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Roadmap on Nanomedicine for the Central Nervous System

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Abstract

In recent years, a great deal of effort has been undertaken with regards to treatment of pathologies at the level of the central nervous system (CNS). Here, the presence of the blood-brain barrier (BBB) acts as an obstacle to the delivery of potentially effective drugs and makes accessibility to, and treatment of, the central nervous system one of the most significant challenges in medicine.
In this Roadmap article, we present the status of the timeliest developments in the field and identify the outstanding challenges and opportunities that exist. The format of the Roadmap, whereby experts in each discipline share their viewpoint and present their vision, reflects the dynamic and multidisciplinary nature of this research area, and is intended to generate dialogue and collaboration across traditional subject areas. It is stressed here that this article is not intended to act as a comprehensive review article, but rather an up-to-date and forward-looking summary of research methodologies pertaining to the treatment of pathologies at the level of the central nervous system.

Contents

Section 1 – Introduction

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Introduction to the Roadmap

The treatment of pathologies at the level of the central nervous system (CNS) remains a difficult challenge in medicine. Accessibility to the diseased sites is highly hindered by the blood-brain barrier (BBB), an anatomical/functional obstacle for many "undesired" toxic substance, but unfortunately also for potentially efficient drugs that, because of the BBB presence, cannot reach their target [1]. The most known and common example is presented by the treatment of Parkinson's disease patients with dopamine, that is hindered by the inability of this neurotransmitter to cross the BBB. Dopamine precursors (such as L-3,4-dihydroxyphenylalanine -L-dopa-) are thus usually exploited, given their relatively higher BBB permeability. Dopamine is just one of the several therapeutic molecule that, despite being effective in the treatment of a pathology affecting the CNS, can not be exploited due to poor targeting abilities [2].

Among the strategies to overcome these limitations, and to successfully deliver drugs to the CNS, a special attention should be paid to the products offered by nanotechnology, that during the recent years developed efficient "Trojan horses" to penetrate the BBB defence walls. Therapeutic molecules can be encapsulated in different kinds of nanoparticles (lipid-based, polymeric, even inorganic) that, opportunely engineered, make possible the crossing of the BBB and the targeting of the diseased area [3]. These approaches have been proposed for a plethora of common neurological disorders, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, brain cancer, yet also to achieve on-demand neuronal stimulation/recording, being the latter particularly important for closed-loop electrical stimulation systems [4]. Nanomaterials can be tailored to implement multiple functions, such as BBB crossing, specific cell or signalling pathway targeting, and responsiveness to endogenous/exogenous stimuli. The latter feature is particular interesting, and it is peculiar of the latest generation of nanomedical products, that, acting as "nanotransducers", can be indeed considered actual active "nanorobots" rather than simple carriers of diagnostic and/or therapeutic agents [5].

Nanomedicine-based treatments of CNS pathologies do not only represent a scientific and technological challenge, but, as easily to figure out, they also own a very important economic and social impact. The Global Burden of Diseases, Injuries, and Risk Factors Study 2016 pointed out as, worldwide, the burden of neurological disorders in terms of absolute number of DALYs (disability-adjusted life-years, *i.e.*, the sum of years of life lost and years lived with disability) continues to increase [6]. Neurological diseases globally represent the leading cause of DALYs (276 million people affected) and the second leading cause of deaths (9 million). The major pathologies contributing to neurological DALYs were found to be stroke (42.2%), migraine (16.3%), Alzheimer's and other dementias (10.4%), and meningitis (7.9%).

According to the World Health Organization, neurological disorders will affect an increasing number of people worldwide in the next decades. Nowadays, accounting for over 6% of the global burden of diseases, they present high economic and social costs [7]. In 2010, the total European cost of brain disorders was estimated to be 798 billion €, divided as follows: 37% healthcare costs, 23% direct non-medical costs, and 40% indirect costs. Of these, 5.2 billion € were spent on brain cancer, 13.9 billion € on Parkinson's disease, 64.1 billion € on stroke, and 105.2 billion € on dementia [8]. In the US, the current estimated annual cost to American society of just nine of the most common neurological diseases is 789 billion USD in 2014, and costs will increase even further over the coming years as the elderly segment of the population will nearly double between 2011 and 2050 [9]. Brain disorders,

therefore, pose an enormous socioeconomic burden to Europe and to the rest of the world.

Sections overview

As mentioned, BBB represents the first challenge in the treatment of CNS diseases: Section 2 is dedicated to advanced BBB models, highlighting their importance in pre-clinical research to maximize the translational success of new drugs and nanomedical products. The most promising advancements in the treatment of stroke and ischemia are introduced by Section 3, with particular attention to innovative approaches involving extracellular vesicles and RNA-based therapies. Section 4 reports on new materials, designs, and strategies adopted for neuronal interfaces, to achieve highly precise neuronal signal modulation, activation, and recording. Section 4 is dedicated to brain cancer, a pathology with an extremely grim prognosis: the importance of smart nanomaterials in glioma treatment is highlighted. Section 6 and 7 are focused on two of the most common neurodegenerative diseases: Parkinsons' and Alzheimer's disease, respectively. Current strategies, challenges, and future perspectives are described with a particular focus on innovative drug delivery systems and routes. Section 8 provides an overview of non-conventional and innovative strategies for deep brain stimulation, trying to overcome limitations of the current state of the art and indicating the challenges such technologies are expected to face in the next years. Section 9 reports on the importance of the evaluation and treatment of inflammatory conditions in the CNS, often associated to important pathological conditions. The possibility to modulate the function of neuro-inflammatory-related cells and preserving neuronal health is shown. Section 10, eventually, provides an overview of immune modulation at CNS level, highlighting its pivotal role in the treatment of CNS cancer and neurodegenerative diseases.

Acknowledgments

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References

1. Terstappen G.C., Meyer A.H., Bell R.D., Zhang W. Strategies for delivering therapeutics across the blood-brain barrier. Nat. Rev. Drug Discov. 20: 362-383 (2021)
2. Haddad F., Sawalha M., Khawaja Y., Najjar A., Karaman R. dopamine and levodopa prodrugs for the treatment of Parkinson's disease. Molecules 23: 40 (2018)
3. Tang W., Fan W., Lau J., Deng L., Shen Z., Chen X. Emerging blood-brain-barrier-crossing nanotechnology for brain cancer theranostics. Chem. Soc. Rev. 48: 2967-3014 (2019)
4. Tanskanen J.M.A., Ahtiainen A., Hyttinen J.A.K. Toward closed-loop electrical stimulation of neuronal systems: A review. Bioelectricity 2: 328-347 (2020)
5. Genchi G.G., Marino A., Grillone A., Pezzini I., Ciofani G. Remote control of cellular functions: The role of smart nanomaterials in the medicine of the future. Adv. Healthc. Mater. 6: 1700002 (2017)
6. GBD 2016 Neurology Collaborators. Global, regional, and national burden of neurological disorders, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 18: 459-480 (2019)
7. World Health Organization, *Neurological Disorders: Public Health Challenges*, Geneva, Switzerland 2006.
8. Olesen J., Gustavsson A., Svensson M., Wittchen H.U., Jönsson B., CDBE2010 study group, European Brain Council. The economic cost of brain disorders in Europe. J. Neurol. 19: 155-162 (2012)
9. Gooch C.L., Pracht E., Borenstein A.R. The burden of neurological disease in the United States: A summary report and call to action. Ann. Neurol. 81: 479-484 (2017)

Section 2 – Microphysiological systems for preclinical testing of drug-loaded nanoparticle transport across the human blood-brain barrier

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Status

The blood-brain barrier (BBB) is a highly selective and semipermeable barrier that separates the blood circulation from the neural cells of the central nervous system (CNS) and represents the tightest barrier in the human body [1]. It consists of endothelial cells which form tight and adherens junctions, surrounded by brain pericytes and astrocytes with their end-feet in contact to the abluminal side of the brain vessels (Figure 1a, 1b). The BBB maintains brain homeostasis, conceiving a physical and functional protection of the brain by regulating the selective active and passive transport of molecules, ions, fluids and cells [2]. The BBB constitutes a nearly-impenetrable obstacle against efficient therapeutic drug delivery into the brain tissue from blood, consequently limiting treatment options and neuro-pharmaceutical development for several brain pathological processes, including neurodegenerative diseases. Indeed, only 3–5% of compounds which pass preclinical *in vitro* and *in vivo* tests are ultimately approved for patients, mostly because of their inability to cross the human BBB *in vivo* [3]. Despite the enormous contribution of *in vivo* animal models to basic and translational research, they show crucial genetic, molecular, immunologic, and cellular differences with respect to humans and, therefore, have limited efficacy in the development of efficient drug treatments against human pathologies [4]. Improving the effectiveness of preclinical models is critical to the reduction of costly failures in clinical trials and improve patients' outcomes. The lack of reliable preclinical models capable of reproducing human anatomical complexity and predicting drug transport through the BBB, in conjunction with insufficient strategies to assist drugs to cross the BBB, significantly contributes to the high failure rate of drug candidates validated in animal models leading to disappointing outcomes in clinical settings [5]. Two main research breakthroughs are needed to overcome the bottleneck in brain therapeutics: i) development of more efficient targeted brain-delivery strategies through the use of innovative nanomaterials including advancement of administration approaches and exploration of delivery routes [6]–[8]; and ii) creation of reliable human BBB models for *in vitro* preclinical investigation to advance the development of drugs efficiently targeting the brain, thus improving outcomes for patients affected by CNS pathologies [9][10].

Current and Future Challenges

The design of innovative strategies to deliver therapeutic agents across the BBB has become a major research topic in neuroscience. Only a few lipophilic small molecules, such as alcohol, caffeine or opioids (morphine, heroin), few analgesics, antibiotics or antipsychotics, typically with a molecular weight below 400-500 Da, can cross the BBB by passive or carrier-mediated mechanisms and reach the CNS at the concentrations needed for treatment. Overall, 98% of small molecule drugs and nearly 100% of biologic drugs do not reach efficacy of treatment, requiring further studies and development [11]. In recent years, advances in bioengineering and biomaterials have generated various strategies to assist drugs to cross the BBB with chemical modification of prodrugs or by the design of innovative carriers. Among them, innovative nanoparticles (NPs)-mediated drug delivery (polymer NPs, liposomes, inorganic systems) emerged as effective and non-invasive systems to treat cerebral diseases [7], [8]. Even though advances in delivery strategies are expected to generate positive results,

testing those molecules and carriers in a model lacking human relevance could eventually lead to similar failures in clinical trials [12].

Modelling the human BBB using *in vitro* models is fundamental to the of study brain physiopathology and biological mechanisms of drug transport to the brain. Two-dimensional (2D) transwell membrane models have been widely employed for BBB *in vitro* modelling. While reproducible and easy to use, these often consist of human primary or immortalized cells plated on a 2D porous membrane, generally display non-physiological permeability, blood flow and shear stress, and fail to replicate key cell-cell or cell-matrix interactions as they lack the anatomical architecture of the *in vivo* brain [13] (Figure 1c, 1d). Hence, the reproduction of fundamental BBB complexity in these 2D systems is limited, including their ability to accurately model genetic expression of junctional proteins and membrane transporters, which makes questionable their predictive value for human response [10]. In this context, there exists an unmet need for innovative *in vitro* models of the BBB that closely mimic *in vivo* brain endothelium in preclinical investigations, to serve as reliable tools to elucidate the role of the BBB in brain pathogenesis or for preclinical drug screening, with the aim of reducing the number of experimental animals, increase the efficiency of pharmacological research, and perform patient-specific studies to develop personalized treatments [4].

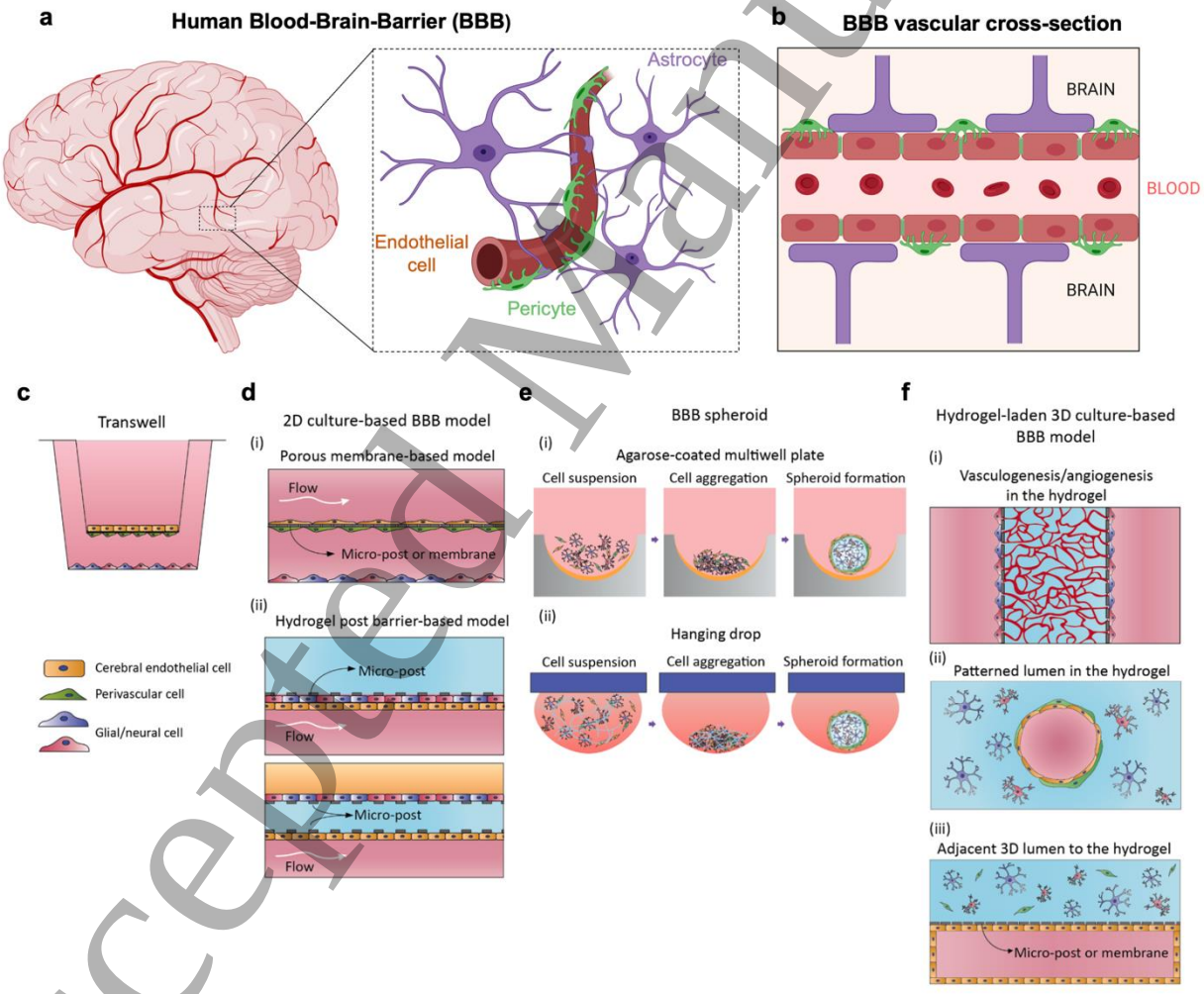

Figure 1. **In vitro Models of the Blood-Brain Barrier.** a) Schematic of the human BBB composed of brain Endothelial cells (ECs, red), capillary overlapped by brain pericytes (PCs, green) and astrocytes (ACs, violet) endfeet and b) Schematic of BBB vascular cross-section. c) Schematic overview of: c) transwell systems, d) 2D culture systems, e) BBB spheroids or organoids and f) 3D culture based microphysiological systems. Reproduced with permission from [13]. Schematic created with Biorender.com.

