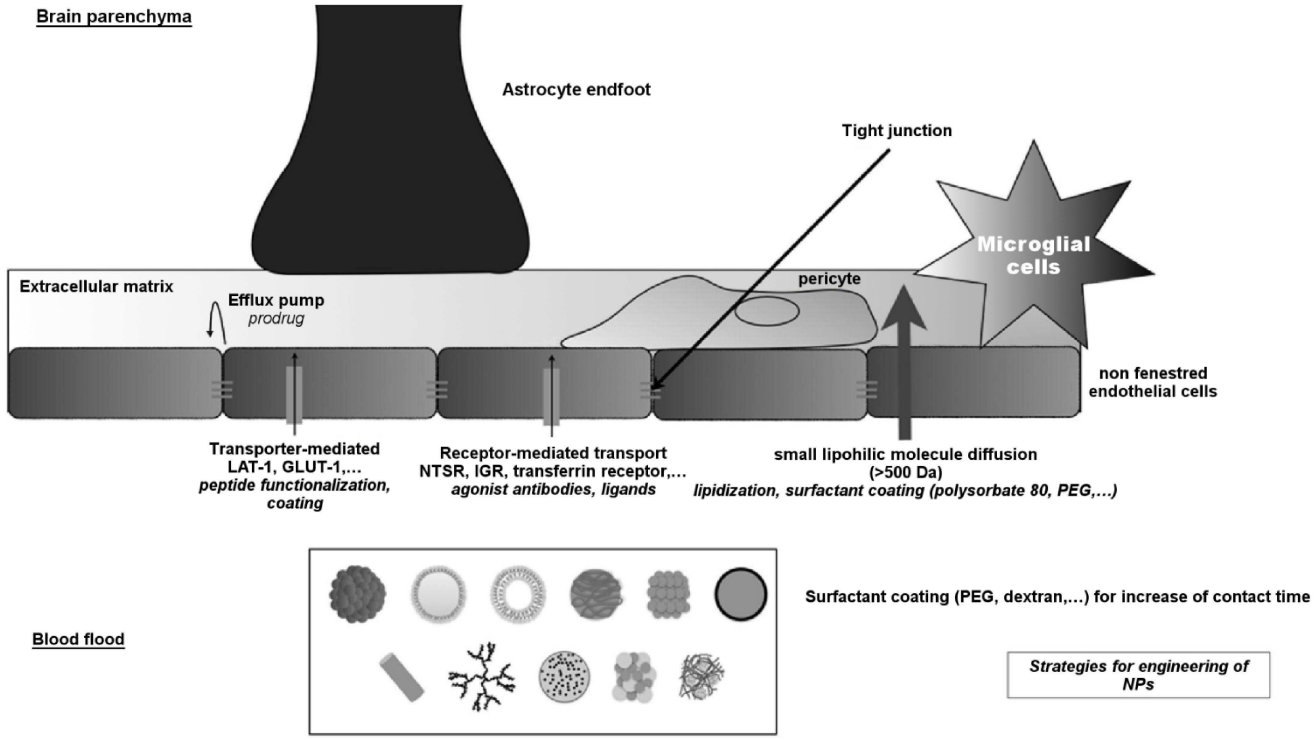


Figure 5

A.