Advances in Science and Technology to Meet Challenges

Nanomaterial-based delivery systems have the capability to improve drug treatment specificity and short- and long-term efficacy. NP physical characteristics, such as size, shape, stiffness, surface and bulk composition can be tailored for optimal drug delivery. In particular, small NPs (<100 nm size) exhibit more favourable delivery across the BBB than larger ones [6]. Furthermore, although rod-shaped NPs have superior ability to cross vessel walls than spherical ones, they show decreased selectivity for brain tissue [14]. Low stiffness NPs have shown a superior ability to cross the vessel wall, including the BBB, than stiffer ones. Additionally, NP interactions with the physiological fluids should be minimized to avoid the corona effect and NP aggregation phenomena as well as to allow NP escape from the immune response. While pegylated NPs have been commonly used, anti-PEG (polyethylene glycol) antibodies have been recently identified [15], suggesting the need for new improved strategies avoiding rapid clearance of NPs for long-term efficacy. In addition, targeting ligands should be used in tandem with antifouling molecules to allow NP crossing of the BBB to reach the target brain cells [16]. To enhance delivery, the surface of NPs has been functionalized with antibodies, carbohydrates and other ligands to facilitate their transport across the BBB via transcellular pathways. As an example, low density lipoprotein receptor related protein-1 (LRP-1) has been identified as a selective receptor on both the BBB endothelial cells and glioma cells: NP surface functionalization with Angiopep-2 (a peptide binding LRP-1) have been shown to facilitate receptor-mediated transcytosis of NPs across the BBB and targeted cargo delivery to glioma cells in the brain [17]. Furthermore, advancements in administration approaches and exploration of alternative delivery routes could improve the efficiency of drug delivery through the BBB. In this context, non-invasive methods, able to deliver drugs bypassing the BBB, to administer drugs bypassing the BBB have been widely studied.

On the other hand, invasive modalities for drug release to the brain have been also proposed, including convection enhanced delivery (CED) for intracranial injection of therapeutics, by generating a pressure gradient at the tip of an infusion catheter to deliver payloads directly into the interstitial spaces of the CNS [18][19]. NP design may be optimised by exploiting *in vitro* preclinical BBB models as testing platforms, as recently demonstrated in previous studies [20][21][22]. Particularly, BBB spheroids, organ-on-chip and micro-physiological systems (MPS) (Figure 1e, 1f) have the potential to more closely recapitulate the microenvironmental characteristics and primary functions of human BBB. With respect to 2D models, MPS provide improved representation of complex dynamic cell interactions, and can reproduce blood flow and the whole 3D tissue structure with its barrier functions. Such characteristics are combined with high-throughput screening ability. Personalized nanomedicine design could also be possible through patient-specific MPS models making use of patient-derived human induced pluripotent stem cells (iPSCs)[10] (Figure 2a-d).

Concluding Remarks

Human BBB models are of great interest to the scientific community and pharma industries for testing drug transport across microvessels in a 3D microenvironment with close similarity to the *in vivo* human brain microvasculature [20]. Advanced microphysiological models using microfluidic technology have demonstrated the potential to accelerate *in vitro* pre-clinical validation and screening of novel drugs and their nanovectors for effective therapeutic treatments [21] [23]. Such systems are expected to facilitate a more comprehensive comparison among different drug candidates for an accurate preclinical assessment of their ability to cross the human BBB (Figure 2e, 3a). Hence, BBB models support the paradigm change in preclinical investigation from animal to alternative testing, according to the "3Rs Principle" (Replacement, Reduction, and Refinement). Important advancements in this field are represented by self-assembled MPSs of the human BBB [20][21][22]. The availability of simple and cost-effective protocols for the design of human BBB models and for their use in drugs and drug-loaded NPs testing is expected to have a high socio-economic impact, reducing the time and cost required for translation of basic science discoveries into clinical settings [13]

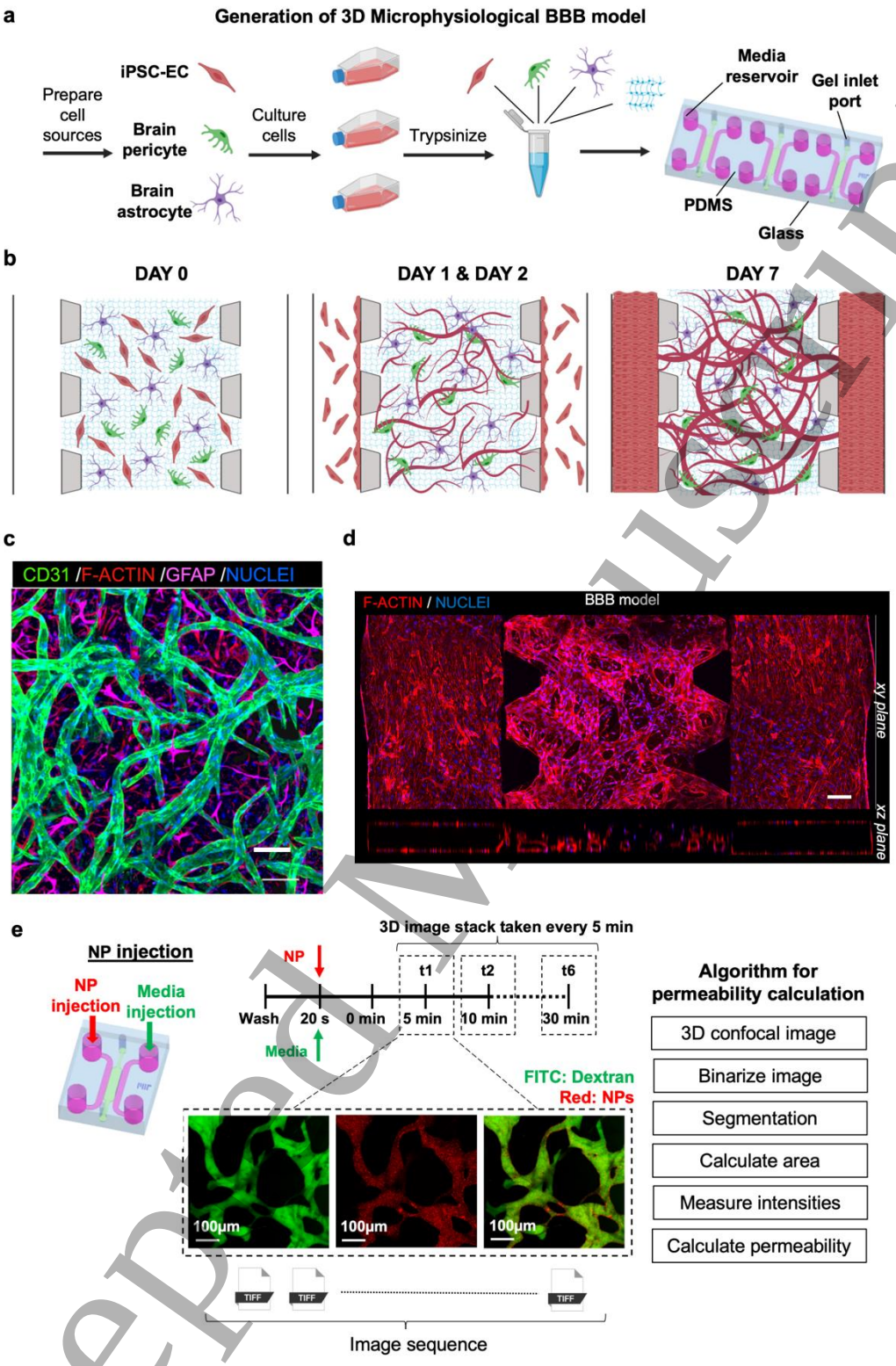

Figure 2. **3D Microphysiological BBB model**. a) Schematic explanation of BBB model and protocol, from 2D culture of induced pluripotent stem cells-derived endothelial cells (iPSC-ECs), brain pericytes (PCs) and brain astrocytes (ACs) to generate a 3D Microphysiological BBB model by self-assembled vasculature within a microfluidic device. The PDMS microfluidic platform was fabricated using soft lithography techniques and designed with inlet ports for injecting cell-gel suspensions, and large medium reservoirs and fluidic channels for culture medium. b) Schematic of dynamic culture of the Microphysiological BBB model over time in a section of 3D microfluidic system. Experimental steps and seeding configuration and of vasculogenesis process of

Microphysiological BBB model including iPSC-ECs + PCs + ACs as self-assembled microvascular network that undergoes maturation within 7 days of culture. 3-dimensional ECs layer covering top, bottom and side surfaces of the fluidic channels. c) Confocal image of self-assembled microvasculature of the Microphysiological BBB model including iPSC-ECs (CD31, green), PCs (F-actin, red) and ACs (GFAP, magenta), and nuclei (DAPI, blue). d) Confocal images of xy and xz (cross-section) planes of the 3D Microphysiological BBB model with iPSC-ECs + PCs + ACs, including EC layers in the side channels. Scale bars 200 µm. e) Schematic and methods of 3D microvascular permeability measurements. Confocal images of transport of NPs microvasculature (in red) are displayed. c, d) Reproduced with permission from [20]. e) Reproduced with permission from [21]. All schematics were created with BioRender.com.

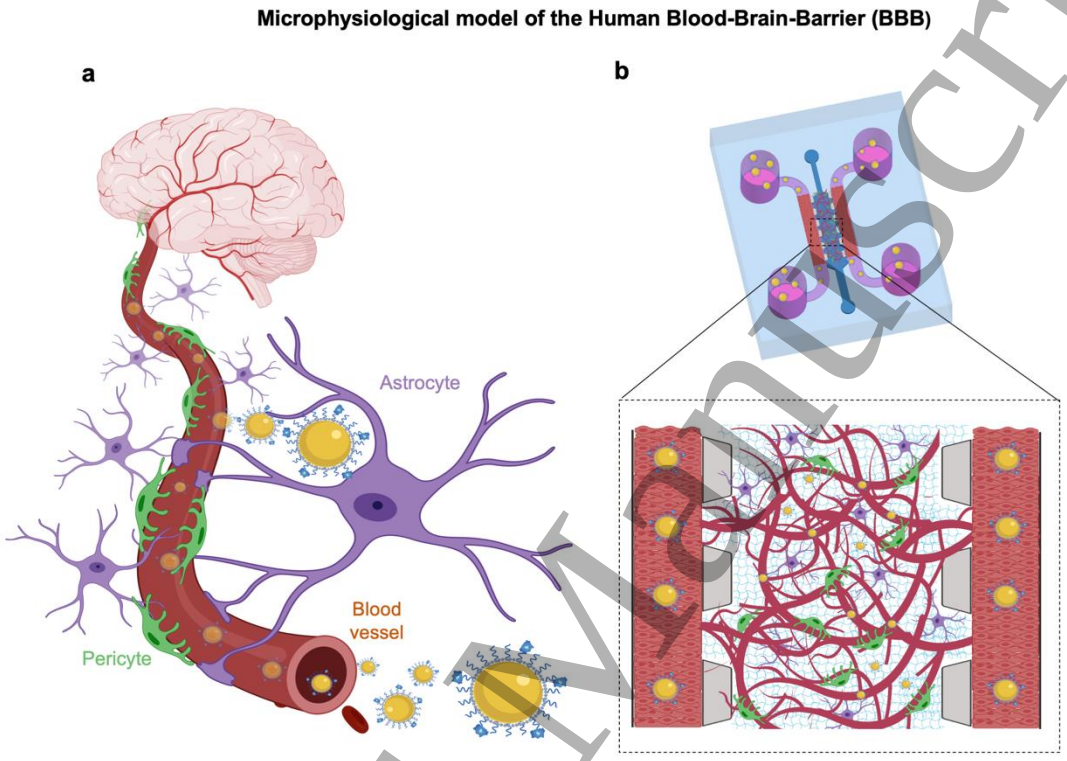

Figure 3. **Preclinical models for testing nanoparticles transporting drugs across the blood-brain barrier using a microphysiological system**. Schematic model of transport of nanoparticle across the blood-brain barrier in vivo and in vitro using microphysiological systems. Schematics created with Biorender.com.

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References

[1] M. D. Sweeney, A. P. Sagare, and B. V. Zlokovic, "Blood–brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders," *Nat. Rev. Neurol.*, 2018.

[2] N. J. Abbott, L. Rönnbäck, and E. Hansson, "Astrocyte-endothelial interactions at the blood-brain barrier," *Nat. Rev. Neurosci.*, vol. 7, no. 1, pp. 41–53, 2006.

[3] W. M. Pardridge, "Treatment of alzheimer's disease and blood–brain barrier drug delivery," *Pharmaceuticals*, vol. 13, no. 11, pp. 1–25, 2020.

[4] R. Cecchelli *et al.*, "Modelling of the blood–brain barrier in drug discovery and development," *Nat. Rev. Drug Discov.*, vol. 6, no. 8, pp. 650–661, 2007.

[5] W. M. Pardridge, "Blood-brain barrier endogenous transporters as therapeutic targets: A new

model for small molecule CNS drug discovery," *Expert Opin. Ther. Targets*, vol. 19, no. 8, pp. 1059–1072, 2015.

[6] Y. Chen and L. Liu, "Modern methods for delivery of drugs across the blood-brain barrier," *Adv. Drug Deliv. Rev.*, vol. 64, no. 7, pp. 640–665, 2012.

[7] J. Kreuter, "Nanoparticulate systems for brain delivery of drugs," *Adv. Drug Deliv. Rev.*, vol. 64, no. SUPPL., pp. 213–222, 2012.

[8] T. Patel, J. Zhou, J. M. Piepmeier, and W. M. Saltzman, "Polymeric nanoparticles for drug delivery to the central nervous system," *Adv. Drug Deliv. Rev.*, vol. 64, no. 7, pp. 701–705, 2012.

[9] S. Jackson, C. Meeks, A. Vézina, R. W. Robey, K. Tanner, and M. M. Gottesman, "Model systems for studying the blood-brain barrier: Applications and challenges," *Biomaterials*, vol. 214, no. December 2018, p. 119217, 2019.

[10] T. Osaki, Y. Shin, V. Sivathanu, M. Campisi, and R. D. Kamm, "In Vitro Microfluidic Models for Neurodegenerative Disorders," *Adv. Healthc. Mater.*, vol. 7, no. 2, pp. 1–29, 2018.

[11] J. L. Mikitsh and A. M. Chacko, "Pathways for small molecule delivery to the central nervous system across the blood-brain barrier," *Perspect. Medicin. Chem.*, no. 6, pp. 11–24, 2014.

[12] M. J. Mitchell, M. M. Billingsley, R. M. Haley, M. E. Wechsler, N. A. Peppas, and R. Langer, "Engineering precision nanoparticles for drug delivery," *Nat. Rev. Drug Discov.*, 2020.

[13] S. Seo, H. Kim, J. H. Sung, N. Choi, K. Lee, and H. N. Kim, "Microphysiological systems for recapitulating physiology and function of blood-brain barrier," *Biomaterials*, vol. 232, no. December 2019, p. 119732, 2020.

[14] D. Furtado, M. Björnmalm, S. Ayton, A. I. Bush, K. Kempe, and F. Caruso, "Overcoming the Blood–Brain Barrier: The Role of Nanomaterials in Treating Neurological Diseases," *Adv. Mater.*, vol. 30, no. 46, 2018.

[15] T. C. Chang, B. M. Chen, J. Y. Wu, T. L. Cheng, and S. Roffler, "Impact of anti-PEG antibody affinity on accelerated blood clearance of pegylated epoetin beta in mice," *Biomed. Pharmacother.*, vol. 146, no. November 2021, p. 112502, 2022.

[16] G. Tosi, J. T. Duskey, and J. Kreuter, "Nanoparticles as carriers for drug delivery of macromolecules across the blood-brain barrier," *Expert Opin. Drug Deliv.*, vol. 0, no. 0, p. 1, 2019.

[17] A. C. Di Polidoro *et al.*, "Theranostic design of angiopep-2 conjugated hyaluronic acid nanoparticles (Thera-ang-chanps) for dual targeting and boosted imaging of glioma cells," *Cancers (Basel).*, vol. 13, no. 3, pp. 1–21, 2021.

[18] W. A. Banks, "From blood-brain barrier to blood-brain interface: New opportunities for CNS drug delivery," *Nat. Rev. Drug Discov.*, vol. 15, no. 4, pp. 275–292, 2016.

[19] A. M. Mehta, A. M. Sonabend, and J. N. Bruce, "Convection-Enhanced Delivery," *Neurotherapeutics*, vol. 14, no. 2, pp. 358–371, 2017.

[20] M. Campisi, Y. Shin, T. Osaki, C. Hajal, V. Chiono, and R. D. Kamm, "3D self-organized microvascular model of the human blood-brain barrier with endothelial cells, pericytes and astrocytes," *Biomaterials*, vol. 180, pp. 117–129, 2018.

[21] S. W. L. Lee *et al.*, "Modeling Nanocarrier Transport across a 3D In Vitro Human Blood-Brain–Barrier Microvasculature," *Adv. Healthc. Mater.*, vol. 9, no. 7, Apr. 2020.

[22] C. Hajal *et al.*, *Engineered human blood–brain barrier microfluidic model for vascular permeability analyses*, vol. 17, no. 1. Springer US, 2022.

[23] J. P. Straehla, C. Hajal, H. C. Safford, and G. S. Offeddu, "A predictive micro fl uidic model of human glioblastoma to assess traf fi cking of blood – brain barrier-penetrant nanoparticles," 2022.

Section 3 – Advanced nanomaterials for ischemic stroke treatment

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Status

Stroke is an acute brain disease caused by the lack of blood flow to brain cells either due to a hemorrhage or occlusion of cerebral blood vessels often leading to the dysfunction of brain cells and neuronal death. Approximately 87 % of stroke cases are ischemic and the remaining 13 % have been reported to be related to haemorrhage [1]. Cerebral ischemia is accompanied by a series of pathophysiological changes including oxidative stress, localized inflammation, neuronal damage and loss of integrity of blood brain barrier (BBB). All these events have significant role in brain damage [2]. Current management strategy for ischemic stroke includes reperfusion to restore blood flow in the brain by administration of tissue plasminogen activator (t-PA) or by performing thrombectomy. But reperfusion is accompanied by generation of reactive oxygen species (ROS) which in turn can initiate inflammatory responses and often leads to tissue damage [3]. Administration of neuroprotectants has shown relatively low efficacy in alleviating reperfusion-induced injury in part because they are not able to tackle the biological complexity after an ischemic insult and due to their low efficacy to penetrate the brain [4, 5]. Hence there is need to search for alternate treatment strategies to maximize clinical efficacy and transport through the BBB. Synthetic or biological nanoparticles (e.g. extracellular vesicles (EVs)) offer potential to deliver therapeutic molecules in ischemic stroke such as neuroprotectants drugs across BBB, enhance their circulation half-life and promote their accumulation at ischemic sites [6-9]. This opinion article will focus on the advanced treatment strategies based on nanomaterials for the management of ischemic stroke.

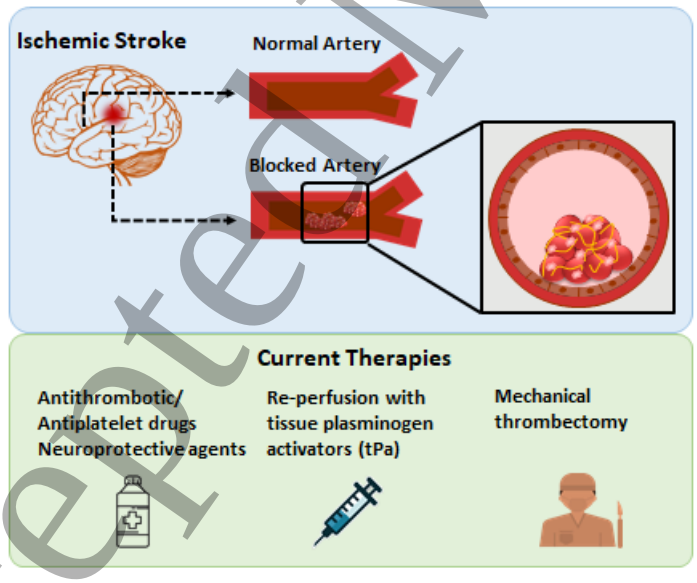

Figure 1: Schematic representation of ischemic stroke and current therapeutic strategies.

Current and Future Challenges

In many cases, patients who survive a stroke event have limited functional recovery due to a limited remodelling and restorative process in the lesion area. Neuroprotective strategies targeting the cascade of cellular and molecular events that lead to ischemic damage, and strategies to promote

post-ischemic regeneration, have been pursued in the last years, although without clinical translation [5, 10]. Neuroprotective strategies targeting the cascade of molecular events that lead to ischemic damage as well as strategies to promote post-ischemic regeneration, have shown potential in preclinical models [11]. These include pharmacological interventions (e.g. free-radical trapping agents; magnesium; NA-1) that target a single molecular target [11]. Nonetheless, so far, no therapy has demonstrated clear efficacy in clinical trials. However, interventions employing a cocktail of factors which can simultaneously target several molecular targets have not been tested in clinical trials. EVs are lipidic vesicles, with a diameter between 50-200 nm, that transport a cocktail of active molecules of the parental cell (DNA, mRNA, miRNAs, enzymes and growth factors) and act as mediators of cell-to-cell communication [12]. EVs present attractive features for therapeutic purposes because they regulate angiogenesis [13], neurogenesis [14] and synaptogenesis [14]. Currently, there is a running clinical trial that aims to evaluate mesenchymal stroma cell-derived EVs in the context of acute ischemic stroke (NCT03384433). Although functional benefits of EVs from different sources have been observed in animal models of ischemic stroke, their local neuroprotective/regenerative potential may be hampered by extremely low levels of accumulation in the brain after systemic delivery (typically below 1% of the initial dose)[15]. Thus, a main challenge is to develop strategies for the increased accumulation of EVs in the brain.

Better understanding of the physicochemical interactions of nanocarriers with BBB is needed to accomplish their clinical translation. Recent reports suggest that nanocarriers can cross BBB more effectively in the venules where blood flow is low and perivascular space is available [16]. Retention and absorption of the nanocarriers from venules can be achieved by active targeting of nanocarriers using ligands specific to receptors of brain endothelial cells in venules. But our understanding of proteins expressed by brain endothelial cells of venules, arteries and capillaries is limited. In addition, permeability of BBB is not uniform throughout and it has been reported that intravenously administered transferrin conjugated gold nanoparticles could preferentially accumulate at neurogenic niches after activation with near infrared (NIR) radiation [17]. This depicts potential of exploring the feasibility of efficient BBB targeting across all parts of the brain such as cerebellum, thalamus, cortex etc. Stroke alters the BBB permeability and better understanding of BBB characteristics after stroke is needed to design more effective formulations for stroke therapy [18].

Advances in Science and Technology to Meet Challenges

Several nanoparticle-based delivery platforms have been investigated for the delivery of neuroprotectants, anti-inflammatory agents and imaging probes for ischemic stroke treatment. Among these, liposomes were one of the first generation of nanoformulations employed. Induction of stealth property to the liposomes by PEGylation and use of active targeting ligands like transferrin depicted efficient drug delivery across BBB [19]. Stimuli sensitive polymer and metal based nanoparticles were explored to enhance drug disposition to ischemic areas utilizing biological stimuli such as high reactive oxygen species (ROS) levels in ischemic regions [6-9]. Research sheds light in to the significance of using synthetic multivalent epitopes with tunable avidity to promote receptor mediated transcytosis across BBB [20]. Moderate avidity of the epitopes towards low density lipoprotein-receptor related protein 1 (LRP1) [21] or transferrin receptor [17] facilitated the transcytosis transport across the BBB. EVs are also emerging as therapeutic delivery vehicles for brain diseases [22]. EVs have been used successfully for the delivery of siRNA into the brain after intravenous injection [23]. A recent study showed that tumour-derived EVs showed enhanced transcytosis across BBB through modulation of the endothelial recycling endocytic pathway. Specifically, EVs reduced expression of rab7 which in turn promoted the efficiency of BBB transport [24]. Despite the exact mechanism by which EVs breach BBB is still unclear these findings can help in designing more efficient EV based formulations for BBB transport. Another significant advance in the BBB targeted drug delivery is the selective targeting of nanoparticles to BBB by labelling the brain

endothelial cell surfaces with specific ligands. Nanoparticles with specific affinity to these ligands can preferentially bind the brain endothelial cell surface thereby minimizing off target effects [25]. Designing an optimal in vitro BBB model mimicking the properties and complexity of BBB is also critical in screening efficient formulations. Researchers have developed promising microfluidics based in vitro BBB model which can be a useful tool to investigate the permeability of drug molecules [26]. Yet, the possibility to perform these screenings in high-throughput while taking in account the heterogeneity of the BBB in terms of composition and flow dynamics remains elusive.

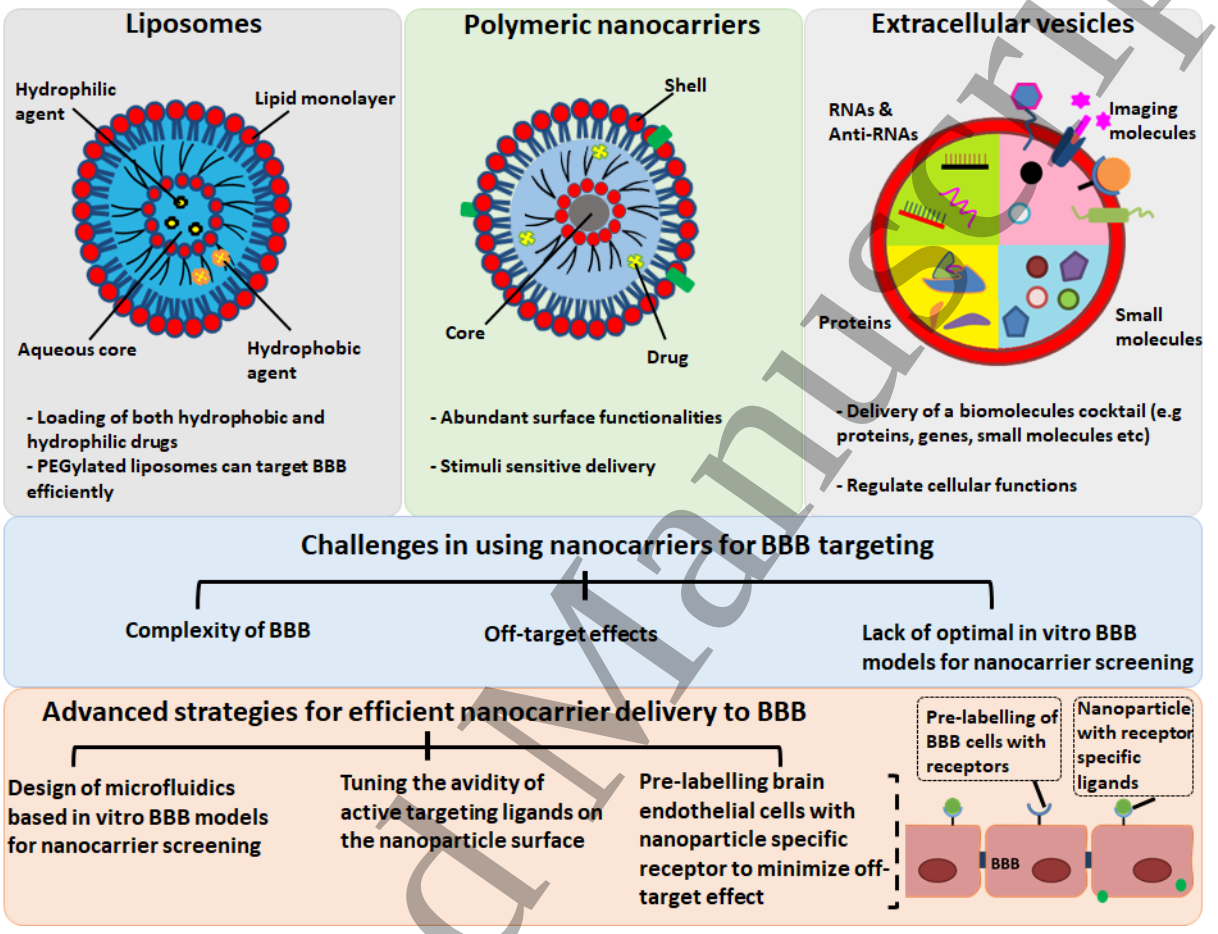

Figure 2: Schematic representation of various nanocarriers for ischemic stroke management, challenges in their application and the recent advanced strategies to facilitate their clinical translation.

Concluding Remarks

Successful clinical translation of various nanoparticle based formulations in ischemic stroke needs thorough understanding of pathogenesis of ischemic stroke and mechanism of interaction of these nanoparticles with ischemic tissues. Route and frequency of administration of nanoparticle based therapeutics and optimization of their therapeutic windows are also important determinants of successful clinical outcome. Apart from commonly employed intravenous injection, alternative routes of administration such as nose to brain delivery can also be explored for drug delivery to ischemic brain areas. Facilitation of the transport of drug molecules across BBB by the nanoparticles also needs to be addressed in more detail with the help of advances in design and development of nanoformulations and in vivo imaging systems such as intravital microscopy. Development of new drug molecules and novel nanoparticle based delivery systems will improve the clinical outcome in the treatment and long-term management of ischemic stroke.

Acknowledgements
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References:

[1] S.S. Virani, A. Alonso, H.J. Aparicio, E.J. Benjamin, M.S. Bittencourt, C.W. Callaway, A.P. Carson, A.M. Chamberlain, S. Cheng, F.N. Delling, M.S.V. Elkind, K.R. Evenson, J.F. Ferguson, D.K. Gupta, S.S. Khan, B.M. Kissela, K.L. Knutson, C.D. Lee, T.T. Lewis, J. Liu, M.S. Loop, P.L. Lutsey, J. Ma, J. Mackey, S.S. Martin, D.B. Matchar, M.E. Mussolino, S.D. Navaneethan, A.M. Perak, G.A. Roth, Z. Samad, G.M. Satou, E.B. Schroeder, S.H. Shah, C.M. Shay, A. Stokes, L.B. VanWagner, N.Y. Wang, C.W. Tsao, E. American Heart Association Council on, C. Prevention Statistics, S. Stroke Statistics, Heart Disease and Stroke Statistics-2021 Update: A Report From the American Heart Association, Circulation 143(8) (2021) e254-e743.
[2] B.C.V. Campbell, P. Khatri, Stroke, Lancet 396(10244) (2020) 129-142.
[3] S. Bhaskar, P. Stanwell, D. Cordato, J. Attia, C. Levi, Reperfusion therapy in acute ischemic stroke: dawn of a new era?, BMC Neurol 18(1) (2018) 8.
[4] M. Fisher, S.I. Savitz, Pharmacological brain cytoprotection in acute ischaemic stroke - renewed hope in the reperfusion era, Nat Rev Neurol 18(4) (2022) 193-202.
[5] S.I. Savitz, J.C. Baron, M. Fisher, S.X. Consortium, Stroke Treatment Academic Industry Roundtable X: Brain Cytoprotection Therapies in the Reperfusion Era, Stroke 50(4) (2019) 1026-1031.
[6] W. Lv, J. Xu, X. Wang, X. Li, Q. Xu, H. Xin, Bioengineered Boronic Ester Modified Dextran Polymer Nanoparticles as Reactive Oxygen Species Responsive Nanocarrier for Ischemic Stroke Treatment, ACS Nano 12(6) (2018) 5417-5426.
[7] J. Shi, W. Yu, L. Xu, N. Yin, W. Liu, K. Zhang, J. Liu, Z. Zhang, Bioinspired Nanosponge for Salvaging Ischemic Stroke via Free Radical Scavenging and Self-Adapted Oxygen Regulating, Nano Lett 20(1) (2020) 780-789.
[8] M. Li, J. Li, J. Chen, Y. Liu, X. Cheng, F. Yang, N. Gu, Platelet Membrane Biomimetic Magnetic Nanocarriers for Targeted Delivery and in Situ Generation of Nitric Oxide in Early Ischemic Stroke, ACS Nano 14(2) (2020) 2024-2035.
[9] Q. Bao, P. Hu, Y. Xu, T. Cheng, C. Wei, L. Pan, J. Shi, Simultaneous Blood-Brain Barrier Crossing and Protection for Stroke Treatment Based on Edaravone-Loaded Ceria Nanoparticles, ACS Nano 12(7) (2018) 6794-6805.
[10] S.I. Savitz, J.C. Baron, M.A. Yenari, N. Sanossian, M. Fisher, Reconsidering Neuroprotection in the Reperfusion Era, Stroke 48(12) (2017) 3413-3419.
[11] M. Tymianski, Novel approaches to neuroprotection trials in acute ischemic stroke, Stroke 44(10) (2013) 2942-50.
[12] G. van Niel, G. D'Angelo, G. Raposo, Shedding light on the cell biology of extracellular vesicles, Nat Rev Mol Cell Biol 19(4) (2018) 213-228.
[13] H. Henriques-Antunes, R.M.S. Cardoso, A. Zonari, J. Correia, E.C. Leal, A. Jimenez-Balsa, M.M. Lino, A. Barradas, I. Kostic, C. Gomes, J.M. Karp, E. Carvalho, L. Ferreira, The Kinetics of Small Extracellular Vesicle Delivery Impacts Skin Tissue Regeneration, ACS Nano 13(8) (2019) 8694-8707.
[14] P. Sharma, P. Mesci, C. Carromeu, D.R. McClatchy, L. Schiapparelli, J.R. Yates, A.R. Muotri, H.T. Cline, Exosomes regulate neurogenesis and circuit assembly, Proceedings of the National Academy of Sciences 116(32) (2019) 16086.