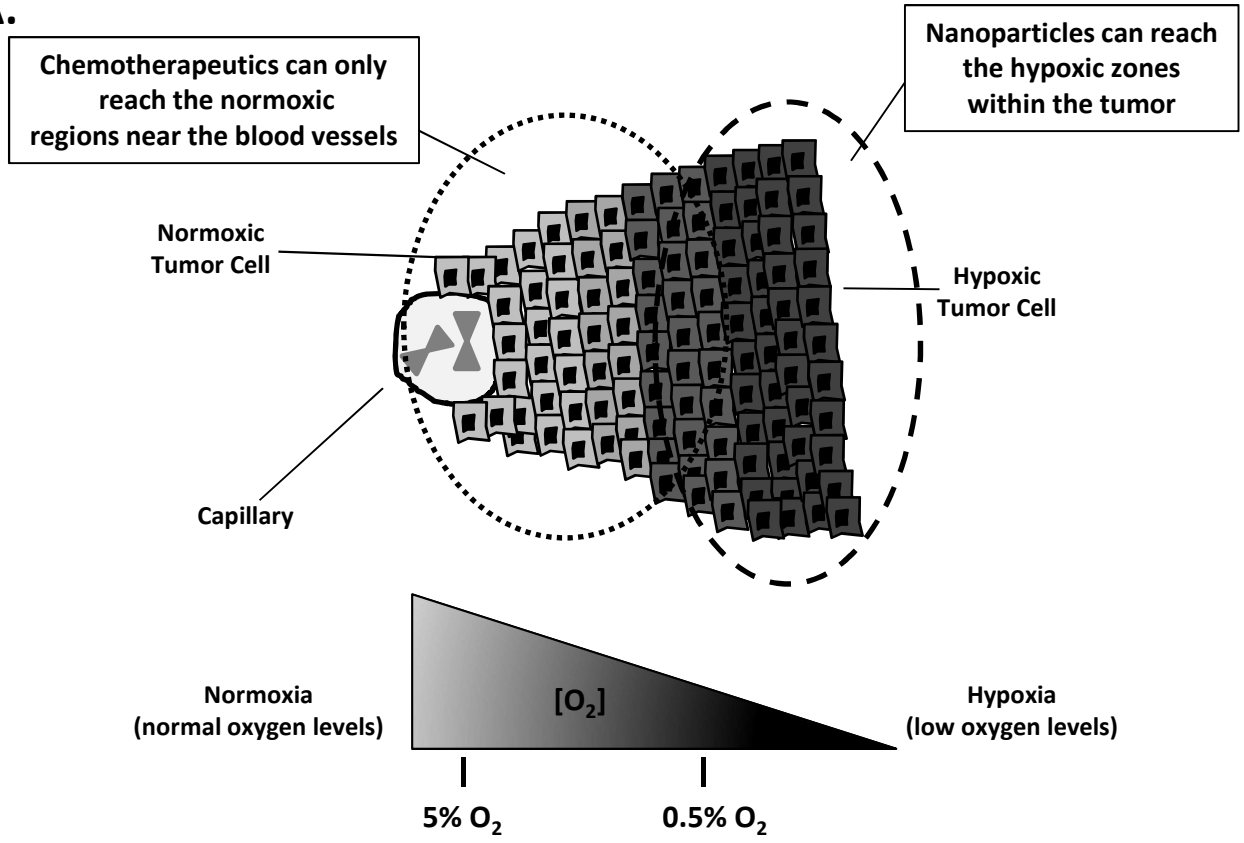

Chemotherapeutics can only reach the normoxic regions near the blood vessels
Nanoparticles can reach the hypoxic zones within the tumor
Normoxic Tumor Cell
Hypoxic Tumor Cell
Capillary
Normoxia (normal oxygen levels)
[O2]
Hypoxia (low oxygen levels)
5% O2
0.5% O2