[15] A. Banerjee, V. Alves, T. Rondao, J. Sereno, A. Neves, M. Lino, A. Ribeiro, A.J. Abrunhosa, L.S. Ferreira, A positron-emission tomography (PET)/magnetic resonance imaging (MRI) platform to track in vivo small extracellular vesicles, Nanoscale 11(28) (2019) 13243-13248.
[16] K. Kucharz, K. Kristensen, K.B. Johnsen, M.A. Lund, M. Lonstrup, T. Moos, T.L. Andresen, M.J. Lauritzen, Post-capillary venules are the key locus for transcytosis-mediated brain delivery of therapeutic nanoparticles, Nat Commun 12(1) (2021) 4121.
[17] C. Praca, A. Rai, T. Santos, A.C. Cristovao, S.L. Pinho, R. Cecchelli, M.P. Dehouck, L. Bernardino, L.S. Ferreira, A nanoformulation for the preferential accumulation in adult neurogenic niches, J Control Release 284 (2018) 57-72.
[18] S. Bernardo-Castro, J.A. Sousa, A. Bras, C. Cecilia, B. Rodrigues, L. Almendra, C. Machado, G. Santo, F. Silva, L. Ferreira, I. Santana, J. Sargento-Freitas, Pathophysiology of Blood-Brain Barrier Permeability Throughout the Different Stages of Ischemic Stroke and Its Implication on Hemorrhagic Transformation and Recovery, Front Neurol 11 (2020) 594672.
[19] G.E. Bruch, L.F. Fernandes, B.L.T. Bassi, M.T.R. Alves, I.O. Pereira, F. Frezard, A.R. Massensini, Liposomes for drug delivery in stroke, Brain Res Bull 152 (2019) 246-256.
[20] D.T. Wiley, P. Webster, A. Gale, M.E. Davis, Transcytosis and brain uptake of transferrin-containing nanoparticles by tuning avidity to transferrin receptor, Proc Natl Acad Sci U S A 110(21) (2013) 8662-7.
[21] X. Tian, D.M. Leite, E. Scarpa, S. Nyberg, G. Fullstone, J. Forth, D. Matias, A. Apriceno, A. Poma, A. Duro-Castano, M. Vuyyuru, L. Harker-Kirschneck, A. Saric, Z. Zhang, P. Xiang, B. Fang, Y. Tian, L. Luo, L. Rizzello, G. Battaglia, On the shuttling across the blood-brain barrier via tubule formation: Mechanism and cargo avidity bias, Sci Adv 6(48) (2020).
[22] M.M. Lino, S. Simoes, F. Tomatis, I. Albino, A. Barrera, D. Vivien, T. Sobrino, L. Ferreira, Engineered extracellular vesicles as brain therapeutics, J Control Release 338 (2021) 472-485.
[23] L. Alvarez-Erviti, Y. Seow, H. Yin, C. Betts, S. Lakhal, M.J. Wood, Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes, Nat Biotechnol 29(4) (2011) 341-5.
[24] G. Morad, C.V. Carman, E.J. Hagedorn, J.R. Perlin, L.I. Zon, N. Mustafaoglu, T.E. Park, D.E. Ingber, C.C. Daisy, M.A. Moses, Tumor-Derived Extracellular Vesicles Breach the Intact Blood-Brain Barrier via Transcytosis, ACS Nano 13(12) (2019) 13853-13865.
[25] D. Gonzalez-Carter, X. Liu, T.A. Tockary, A. Dirisala, K. Toh, Y. Anraku, K. Kataoka, Targeting nanoparticles to the brain by exploiting the blood-brain barrier impermeability to selectively label the brain endothelium, Proc Natl Acad Sci U S A 117(32) (2020) 19141-19150.
[26] C. Hajal, G.S. Offeddu, Y. Shin, S. Zhang, O. Morozova, D. Hickman, C.G. Knutson, R.D. Kamm, Engineered human blood-brain barrier microfluidic model for vascular permeability analyses, Nat Protoc 17(1) (2022) 95-128.

Section 4 – Nanotechnology-based neuronal interfaces

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Status

Implantable Neural Interfaces (INIs) are devices capable to stimulate or record nervous system activity to restore a lost or compromised neurophysiological function. INIs function requires the intimate contact of the device with the nervous tissue to modulate neuronal activity for the treatment and diagnosis of cognitive or sensory-motor disorders [1]. A wide plethora of INIs examples has been presented nowadays, ranging from deep brain stimulation electrodes for mental disorders management (including Parkinson's disease), cochlear implants, retinal prostheses, and electrocorticogram (ECoG) electrodes to record brain activity. Furthermore, research on microfabrication technique enabled progress in electrode miniaturization, reaching extremely high spatial resolution. In this regard, Utah array, a silicon-based INI capable of dense sampling of multiple brain regions simultaneously, flexible polyimide-based microelectrodes with improved cells/implant interface and micromagnetic stimulation technology guarantee better integration with surrounding tissue [2, 3].

However, there is a common bottleneck that impedes long term usage of this technology: geometry and physiochemical properties of INIs materials differ completely from those of the tissue with which they must interface. This occurrence causes INI electrical performances drop due to fibrotic tissue encapsulation. Furthermore, tissue damage due to chronic inflammation response and micromotion at the tissue/electrode interface are other severe consequences of the consistent mechanical mismatch between INIs and the brain tissue. Moreover, most of the present INIs typologies requires percutaneously cabled electronics to connect the electrode with the power source. This poses further biocompatibility and encumbrance issues and a second invasive surgery to remove the implant is needed to avoid the risk to worsen nervous tissue damage.

There has been a tremendous effort in the last decade to overcome these limitations to envision long-term usage of INIs. Research on material science is allowing remarkable improvement to enhance INIs biocompatibility by developing new polymeric conductive materials, nature-derived compounds, and hybrid materials to reduce chronic inflammation response and to further miniaturize the implant. Efforts have been also made to avoid cable communication, by developing new wireless-controlled devices. However, despite these progresses INIs technology is still far from a successful long-term use and more efforts are needed to enhance its clinical relevance.

Current and Future Challenges

In the recent years Nanotechnology is giving a significant contribution in overcoming the lack of long-term efficiency of INIs (Figure 1). Nanoscale materials and nanofabrication techniques could in this sense operate a sort of revolution that would allow to build the *ideal INI*: a device capable to modulate neuronal activity with single-neuron resolution for long-term usage that does not affect the integrity of the nervous tissue and capable of wireless control. As an example, nanomaterials as carbon nanotubes (CNT) and graphene have been reported to successfully improve electrode signal-to-noise ratio and to enhance charge injection capacity, when used as conductive materials in electrode fabrications [4]. By employing such nanostructures, enhancement of mass transport phenomena with radial diffusion occurs, which results in improved sensitivity of the electrode due to. Moreover,

nanostructures such as gold and platinum nanoparticles and zinc oxide nanowires exploited as electrode nano-coatings have been reported to ameliorate recording sensitivity by reducing electrical impedance due to their high surface-to-volume ratio [5, 6]. In addition to electrical performances, nanotechnology advances cold help to improve INIs flexibility, and electrode/tissue interface properties as well. Conductive polymers (CPs) are another class of nanostructured materials that combine biocompatibility, similar mechanical properties respect to native tissue and metal-like conductivity. These materials can be employed as substitute to bare metals as conductive elements of INIs as demonstrated by studies that reported the fabrication of highly flexible, porous and injectable hydrogel using poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) as CPs [7 - 9].

These reported examples illustrated some of the most promising research areas that demonstrate the impact of nanotechnology in overcoming the bottleneck of current INIs technology. However, in our opinion nanotechnology could represent the most promising future challenge in NIs technology, giving the opportunity to even change its actual paradigm: by employing nanostructured entities as the INI itself instead of probes, electrodes, or flexible structures. In this regard, scaling down materials dimensions allows the exploitation of new physical properties of the matter, because different energies such as electrical, optical, thermal, and mechanical all converge at the nanoscale, enabling energy conversion and transduction from an energy type to another.

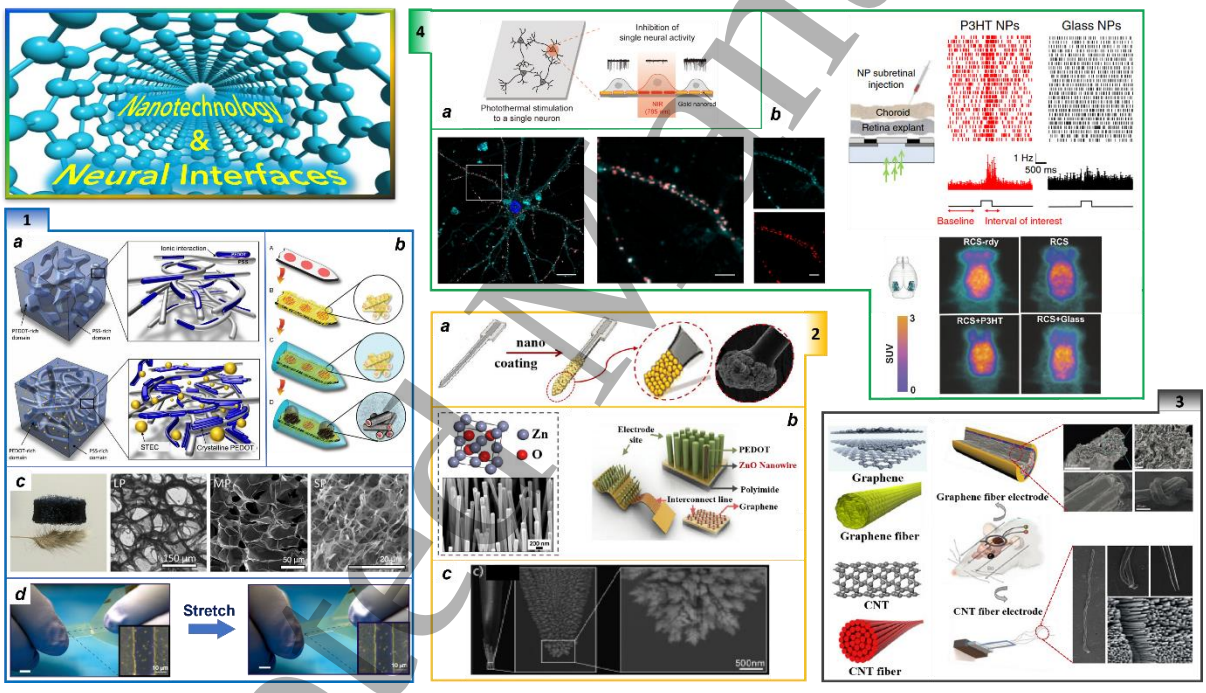

Figure 1. Recent advances in Nanotechnology for improving Neural Interfaces performances. 1) Incorporation of nanostructured CPs (PEDOT:PSS) in INIs design. Schematic illustration of PEDOT:PSS conductivity and stretchability enhancement using ionic coumpunds (STEC) (adapted from [6]) (a). Fabrication steps of PEDOT:PSS/alginate hydrogel as coating for INIs (reproduced from [2]) (b). Freeze-dried PEDOT:PSS foam and its 3D microstructure as highly stretchable and conducting hydrogel (adapted from [8]) (c). PEDOT:PSS based stretchable microelectrode maintaining its integrity during strain (adapded from [7]) (d). 2) Incorporation of metallic nanostructrured materials in INIs design. Gold nanoparticles-based nanocoating to lower electrode impedence (adapted from [3]) (a). Crystalline structure of ZnO nanowies and their use to enhance electrode electrical performance (adapted from [3]) (b). SEM image of the tip of a microelectrode functionalized with Pt nanoparticles (adapted from [3]) (c). 3) Incorporation of carbon-based nanostructured materials in NIs desing (adapted from [3]). 4) Examples of nanostructured materials used as wireless nanotransducers for neuromodulation. Gold nanorods-based thermal inhibition of neural circuits upon NIR laser illumination

(adapted from [11]) (a). Subretinal implantation of polymeric-based photoconductive nanoparticles to stimulate retinal neurons *in vivo* (adapted from [10]) (b).

Advances in Science and Technology to Meet Challenges

Switching from electrodes to nanostructured materials as the INI core represents the most promising challenge in this technology. In the past years there has been a strong interest to investigate the use of nanostructured materials as neuromodulating structures.

Nanostructured materials could work as nanotransducers, by converting an external energy source to another type of energy to stimulate or sense neuronal activity at the cellular level [9]. Importantly, this phenomenon occurs in wireless configuration, avoiding the needs of cables and bulky connectors. Several class of nanotransducers for wireless neuromodulation have been presented nowadays (Figure 2). Optoelectrical nanotransducers such as quantum dots and conjugated polymer nanoparticles have been demonstrated to trigger neuromodulating effect upon light conversion into electrical potential. A recent example showed that subretinal injection of poly[3-hexylthiophene] (P3HT) nanoparticles allowed light-evoked stimulation of retinal neurons and rescue visual functions in a rat model of retinitis pigmentosa [10].

Temperature has been reported to modulate nervous activity by stimulating or inhibiting it. Although its mechanism is not fully understood, changes in membrane capacity and the activation of temperature-gated ion channels are thought to play a crucial role in triggering temperature-mediated neuromodulating effect. Gold nanoparticles have been already demonstrated to efficiently convert NIR light into local heat to stimulate or inhibit action potential propagation [11]. Other promising examples of wireless neuromodulation are piezoelectric nanoparticles. These nanostructures can convert external mechanical force, such as ultrasound, into local electric fields to modulate voltage-dependent ion channels, in order to trigger action potential. In this regard, barium titanate nanoparticles have showed neuromodulating potential, as local currents generated by ultrasound can induce Ca^{2+} influx into cell cytoplasm [12].

All these nanomaterials show remarkable potential to change the current paradigm of INIs technology. For this reason, future research is strongly needed to develop strategies to efficiently target these nanostructures across the brain, overcoming physiological barriers such as the blood-brain-barrier. Furthermore, more studies are also needed to understand nanomaterials interactions with immune system, as well as the mechanisms of cellular interaction and clearance of these nanostructures. This is of fundamental importance to envision long-term usage and future clinical translation of nanotransducers-based neuromodulation.

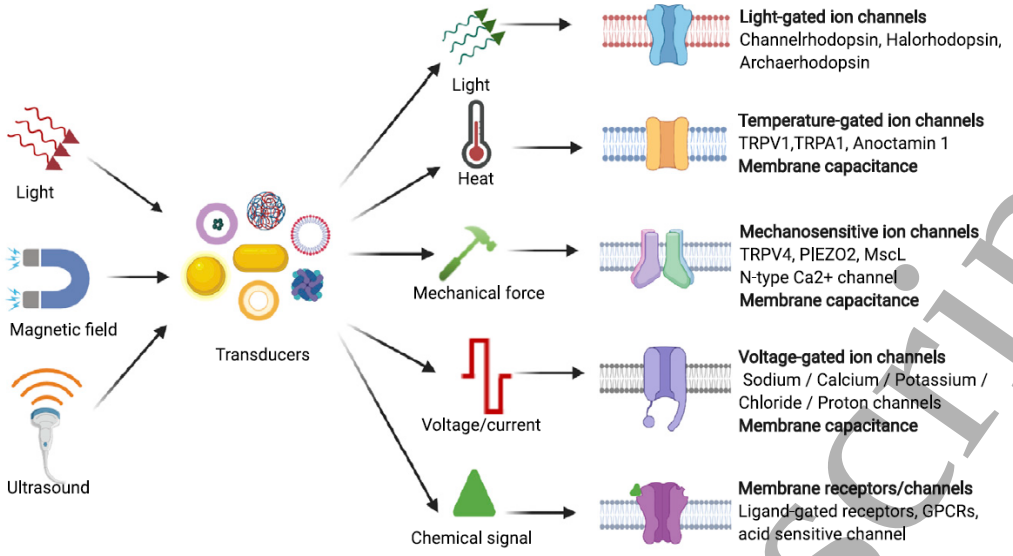

Figure 2. Examples of nanostructured materials used as nanostransducer for wireless neuromodulation and their working principle (adapted from [9])

Concluding Remarks

Scaling down materials dimensions allows to explore extraordinary physical properties only exhibited at the nanoscale. The employment of nanostructured and hybrid materials (such as carbon-based materials and CPs) has already been shown to enhance INIs electrical performances and to reduce the mechanical mismatch at the electrode/tissue interface, which is primarily responsible for their lack of efficiency in chronic experiments.

In our opinion, the most promising contribution driven by Nanotechnology to extend the clinical relevance of INIs will be given using nanostructured materials as wireless nanotransducers for neuromodulation. Future studies to better understand their cellular interactions and brain targeting will be of primarily importance to understand the correct dose to trigger the desired effect and the most appropriate route of administration to reach the target neurons. Furthermore, development of portable energy sources and closed-loop control devices for energy transduction will allow the use of this technology even outside of healthcare facilities.

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References

[1] Redolfi Riva E and Micera S 2021 Progress and challenges of implantable neural interfaces based on nature-derived materials. *Bioelectronic Medicine 7* 1-10

[2] Abidian M R and Martin D C 2009 Multifunctional nanobiomaterials for neural interfaces. *Adv. Funct. Mater.* 19(4), 573-585

[3] Park H J et al 2013 Activation of the central nervous system induced by micro-magnetic stimulation. *Nat commun* 4(1) 1-9.

[3] Liu S *et al* 2020 Micro-and nanotechnology for neural electrode-tissue interfaces. *Biosensors and Bioelectronics* 112645

[4] Young A T *et al* 2018 Neuro-Nano Interfaces: Utilizing Nano-Coatings and Nanoparticles to Enable Next-Generation Electrophysiological Recording, Neural Stimulation, and Biochemical Modulation. *Adv funct mater*, 28(12) 1700239.

[5] Ryu M *et al* 2017 Enhancement of interface characteristics of neural probe based on graphene, ZnO nanowires, and conducting polymer PEDOT. *ACS Appl. Mater. Interfaces* 9 12 10577–10586
[6] Liang Y *et al* 2021 PEDOT: PSS-Based Bioelectronic Devices for Recording and Modulation of Electrophysiological and Biochemical Cell Signals. Adv Healthc Mater, 10(11) 2100061.
[7] Liu Y *et al* 2019 Soft and elastic hydrogel-based microelectronics for localized low-voltage neuromodulation. *Nat. biomed. Eng.* 3 58-68
[8] Chen G *et al* 2019 Strain-and strain-rate-invariant conductance in a stretchable and compressible 3D conducting polymer foam. *Matter* 1 205-218
[9] Li X *et al* 2021 Nanotransducers for wireless neuromodulation. *Matter* 4 1484-1510
[10] Maya-Vetencourt J F *et al* 2020 Subretinally-injected semiconducting polymer nanoparticles rescue vision in a rat model of retinal dystrophy. *Nat. Nanotechnol.* 15 698-708
[11] Yoo S Park *et al* 2018 Single-cell photothermal neuromodulation for functional mapping of neural networks. *ACS nano* 13(1) 544-551
[12] Marino A *et al* 2015 Piezoelectric nanoparticle-assisted wireless neuronal stimulation. *ACS Nano* 9 7678-7689

Section 5 - Brain cancer nanomedicine: State of the art and challenges

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Status

Brain cancer represents one of the most difficult conditions to treat. In particular, glioblastoma multiforme (GBM), which represent 47.7% of brain tumors, is one of the deadliest tumors, and is characterized by an extremely dismal prognosis. [1] The current gold standard treatment for GBM includes surgery, chemotherapy and radiotherapy; in particular, the Stupp protocol, based on the administration of the drug temozolomide in combination with radiotherapy, is the most common therapeutic regimen. [2] Another therapeutic approach consist of the application of a carmustine-loaded biodegradable wafer (Gliadel®) directly in the resection cavity during surgery. [3] Nevertheless, despite the efforts, GBM prognosis is still very poor, with an average 5-year survival rate of 5.6 %. [1] This poor outcome is related to the complex nature of brain cancer and the therapeutic challenges related to it, that conventional therapies fail to address in an efficient way. Therefore, there is an urgent need of new approaches to improve efficacy, while reducing the severe side effects related to the aspecific distribution of conventional drugs within the body after systemic administration. In this sense, a lot of effort has been put in designing drugs that are highly bioavailable and that specifically target cancer cells. Owing to their peculiar physicochemical properties (high surface-to-volume ratio, sizes in the nanoscale), tunable morphology and composition, easy preparation protocols and possible surface functionalization, biocompatible nanoparticles emerged as a promising tool to treat cancer, creating a new branch of nanotechnology, called nanomedicine. Nanoparticles offer several advantages with respect to conventional chemotherapy: 1) they can encapsulate hydrophobic molecules that are difficult to administer in biological fluids, improving their solubility and biocompatibility; 2) they can release drugs in a controlled manner and, in some cases, the release can be triggered by external stimuli; 3) they can be easily conjugated to ligands that are specific for cancer cells, imparting targeting abilities to the nanoparticles and favoring their accumulation in tumor tissues. [1] Besides being a delivery agent for drugs or other active compounds, nanoparticles can also have an active role in cancer medicine, being themselves therapeutic or contrast agents (superparamagnetic iron oxide nanoparticles, gold nanoparticles or quantum dots, for instance). Currently, there are a plethora of nanoparticles that can be employed to treat brain cancer, and, depending on their composition, they can be classified as organic (*e.g.*, liposomes, polymeric nanoparticles or nanostructured lipid carriers) or inorganic nanoparticles.

Current and Future Challenges

Brain cancer presents several therapeutic challenges that make its treatment very problematic. First of all, the blood-brain barrier (BBB) that normally protects the brain from harmful substances, represents one of the main obstacles for the delivery of therapeutic compounds to the brain, since most of the conventional drugs are unable to efficiently cross it. [4] On the other hand, nanoparticles can be functionalized with ligands that interact with receptors overexpressed on endothelial cells and that can trigger active transport mechanisms, such as receptor-mediated transcytosis (RMT). [4] For instance, peptides derived from the specific amino acid sequence of the apolipoprotein E (ApoE) bind to the low-density lipoprotein receptor of capillary endothelial cells; thus, nanoparticles functionalized with ApoE have a higher BBB crossing efficiency. [5],[6]

Another goal in nanomedicine is to favor the accumulation of therapeutics within diseased tissues, in order to reduce side effects on the healthy ones. Nanoparticles are known to preferentially accumulate in tumors *via* passive targeting exploiting the so-called enhanced permeation and retention (EPR) effect. This phenomenon depends on the size of the nanoparticles, on the abnormal vascular architecture around tumors, that favors extravasation, and on the lack of a proper lymphatic

drainage. [7] However, passive targeting is difficult to control, with consequent poor drug diffusion and aspecific accumulation in liver and spleen and it can induce multidrug resistance (MDR). [8] Active targeting, instead, relies on the interaction between a ligand (*e.g.*, antibodies, peptides, small molecules or aptamers) attached to the nanoparticles surface and a receptor overexpressed by the target cells. For instance, nanoparticles functionalized with the peptide angiopep-2 can selectively target GBM cells and have improved BBB crossing abilities.[9] More recently, a new strategy exploiting the ability of cancer cells to recognize each other has been developed. In fact, tumor cells can form multicellular aggregates thanks to the interaction between specific proteins in hemophilic adhesion domains of plasma membranes, or between tumor-specific binding proteins. [10] By mimicking this natural tendency of cancer cells to homotypic recognition, coating nanoparticles with cancer cell membranes extracts could significantly increase their uptake in the tumor cells. This targeting strategy, referred as ”homotypic targeting“, accounts for the intricate interactions that requires simultaneous binding of different ligands to efficiently target cancer cells. [11]

Another future challenge in brain cancer nanomedicine will be to design nanoparticles able to exert a therapeutic action when remotely activated by an external stimulus (*e.g.*, magnetic fields, light irradiation and ultrasound). This will guarantee a less invasive and “on demand” treatment, activated only when the nanoparticles are effectively located in the tumor area, avoiding potential harmful effects on healthy cells.

Advances in Science and Technology to Meet Challenges

In recent years, nanomedicine has made huge progresses in overcoming the main issues related to the treatment of brain cancer. As mentioned before, the improvement of the BBB crossing abilities of nanoparticles is one of the biggest challenges to meet. Several nanoparticles functionalized with ligands interacting with receptors on endothelial cells surface (transferrin, lactoferrin, insulin and low-density lipoprotein receptors) that activate RMT have been develop to enhance their crossing efficiency. [2] Another proposed solution is to exploit immune cells (*e.g.,* neutrophils, monocytes, and macrophages) to activate cell-mediated transcytosis; for instance, drug-loaded liposomes can be absorbed by immune cells circulating in the blood and transported through the BBB towards the inflammation site in the brain exploiting immune cells properties called diapedesis and chemotaxis. [12]

In order to improve the accumulation of nanomaterials in tumors, researchers have proposed several strategies. Among them, functionalization with peptides or aptamers interacting with receptors overexpressed on tumor cells has demonstrated to be an effective targeting strategy. Nevertheless, when dealing with genetic heterogeneous tumors such as GBM, approaches relying on one or two single interactions are often inefficient. In these cases, homotypic targeting has shown very promising results in the accumulation of therapeutics in the tumor site. For instance, boron nitride nanotubes loaded with doxorubicin and coated with GBM cell membrane extracts were shown to selectively target GBM cells, while the uptake and, as a consequence, the cytotoxicity on other healthy cells used in the study were not observed (Figure 1). [13]

New nanoparticles exerting an anticancer action only when activated by an external stimulus have been also developed. Nanostructured lipid carriers loaded with superparamagnetic iron oxide nanoparticles were able to induce apoptosis in GBM cells through hyperthermia-induced lysosomal membrane permeabilization after a chronic stimulation with a proper alternated magnetic field. [7] An innovative approach to treat brain cancer is represented by the ultrasound stimulation of organic piezoelectric nanoparticles. Treatment with these nanoparticles loaded with a drug, followed by chronic ultrasound stimulation, led to the activation of cell apoptosis and anti-proliferation pathways, induction of cell necrosis, inhibition of cancer migration, and reduction of cell invasiveness in drug-resistant GBM cells (Figure 2). [6]

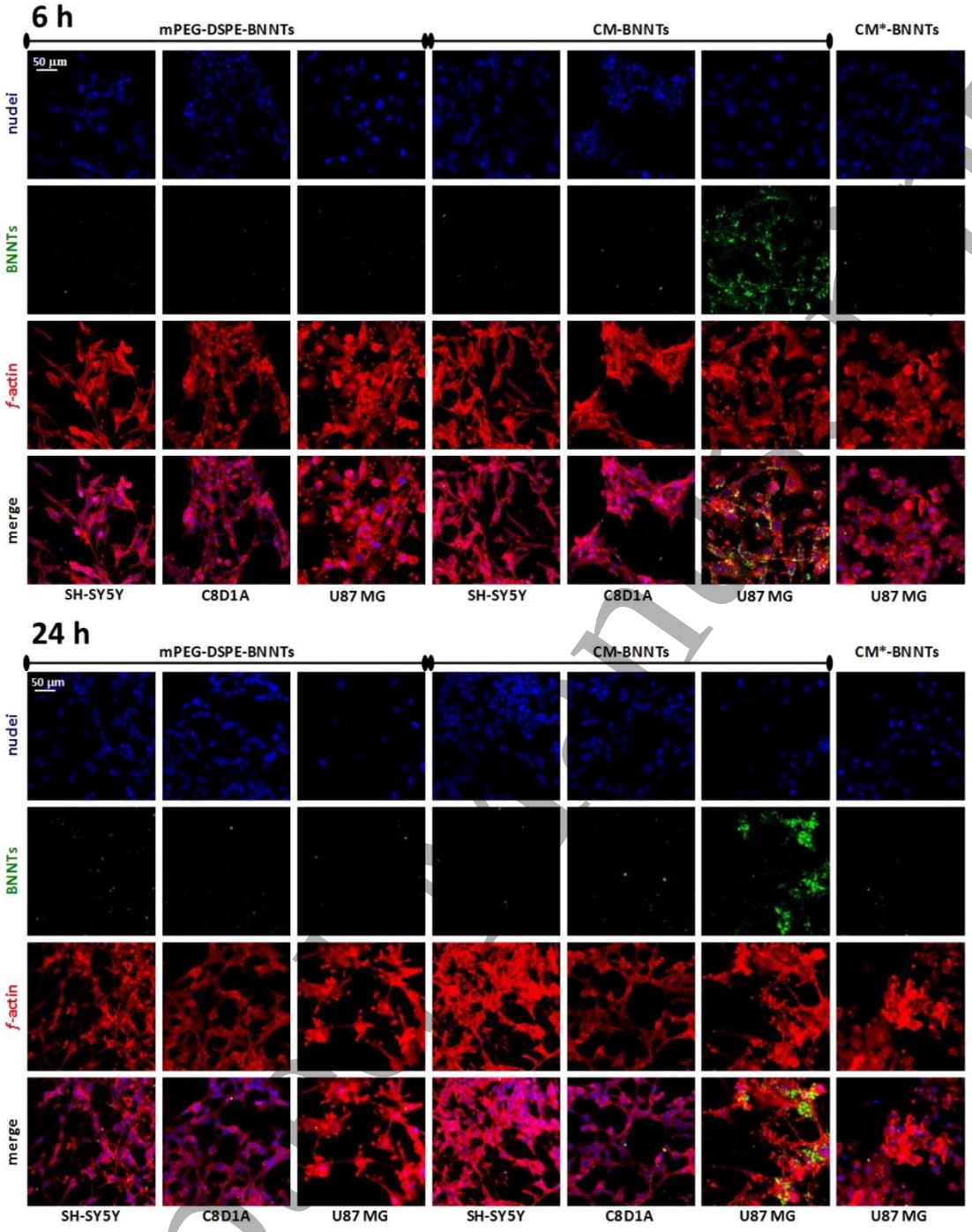

Figure 1. Targeting investigation of cell-membrane coated boron nitride nanotubes (CM-BNNTs) as compared to BNNTs coated with a conventional lipid (mPEG-DSPE-BNNTs), or with cell membrane extracts deprived of the proteins (CM*-BNNTs) at different time points. Confocal acquisitions of SH-SY5Y derived neurons, C8D1A astrocytes, and U87 MG cells (a glioblastoma model) incubated with 100 µg/ml of mPEG-DSPE-BNNTs, CM-BNNTs, or CM*-BNNTs (f-actin in red, BNNTs in green, nuclei in blue) shows that CM-BNNTs are able to selectively target GBM cells, while uptake by other cell lines is almost negligible. mPEG-DSPE-BNNTs or CM*-BNNTs are poorly uptaken by all the cell lines used in the study, highlighting the importance of cell membrane proteins in the homotypic targeting mechanism. (Reproduced from [13], Copyright 2020, with permission from Elsevier).