Figure 6 Perspective model

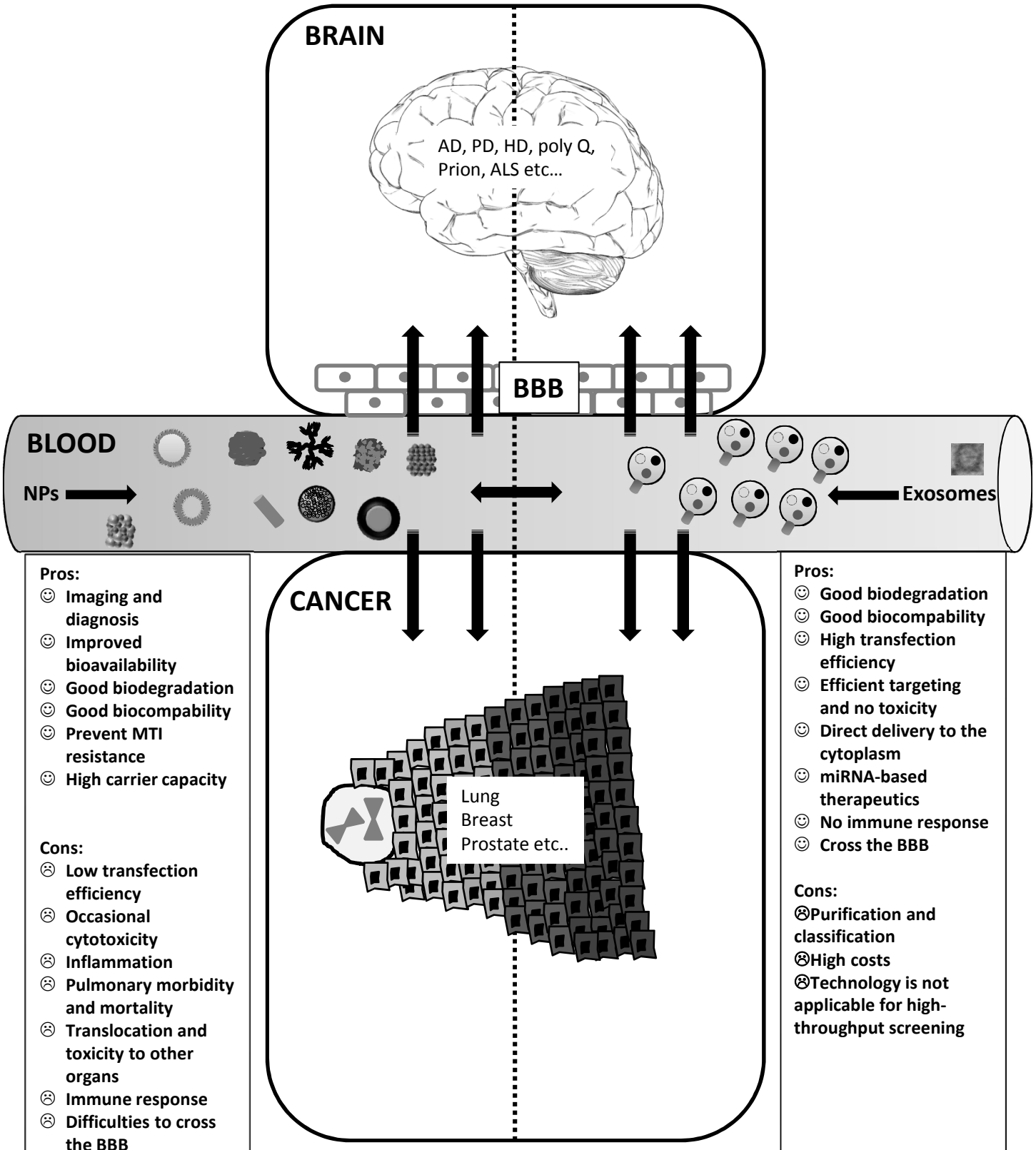


Table 1 Types of nanoparticles and their potential uses in nanomedicine

Type of nanoparticle	Size range (nm)	Therapy/Disease	Reference
Solid lipid NPs	50-1000	ALS, AD, HD, various cancers	(281-285)
Liposomes	25-205	AD, Kaposi sarcoma, breast cancer, melanoma	(173, 286-290)
Polymeric NPs	10-100	Cancer, Poly Q	(219, 291)
Gold NPs	4-40	Cancer	(292-295)
Quantum dots	3-30	Cancer, ND	(24, 296)
Carbon nanotubes	0.4-3 (d) x 20-1000 (l)	Cancer, AD,	(297-301)
Dendrimers	2-10	Breast and colon cancer, Prion	(302-305)
Iron oxide NPs	5-200	AD, ALS	(306-308)
Cerium oxide NPs	5-400	AD, cancer	(309, 310)
Mesoporous silica NPs	30-280	Cancer	(311-315)
nano-Hydroxyapatite	2.5-200	Cancer	(52-54)
Nanogels	20-200	Melanoma, breast and pancreatic cancer, ND	(56, 88, 316, 317)