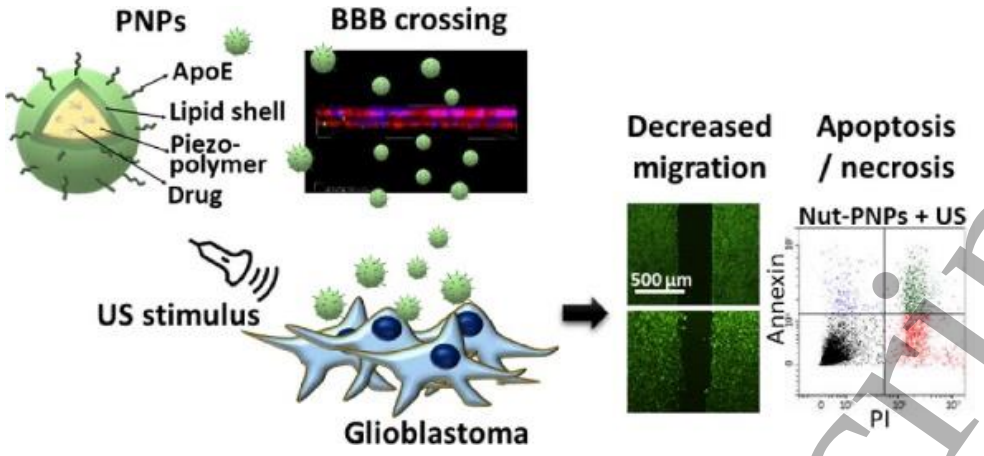

Figure 2. Piezoelectric hybrid lipid-polymeric nanoparticles, composed of the piezoelectric polymer poly(vinylidene fluoride-trifluoro ethylene) and of a lipid shell of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)], encapsulating the non-genotoxic drug, nutlin-3a, and functionalized with a ApoE-derived peptide, that enhances the BBB crossing abilities of the nanoparticles. Upon ultrasound stimulation, the nanoparticles were able to reduce cell migration, thanks to a reduce *f*-actin/*g*-actin ratio, and to foster apoptotic and necrotic events (Reproduced from [6],Copyright 2021, with permission from Elsevier).

Concluding Remarks

Nanomedicine has shown promising results for the treatment of brain cancer by offering several approaches to cross the BBB, improving the systemic delivery of drugs and favoring their accumulation in tumor tissues. Nevertheless, in the future, research will have to focus on making the treatment specific for the patient, moving towards precision medicine. Since brain tumors are extremely heterogeneous between different patients, it would be desirable to be able to develop nanotherapeutics that can be adapted to the specific needs of the patients. This will increase treatment efficacy, while reducing side effects. Nanotherapeutics should be tested directly on patient-derived cancer cells in order to choose the best therapeutic approach and/or should contain features of the patient's cancer cells (*e.g.*, coating with cell membrane extracts) to adapt the targeting abilities to the specific membrane proteins expression of the tumor.

References

[1] A. G. Bhargav, S. K. Mondal, C. A. Garcia, J. J. Green, and A. Quiñones-Hinojosa, "Nanomedicine Revisited: Next Generation Therapies for Brain Cancer," *Adv. Ther.*, vol. 3, no. 10, p. 2000118, 2020, doi: 10.1002/ADTP.202000118.

[2] R. Stupp, W.P. Mason, M.J. van den Bent, M. Weller, B. Fisher, M.J.B. Taphoorn, K. Belanger, A.A. Brandes, C. Marosi, U. Bogdahn, J. Curschmann, R.C. Janzer, S.K. Ludwin, T. Gorlia, A. Allgeier, D. Lacombe, J.G. Cairncross, E. Eisenhauer, R.O. Mirimanoff, "Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma" *N. Engl. J. Med.* vol. 352, pp. 987-996, 2005, doi: 10.1056/NEJMoa043330.

[3] FDA. GLIADEL® WAFER (carmustine implant), for intracranial use www.fda.gov/medwatch. (accessed Sep 18, 2022).

[4] S. Ding, A. I. Khan, X. Cai, Y. Song, Z. Lyu, D. Du, P. Dutta, Y . Lin, "Overcoming blood–brain barrier transport: Advances in nanoparticle-based drug delivery strategies," *Mater. Today*, vol. 37, pp. 112–125, 2020, doi: 10.1016/J.MATTOD.2020.02.001.

[5] F. Re, I. Cambianica, C. Zona, S. Sesana, M. Gregori, R. Rigolio, B. La Ferla, F. Nicotra, G. Forloni, A. Cagnotto, M. Salmona, M. Masserini, G. Sancini, "Functionalization of liposomes with ApoE-derived peptides at different density affects cellular uptake and drug transport across a blood-brain barrier model," *Nanomedicine Nanotechnology, Biol. Med.*, vol. 7, no. 5, pp. 551–559, 2011, doi: 10.1016/j.nano.2011.05.004.

[6] C. Pucci, A. Marino, O. Şen, D. De Pasquale, M. Bartolucci, N. Iturrioz-Rodríguez, N. di Leo, G. de Vito, D. Debellis, A. Petretto, G. Ciofani, "Ultrasound-responsive nutlin-loaded nanoparticles for combined chemotherapy and piezoelectric treatment of glioblastoma cells," *Acta Biomater.*, 2021, doi: 10.1016/J.ACTBIO.2021.04.005.

[7] C. Martinelli, C. Pucci, and G. Ciofani, "Nanostructured carriers as innovative tools for cancer diagnosis and therapy" *APL Bioengineering*, vol. 3, no. 1, p. 011502, 2019, doi: 10.1063/1.5079943.

[8] B. Mansoori, A. Mohammadi, S. Davudian, S. Shirjang, B. Baradaran, "The Different Mechanisms of Cancer Drug Resistance: A Brief Review." *Adv Pharm Bull.,* vol. 7, no. 3, pp. 339-348, doi: 10.15171/apb.2017.041.

[9] C. Pucci, D. De Pasquale, A. Marino, C. Martinelli, S. Lauciello, and G. Ciofani, "Hybrid Magnetic Nanovectors Promote Selective Glioblastoma Cell Death through a Combined Effect of Lysosomal Membrane Permeabilization and Chemotherapy," *ACS Appl. Mater. Interfaces*, vol. 12, no. 26, pp. 29037–29055, 2020, doi: 10.1021/acsami.0c05556.

[10] R.H. Fang, A.V. Kroll, W. Gao, and L. Zhang, "Cell Membrane Coating Nanotechnology" *Adv. Materials*, vol. 30, no. 23, pp. 1-34, 2018, doi: 10.1002/adma.201706759.

[11] R. H. Fang, C. J. Hu, B. T. Luk, W. Gao, J. A. Copp, Y. Tai, D. E. O'Connor, L. Zhang, "Cancer cell membrane-coated nanoparticles for anticancer vaccination and drug delivery," *Nano Lett.*, vol. 14, no. 4, pp. 2181–2188, 2014, doi: 10.1021/nl500618u.

[12] S. Jain, V. Mishra, P. Singh, P. K. Dubey, D. K. Saraf, and S. P. Vyas, "RGD-anchored magnetic liposomes for monocytes/neutrophils-mediated brain targeting," *Int. J. Pharm.*, vol. 261, no. 1–2, pp. 43–55, Aug. 2003, doi: 10.1016/S0378-5173(03)00269-2.

[13] D. De Pasquale, A. Marino, C. Tapeinos, C. Pucci, S. Rocchiccioli, E. Michelucci, F. Finamore, L. McDonnell, A. Scarpellini, S. Lauciello, M. Prato, A. Larrañaga, F. Drago, G. Ciofani, "Homotypic targeting and drug delivery in glioblastoma cells through cell membrane-coated boron nitride nanotubes," *Mater. Des.*, vol. 192, p. 108742, 2020, doi: 10.1016/j.matdes.2020.108742.

Section 6 - Nanoparticle-based developments for Parkinson's disease treatment

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Status

Degeneration and loss of dopaminergic neurons in the substantia nigra pars compacta, and subsequent reduction of dopamine (DA) levels in striatum, are associated with motor symptoms that characterize Parkinson's disease (PD). In fact, since PD is a multifactorial disease where both genetic and non-genetic factors are involved, the most prominent mechanisms related to the development of this disease include the accumulation of misfolded proteins aggregates (i.e. α-synuclein, ubiquitin, PTEN-induced kinase-1 (PINK1), parkin, and other proteins), failure of protein clearance pathways, mitochondrial damage, oxidative stress, excitotoxicity, neuroinflammation, and genetic mutations [1]. Several treatments are available, but none of them is notably effective to reduce neuronal loss and restoring DA levels. It has been introduced some promising alternative strategies, such as stem cell transplantation and gene therapy. However, most of them are still under investigation and safe/efficacy need to be adequately addressed before clinical trials. During the last years, extensive studies to understand the molecular signaling pathways involved in PD, the involvement of molecular chaperones, autophagy-lysosomal pathways, and proteasome systems, have been addressed. In addition, emerging therapies such as pharmacological manipulations, or surgical procedures, are proposed as alternative treatments.

The incorporation of conventional drugs into nanoparticles (NPs), the so-called nanocarriers, has represented a step forward in conventional medicine for the treatment of different diseases and specifically of PD [2]. Of the many advantages, NPs stabilize hydrophobic drugs and facilitate crossing the Blood-Brain-Barrier (BBB) of different nowadays active systems such as enzymes, proteins and even dopamine, seeding novel drug therapies beyond the gold-standard L-Dopa therapy (figure 1). Nanocarriers also offer major pharmacokinetics advantages such as controlled drug release over time while avoiding early metabolism and phagocytosis, facilitate targeting to specific cells (improving efficacy, safety, sensitivity and personalization) and allow for the delivery to the brain of combined drugs, antioxidant agents, neurotrophic, and neuroprotective factors, as well as antiapoptotic factors, or even gene therapy. All these advantages allow us to predict a great advance in this field for the years to come though further studies are still needed, especially to overcome the BBB. In this context, the intranasal administration has been proposed over the last years as a novel route to bypass the BBB and reduce systemic side effects with respect to oral or systemic administration [3]. Several nanoformulations have been already developed showing an enhancement of nose-to-brain drug transport for neurodegenerative diseases [4], including coordination polymer nanoparticles for intranasal dopamine replacement in PDs [5]. Based on the intranasal physiognomy, there are two possible passages from nose to brain (i.e. the olfactory nerves that end up at the olfactory bulb and the maxillary branch of the trigeminal nerve). The goal of different studies is to track the translocation of nanoparticles and the payloads along these nose-to-brain pathways.

Current and Future Challenges

As previously stated, nanotechnology is a promising approach to facilitate PD patients management and design more selective and effective therapies. Moreover, it could be very important to understand

the pathophysiology of the disease, achieve earlier stages of diagnosis, and offer better treatment options. Though, major challenges are still to be faced before its implementation becomes true:

- *Nanocarrier improvement*. Future developments involve biodegradable therapeutic nanocarriers with optimized drug encapsulation yields and controlled release in response to external stimuli.
- *Drug research*. Further developments in using novel DA drugs (e.g. use of IPX066, XP21279, and Opicapone) or non-DA drugs (e.g. α2-adrenergic antagonists, serotonergic, or adenosine A2a antagonists), or even the use of micro-RNA or Si-RNA approach to inhibit mRNA of misfolded protein aggregates, which may offer beneficial effects in late-stage developments of motor symptoms in PD, are needed; The exhaustive study of the PD mechanisms and use of novel gene editing techniques (e.g. CRISP-Cas9) for correction of mutated genes involved in PD, will improve the election of the adequate drug and therapy design for an effective PD treatment.
- *Intranasal administration.* Further research is required to better understand the drug passage mechanism through the intranasal route to specific areas of the brain depending on the NP formulation and its membrane permeability, mucocilliary clearance, or enzymatic degradation [6].
- *Targeting*. The specific targeting of dopaminergic neurons would be strongly desired to reduce secondary effects while ensuring a proper biodistribution (drug bioavailability) [7].
- *Side-effects*. Although nanocarriers overall reduce the toxicity of the free drugs, nanoparticles can induce the triggering of inflammation, oxidative stress, and gene overexpression [8].

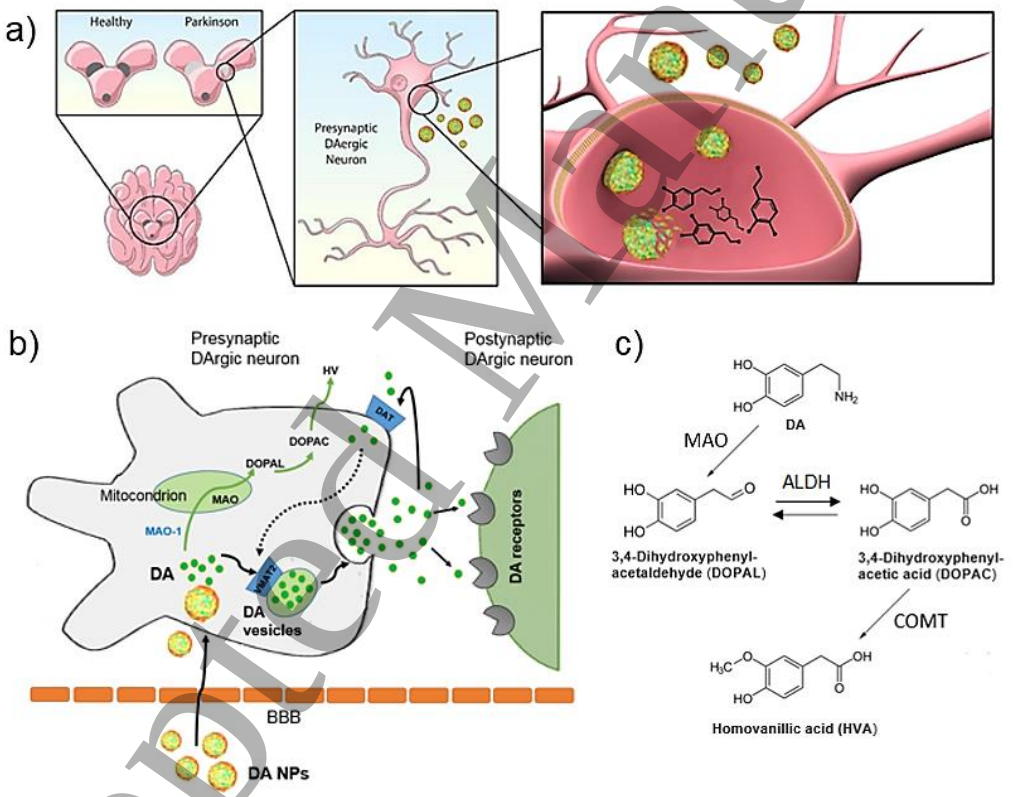

Figure 1. a) Proposed model for DA-based replacement therapy and stimulation of dopaminergic (DArgic) neurons in Parkinson's disease using nanoparticles; b) Pharmacokinetics of dopamine metabolism inside presynaptic neurons after crossing the BBB, the nanocarriers release DA inside DArgic neurons or external DA is re-uptaken by DA transporters (DAT) or metabolized; c) Principal degradation pathways of DA in DArgic neurons. Abbreviations: catechol-O-methyltransferase (COMT), monoamine oxidase (MAO), monoamine oxidase inhibitor (MAO-I), aldehyde dehydrogenase (ALDH), vesicular monoamine transporter type 2 (VMAT2)

Advances in Science and Technology to Meet Challenges

Until now, several nanocarrier systems have been successfully applied such as chitosan nanoparticles, target functionalized liposomes, (bio)hydrogels, carbon nanotubes, graphene-based nanosystems, polymeric nanocapsules or magnetic nanoparticles [9]. For most of them, size, shape and charge can be systematically tuned, though much work is still needed to develop responsiveness in front of pH changes, enzymatic action or redox stimuli. Exploration of novel and disruptive nanocarriers would also benefit the field and offer rational designs for drug transport and effective and selective drug release in the site of action, minimizing doses and side effects as well as increasing the colloidal and chemical stability of the NPs. The development of novel and non-invasive administration routes, such as nose-to-brain delivery, opens up a host of possibilities to reach the brain in an effective manner. In this sense, the use of a suitable mucoadhesive coating on the nanoparticles (e.g. mucoadhesive polymers, gelatins, hydrogels) would favored the mucoadhesion enhancing the retention time in nasal cavity and reducing mucociliary clearance (figure 2)

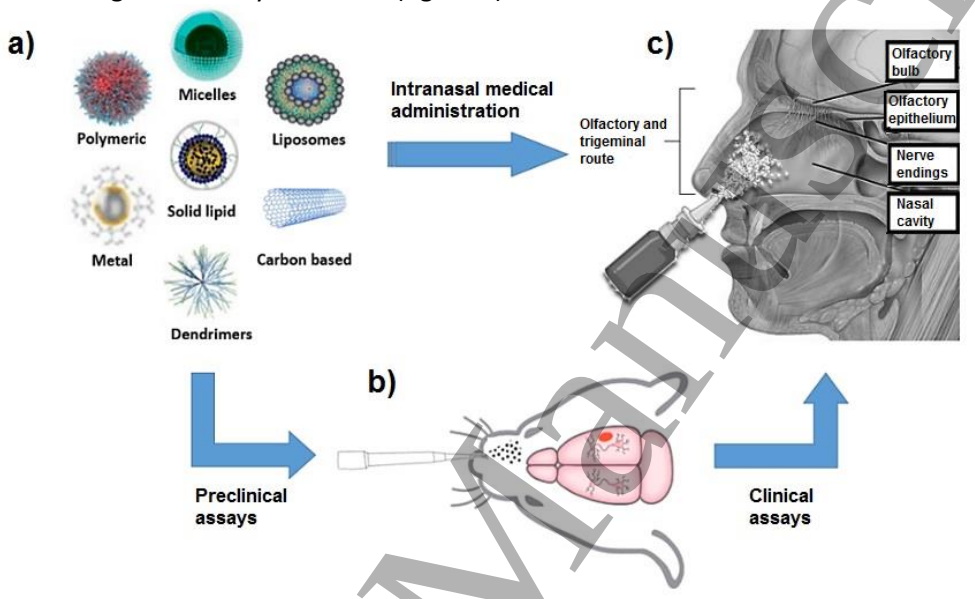

Figure 2. a) Different nanocarriers developed for other administration routes can be modified for nose-to-brain administration. For the validation of the resulting nanoparticles, it is essential to investigate pathogenic pathways at the whole-organism level in PD animal models (b) for further optimization of the nanoformulation before its clinical use (c).

Apart from pharmacological treatments, the monitoring of dopamine levels *in vivo* and in real time is necessary to understand its physiological roles. In this sense, different sensors and biosensors have been developed, as those based in measuring neurotransmitters with fast-scan cyclic voltammetry (FSCV) using multielectrode arrays [10]. These studies help to better understand the complex brain heterogeneity, the dynamic neurochemical environment, and how disease states or drugs affect separate brain areas concurrently.

Concluding Remarks

The limited efficacy of drugs to treat neurodegenerative diseases has become a major challenge over the years due to different physiological factors such as enzymatic degradation, systemic clearance, peripheral side effects and reduced bioavailability. The encapsulation of drugs within NPs has shown a clear improvement in this respect. The use of nanoformulated systems allow using new insoluble or chemically unstable drugs (or a combination of them) as well as design an adequate biodistribution and targeting. So far, many examples have been described in the literature, some of them even reaching clinical phases, leading us to expect very promising results soon even though the development of these new nanopharmaceuticals is at a very early stages. A very relevant aspect of this research is the drastic limitations imposed by the BBB to improve current therapies. In this scenario, the investigation of alternative administration modes to bypass or avoid the BBB crossing,

such as intranasal administration, is an important aspect to improve PD treatment. In fact, a large number of active principles have already being successfully delivered through this non-invasive route to the central nervous system. If clinical studies support such preclinical data, intranasal drug delivery may revolutionize treatments for brain disorders. Finally, we would not like to end this section without mentioning precision medicine therapies as one of the greatest expectations for the future in this area.

Acknowledgements

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References

[1] Maiti P, Manna J, and Dunbar G L 2017 Current understanding of the molecular mechanisms in Parkinson's disease: Targets for potential treatments. *Transl. Neurodegener.* 6, 28.

[2]Baskin J, Jeon J E and Lewis S J G 2021 Nanoparticles for drug delivery in Parkinson's disease. *Journal of Neurology* 268, 1981–94.

[3] Gambaryan P Y, Kondrasheva, I G, Severin E S, Guseva A A and Kamensky A A 2014 Increasing the efficiency of Parkinson's Disease treatment using a poly(lactic-co-glycolic acid) (PLGA) based L-DOPA delivery system *Exp Neurobiol* 23(3), 246–52.

[4] Khan A R, Yang X, Fu M and Zhai, G. 2018 Recent progress of drug nanoformulations targeting to brain *J. Control Release* 291, 37–64.

[5] García-Pardo J et al. 2021 Bioinspired Theranostic Coordination Polymer Nanoparticles for Intranasal Dopamine Replacement in Parkinson's Disease *ACS Nano* 15, 8592−8609.

[6] Yu S, Xu X, Feng J, Liu M and Hu, K Chitosan and chitosan coating nanoparticles for the treatment of brain disease *Int. J. Pharm.* 2019, 560, 282–293.

[7] Jin G-Z, Chakraborty A, Lee J-H, Knowles J C and Kim H W 2020 Targeting with nanoparticles for the therapeutic treatment of brain diseases *Journal of Tissue Engineering* 11, 1–13

[8] Vinod C and Jena S 2021 Nano-Neurotheranostics: Impact of Nanoparticles on Neural Dysfunctions and Strategies to Reduce Toxicity for Improved Efficacy *Front. Pharmacol.* 12, 612692.

[9] Paul A, Yadav KS 2020 Parkinson's disease: Current drug therapy and unraveling the prospects of nanoparticles *J. Drug Delivery Sci. Technol.* 58, 101790.

[10] Rafi H, Zestos A.G 2021 Multiplexing neurochemical detection with carbon fiber multielectrode arrays using fast-scan cyclic voltammetry *Anal. Bioanal. Chem.* 413, 6715–6726.

Section 7 – Nanomedicine advances in Alzheimer's disease treatment

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Status

Alzheimer's disease (AD) is an age-related, irreversible form of dementia characterized by the progressive degeneration of cognitive performance [1]. In developed countries, about 50 million people are affected by dementia and the incidence of AD will increase because of the ongoing increase of the aged population [2]. This makes AD a growing concern that harms the global health care system. The cardinal features of Alzheimer pathology are the cerebral accumulation of β-amyloid (Aβ) peptide in toxic oligomers and amyloid plaques, neurofibrillary tangles of aggregated tau protein, synaptic dysfunction, neuronal death, and cognitive impairment. However, AD is a multifactorial disease with several additional pathogenic mechanisms, including inflammation, oxidative damage, iron dysregulation, mitochondrial dysfunction, and altered cholesterol metabolism [1, 3]. Almost all current AD treatments merely delay the onset of symptoms, without modifying the course of the disease.

The majority of disease-modifying treatments (DMTs), i.e. those proven to alter the underlying disease pathology or disease course, are nearly every designed to specifically target Aβ. Antibodies tackling Aβ are in advanced clinical trials, and Aducanumab, which clears Aβ, was approved by the US Food and Drug Administration (FDA) in 2021, not without controversy. Indeed, the scarce beneficial effects on cognitive decline reported by treated patients drove the European Medicines Agency (EMA) to the refusal of the marketing authorisation for Aducanumab.

Overall, the development of DMTs for AD is complicated by the presence of the blood-brain barrier (BBB), which prevents certain drugs and large-molecule therapeutics from entering the brain.
Therefore, strategies boosting the passage of drugs able to modulate multiple disease-associated pathways across the BBB represent a key opportunity to treat AD.

Nanotechnology has emerged as an exciting and promising strategy to treat neurological disease, with the potential to fundamentally change the way to approach brain-targeted therapeutics. In this field, nanoparticles (NPs) can be tuned by controlling their physiochemical properties, such as size, shape, surface charge, and hydrophobicity to cross the BBB, to bind Aβ, to enhance their anti-amyloid capacity and/or to deliver DMTs to the brain. Accordingly, one phase 2 trial based on the intranasal administration of APH-1105, an alpha (α)-secretase modulator formulated in nanoparticles, is being estimated to start in June 2023. Its safety, tolerability, and efficacy are being evaluated for the treatment of subjects with mild-moderate AD [4].

However, further advances in NPs engineering could be a breakthrough in AD treatment, taking into account the timing of interventions and the population to be recruited [5].

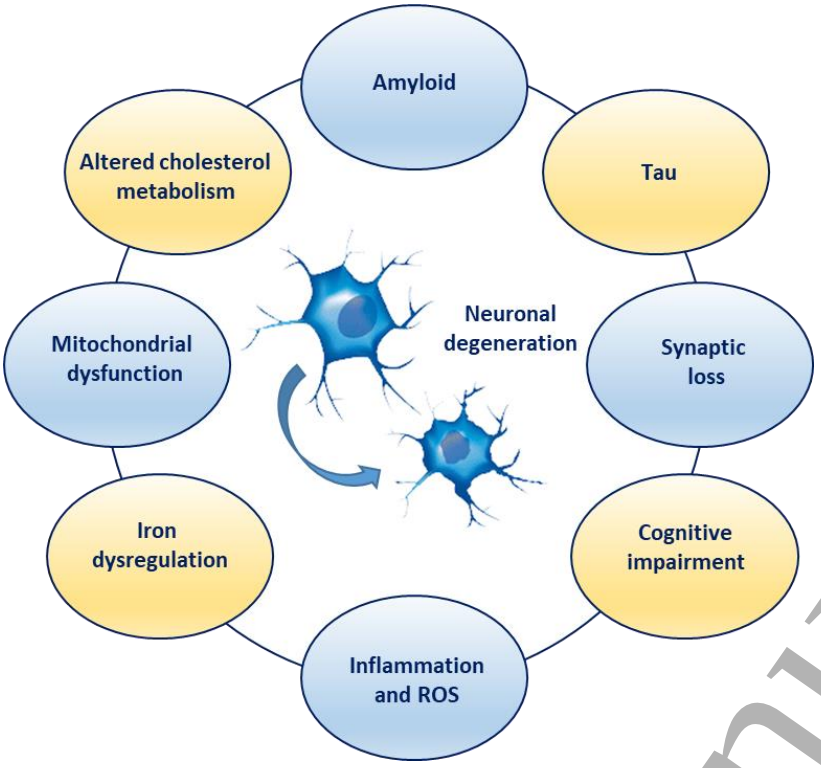

Figure 1. Alzheimer's disease is a multifactorial disorder. Multitarget drugs have been increasingly sought after over the last decades, however strategies to enhance their entry in the brain thus enhancing the therapeutic efficacy are sought.

Current and Future Challenges

There are several issues in seeking effective medicines for AD. One of the biggest obstacles is the presence of the BBB, a formidable challenge to the delivery drugs into the brain. Several compounds have not shown efficacy in clinical trials, because they generally fail to cross the BBB, and the use of NPs as drug delivery system offers an alternative approach for promising and innovative therapeutic solutions for AD.

The NPs, suitably functionalized and with a long blood-residency time, can cross the BBB and release active molecules at target sites in the brain, minimizing side effects. It has been demonstrated that the concentration of drug that reaches the brain is higher if it is formulated in NPs, rather than when administered alone [6]. For example, it was shown that the concentration of rivastigmine that reaches the brain is about 4 times higher when embedded in NPs than when it is free [7]. Also the administration route of NPs may affect the bioavailability of the drug delivered into the body. For example, nasal administration route is the most practical and non-invasive method to administer drugs. However, the nasal cavity has enzymes that can could affect the bioavailability of the drug and NPs [8].

An additional challenge for AD therapy is the multifactorial nature of the disease. Indeed, the different pathogenic mechanisms involved in AD, other than the most known disease hallmarks, make the situation more complicated, impacting on the strategy adopted.

Another issue could be the potential 'ancillary effect' of NPs, which is affected by the inability of the various clearance systems to remove them from the brain, and by their physicochemical properties that could interfere with physiological pathways, as seen for inorganic or metallic NPs. Another limitation could be the expensive process that leads to the production of NPs, because specific

materials, instruments, and optimal conditions are needed in order to obtain specific multifunctional NPs.

Advances in Science and Technology to Meet Challenges

The application of nanotechnology in the treatment of AD includes NPs targeting Alzheimer's β-amyloid or NPs active on other pathways involved in AD pathogenesis or progression.
Among the most promising NPs designed to tackle Aβ, we can cite:

i) PLGA-NPs embedding the peptide (iAβ5) able to inhibit the Aβ fibrillogenesis, and surrounded with anti-transferrin receptor monoclonal antibody (OX26) and anti-Aβ (DE2B4) useful to deliver the drug across the BBB [9].
ii) Liposomes functionalized with phosphatidic acid (PA) and surface decorated with a modified peptide derived from the receptor binding domain of apolipoprotein E (mApoE), that have the dual ability to affect $A\beta_{42}$ aggregation/disaggregation processes and to cross the BBB [10].
Among the NPs designed to target AD-related pathways, we can cite:
i) NPs with antioxidant activity useful to counteract ROS-mediated cerebrovascular dysfunctions, which are considered worsening factors in the progression of AD [11].
ii) Discoidal NPs functionalized with apolipoprotein A-I suitable as a potential supportive treatment to compensate the depletion of cerebral HDLs occurring in AD [3].
iii) Selenium NPs encapsulated PLGA nanospheres with curcumin to decrease Aβ-dependent inflammation [12].

The application of nanotechnologies includes also NPs for AD diagnosis. Polymeric NPs and SPIONs developed for the imaging of amyloid aggregated, have demonstrated great potential in this field [13]. A new strategy is theranostics that combines diagnosis with therapeutic approaches in order to have a unique device that recognizes and identifies the biomarkers of the disease, for example Aβ plaques or the tau tangles, and at the same time reaches them and releases specific drugs.

In general, the attention has focused on lipid nanoparticles, because as being made-up of lipids, they are biocompatible and tolerated by our body. They show several advantages over other systems, including an easy large-scale production, biodegradability and biocompatibility materials, low toxic potential, the ability to control or modify drug release, and the ability to incorporate hydrophilic and lipophilic drugs.

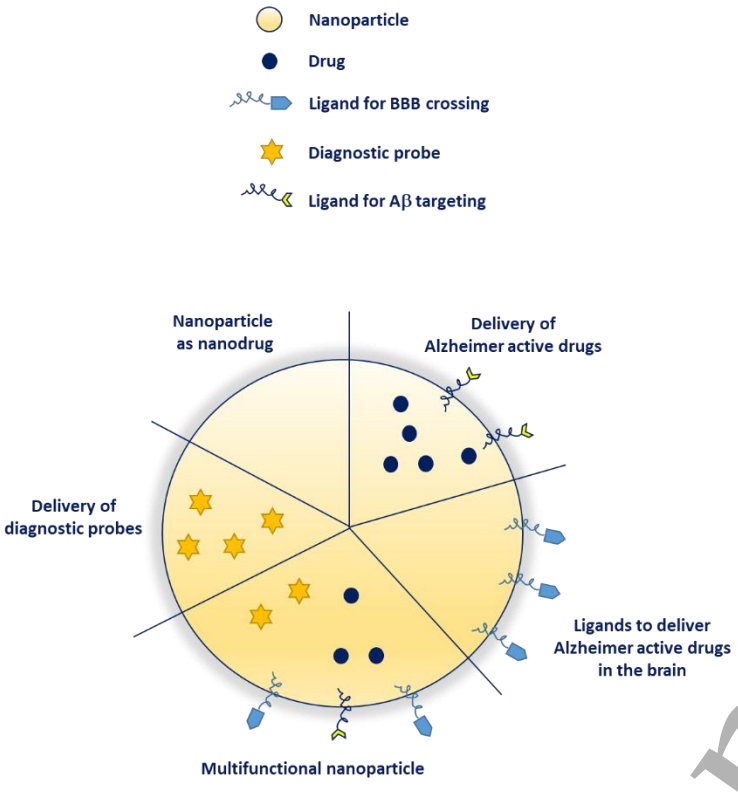

Figure 2. Multifunctional nanoparticles. Nanoparticles with multiple properties (moieties) can be designed to deliver drugs active on AD in the brain.

Concluding Remarks

Achieving sufficient delivery across the BBB is a clinical need in the development of drugs to treat cerebral disorders, because many biopharmaceuticals are largely excluded from the brain after peripheral administration. When it comes to treating AD, the *per se* difficult drug discovery enterprise becomes a titanic challenge, because it is a multifactorial disorder.

Different big pharma are retreating from drug development for AD because of the negative results of more than 200 investigational programs failed in the last decade. Nevertheless, the recent (2021) FDA approval of Aducanumab in mild-moderate AD demonstrate that Aβ-centered strategy is still alive, revitalizing the AD drug development and opening new scenarios for nanotechnologies. In fact, pharmaceutical and biotechnology companies are joining forces with academic institutions and public–private consortia active in this field to develop nanotechnologies against AD.

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References

1. Burns A and Iliffe S 2009 Alzheimer's disease *BMJ 338 b158*
2. Alzheimer's Statistics 2021 www.alzheimers.net/resources/alzheimers-statistics/
3. Sierri G, Dal Magro R, Vergani B, Leone BE, Formicola B, Taiarol L, Fagioli S, Kravicz M, Tremolizzo L, Calabresi L, Re F 2021 Reduced Levels of ABCA1 Transporter Are Responsible

for the Cholesterol Efflux Impairment in β-Amyloid-Induced Reactive Astrocytes: Potential Rescue from Biomimetic HDLs *Int. J. Mol. Sci. 23(1) 102*

4. Pardo-Moreno T, González-Acedo A, Rivas-Domínguez A, García-Morales V, García-Cozar FJ, Ramos-Rodríguez JJ, Melguizo-Rodríguez L 2022 Therapeutic Approach to Alzheimer's Disease: Current Treatments and New Perspectives *Pharmaceutics 14 1117*
5. Mehta D, Jackson R, Paul G, Shi J, Sabbagh M 2017 Why do trials for Alzheimer's disease drugs keep failing? A discontinued drug perspective for 2010-2015 *Expert Opin. Investig. Drugs 26(6) 735–739*
6. Taiarol L, Bigogno C, Sesana S, Kravicz M, Viale F, Pozzi E, Monza L, Carozzi VA, Meregalli C, Valtorta S, Moresco RM, Koch M, Barbugian F, Russo L, Dondio G, Steinkühler C, Re F 2022 Givinostat-Liposomes: Anti-Tumor Effect on 2D and 3D Glioblastoma Models and Pharmacokinetics *Cancers 14 2978*
7. Wilson B, Samanta MK, Santhi K, Kumar KPS, Paramakrishnan N, Suresh B 2008 Poly(n-butylcyanoacrylate) nanoparticles coated with polysorbate 80 for the targeted delivery of rivastigmine into the brain to treat Alzheimer's disease *Brain Res. 1200 459-68*
8. Ling TS, Chandrasegaran S, Xuan LZ, Suan TL, Elaine E, Nathan DV, Chai YH, Gunasekaran B, Salvamani S 2021 The Potential Benefits of Nanotechnology in Treating Alzheimer's Disease *Biomed Res Int 2021 5550938*
9. Loureiro JA, Gomes B, Fricker G, Coelho MAN, Rocha S, Pereora MC 2016 Cellular uptake of PLGA nanoparticles targeted with anti-amyloid and anti-transferrin receptor antibodies for Alzheimer's disease treatment *Colloids Surf. B Biointerfaces 145 8-13*
10. Mancini S, Balducci C, Micotti E, Tolomeo D, Forloni G, Masserini M, Re F 2017 Multifunctional liposomes delay phenotype progression and prevent memory impairment in a presymptomatic stage mouse model of Alzheimer disease *J. Control Release 258 121-129*
11. Dal Magro R, Vitali A, Fagioli S, Casu A, Falqui A, Formicola B, Taiarol L, Cassina V, Marrano CA, Mantegazza F, Anselmi-Tamburini U, Sommi P, Re F 2021 Oxidative Stress Boosts the Uptake of Cerium Oxide Nanoparticles by Changing Brain Endothelium Microvilli Pattern *Antioxidants 10(2) 266*
12. Huo X, Zhang Y, Jin X, Li Y, Zhang LJ, Biology PB 2019 A novel synthesis of selenium nanoparticles encapsulated PLGA nanospheres with curcumin molecules for the inhibition of amyloid β aggregation in Alzheimer's disease *Journal of Photochemistry and Photobiology B: Biology 190 98–102*
13. Binda A, Murano C, Rivolta I 2020 Innovative Therapies and Nanomedicine Applications for the Treatment of Alzheimer's Disease: A State-of-the-Art (2017–2020) *Int. J. Nanomedicine 15 6113-6135*

Section 8 - Nanoparticle-mediated deep brain stimulation

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8.1 Status

Deep brain stimulation (DBS) is an effective and standard treatment for a range of neurological disorders, most commonly for Parkinson's disease and essential tremor. Clinical DBS implants require invasive surgery, and can be associated with various risks due to the large implanted devices. Recently, in order to mitigate these risks, interest in smaller, less invasive methods for stimulation have led to a focus on the use of nanoparticles. Various materials have been explored for these nanoparticles, which can act as transducers of external stimuli such as magnetic fields, ultrasound, or light. In addition, considerable research has been conducted in less invasive nanoparticle delivery routes, such as through the blood-brain barrier (BBB) and via cell- or tissue-specific nanoparticle surface modifications. As nanoparticles used specifically for DBS is a rather new and less explored field, this section will address nanomaterials used for neuronal stimulation, that may be applied to DBS in the future.

8.2 Current and Future Challenges

In clinical DBS, an electrode in the deep brain is wired under the skin to a battery-powered device that provides electrical signals for neural stimulation. Despite the long history and success of clinical DBS, these devices are susceptible to hardware damage and infection risk, and thus often require corrective surgery [1]. The battery, which is implanted under the skin, must also be replaced at regular intervals. As a result, fewer patients undergo this treatment than who would be medically suitable for it [2, 3]. To overcome the drawbacks of a larger implanted electrode, new strategies have been presented in preclinical research in recent years in the form of nanoparticle-mediated neuromodulation.

A common strategy in the earliest nanoparticle neuromodulation technologies is to utilize genetic engineering to introduce heat-, mechano-, or light-responsive ion channels into neurons. Magnetothermal stimulation was among the earliest of these, first showing stimulation of deep brain tissue in mice using 22 nm magnetic nanoparticles and heat-sensitive ion channel TRPV1. To enhance colloidal stability and improve biocompatibility nanoparticles were coated with poly(acrylic acid) and poly(ethylene glycol) (PEG) [4] Using this approach, magnetothermal DBS has also shown alleviation of Parkinson's disease symptoms in mice [5]. Magnetothermal stimulation can also be used for neuronal suppression. Using superparamagnetic $MnFe_2O_4$ - $CoFe_2O_3$ core-shell nanoparticles (12.9 ± 1.4 nm) to provide heating, Munshi *et al.* inserted a temperature sensitive chloride channel, which when heated, causes Cl^- influx and hyperpolarization with 2 s latency(Figure 1c). To improve suspension properties nanoparticles were coated with poly-isobutylene-maleic anhydride which could be futher functionalized with required macromolecules [6].

Mechanosensitive ion channel introduction also enables remote magnetic stimulation, but via the application of mechanical force. Ferromagnetic nanoparticle "m-Torquer" with an overall dimension of 500 nm was used to remotely apply torque to a Piezo-1 mechanosensitive cation channel *in vivo,* as verified by c-fos staining. Piezo-1 was modified with a Myc-tag, and m-Torquer was PEGylated and conjugated with an anti-Myc antibody to enable force transduction (Figure 1a, b) [7] Another mechanosensitive ion channel, TRPV4, which is naturally expressed in dorsal root ganglion (DRG) cells, was used for transduction of neural stimulation via torque from ferrimagnetic iron oxide nanodiscs with sizes varying from 98-226 nm (MNDs). These nanodiscs were modified with oleic acid which was

functionalized with amphiphilic poly(maleic anhydride-alt-1-octadecene) for better colloidal stability. MNDs use the transition from vortex to in-plane magnetization, such that they have a near-zero net magnetic moment in the absence of a magnetic field, and therefore less risk of aggregation. Despite the advantages of using endogenous ion channels and MNDs, one drawback of this approach highlighted by the authors was the difficulty of delivery of MNDs due to their substantial size [8].

Another strategy in neural modulation is nanoparticles that use light to stimulate the brain. The photoelectric approach uses light as the input source of energy, and is converted into electrical signals by nanomaterials [9, 10]. Conversely, in optogenetics, light is the stimulus signal (i.e. transmitted from the nanoparticles to neurons), which requires genetic transduction to express light-sensitive ion channels in cells [11]. The spatial selectivity of light enables even single neuron resolution. The disadvantage, however, is that the penetration depth of the light often does not reach the areas of the brain that need to be targeted for DBS.

Overall, wireless signal transduction via nanoparticles allows flexibility in input energy sources to produce neural stimulation. However, nanoparticles have their own challenges, which need to be overcome. Additionally, studies in primates, and long-term studies have yet to be conducted, which will be necessary for successful to translation of nanoparticle DBS therapy to humans.

8.3 Advances in Science and Technology to Meet Challenges

The earliest technologies in nanoparticle-based neural stimulation opened up a field in neuroengineering which had previously been reserved only for centimeter-scale, battery-operated, and surgically-implanted devices. While the translational potential of some early nanoparticle technologies is limited, we will now present the progress that has been gained in recent years.

Upconversion nanoparticles (UCNP) can be used to overcome the limited penetration depth of light with visible or ultraviolet wavelengths. This was achieved by Yadav *et al*. by doping 39 ± 1.5 nm silica nanoparticles with lanthanides (Yb^{3+}, Tm^{3+}) that absorb light in the near-infrared region. Longer input wavelengths can thus reduce power loss with increasing tissue penetration depth (Figure 1d). However, this technology still required transduction with the light-sensitive ion channel channelrhodopsin-2 (ChR2) [11]. Also, while longer, infrared wavelengths may extend penetration depths, this is still insufficient to reach deep brain tissue from outside the human skull.

An approach also based on light as an input stimulus, which does not rely on genetic modification, was developed by using semiconducting 280.5 nm silicon nanowires (SiNWs). Here, SiNWs were stimulated with a focused light beam after spontaneous uptake into cultured oligodendrocytes. Photoelectric stimulation of the oligodendrocytes led to downstream electric stimulation of cocultured DRGs (Figure 1e) [9]. In this case, it was shown that the intentional uptake of the nanoparticles into the cells did not affect viability, did not interfere with cell mitosis, and did not affect the functionality of the nanoparticles [9]. Whether this is also the case with other nanoparticles where uptake is unintended remains to be elucidated, as well as if uptake is advantageous in electrophysiological stimulation.

Another example of photoelectric stimulation, in which light is converted into an electrical stimulus, is the use of conjugated polymer nanoparticles (P3HT NPs) in the retina. Using a rat model of degenerative retinal disease, it was shown that spared retinal neurons could be stimulated by light using these nanoparticles which have a diameter of 300 nm. The effect lasted up to 8 months after a single injection, restoring the response to visual stimuli as well as cortical and subcortical activity. [10]

Like all light stimulation-based technologies, the disadvantage of using SiNWs and P3HT NPs for DBS is the loss of power with increasing penetration depth into the deep brain, as light needs to be delivered in close proximity of the desired target for stimulation. However, the longevity of P3HT NPs

still provides some insight with regard to long-term functionality of nanostimulators. Not only was functionality proven eight months after the first injection, but also the nanoparticle distribution did not quantitatively change, and thus did not get cleared over time (Figure 2 a,b) [10]. The previously described magnetothermal approach by Chen *et al.* was also able to confirm their functionality one month after nanoparticle injection [4].

Ultrasound-induced electrical stimulation transduced by piezoelectric materials is a growing technology in neuroengineering, but new and much less well-studied at the nanoscale. A dual targeting system, consisting of 300 nm barium titanate nanoparticles (BTNPs) coated with a copolymer of a phosphatidylethanolamine and PEG coupled with an antibody against the transferrin receptor, enabled crossing of a BBB model, and selective cell uptake in glioblastoma cells (Figure 2c, d) [12]. As the BTNPs have demonstrated neuronal stimulation previously, [13] they could be used in the future as intravenously injectable neural stimulators. This surface modification approach also provides insight into how other nanostimulators can be transported across the BBB to provide a less invasive method of nanoparticle delivery. In addition, this demonstrates a possibility for specific stimulation of individual cell or tissue types that does not rely on prior genetic modification of the cells being stimulated [4, 6-8, 11, 12].

Similarly, magnetoelectric (ME) neurostimulator materials are a growing field, and also under-studied at the nanoscale. Like BTNPs, magnetoelectric materials use piezoelectric transduction, but in combination with magnetostrictive materials, in order to achieve magnetic-to-electric signal transduction. ME materials have recently demonstrated by stimulation of the subthalamic region through nanoparticles of size 224-27nm in mice, which promoted behavioral change (Figure 1f, g) [14].

An *in vivo* study with live mice as a new approach to solve depressive symptoms employed not a continuous magnetic field, but instead exposure to pulses at a low (10 Hz) frequency. This was conducted with superparamagnetic iron oxide nanoparticles, injected into the left pre-limbic cortex. These nanoparticles were coated with polyglucose sorbitol carboxymethylether and had dimensions of 9 and 30 nm. The combined mechanisms/effects increased the susceptibility of the cell membrane under pulsed stimulation. Using this mechanism, symptoms of depression in mice improved, with the therapeutic effect demonstrated using various biomarkers. [15]

Overall, the new but growing interest in less invasive neuromodulation has provided a good starting point for the field of nanoscale neural stimulators. Nonetheless, some technologies are only beginning to be understood, and further research is needed to address unanswered questions. Among these is the selective stimulation of single neurons or tissue types without genetic modification, which has so far achieved only early findings. Research is also needed to determine what, if any, effects there are on other areas of the brain away from the stimulation site, and what the physiological side effects are. In addition, research is being conducted on less invasive methods of nanoparticle delivery beyond injection, such as through the BBB.

The long-term effects of nanoscale neurostimulators in the brain are not yet well studied. To date, there have been few studies on how the distribution of nanoparticles changes, or whether the nanomaterials degrade or lose function over time. In addition, it is often unclear what, if any, options are available to remove nanoparticles if necessary. Further research is also needed on whether nanoparticles are taken up by cells over time, and what the consequences of this are.

8.4 Concluding Remarks

The need for less invasive methods of neural stimulation has driven the relatively new field of nanoparticles for DBS. Injectable nanoparticles have to potential to significantly reduce the adverse effects of invasive devices that typically require wires and batteries. At the same time, their use brings

a number of challenges, ranging from synthesis to long-term viability. Presumably, there will be more than one technique for translational applications in the future, adapted to the needs of patients and the goals of therapy.

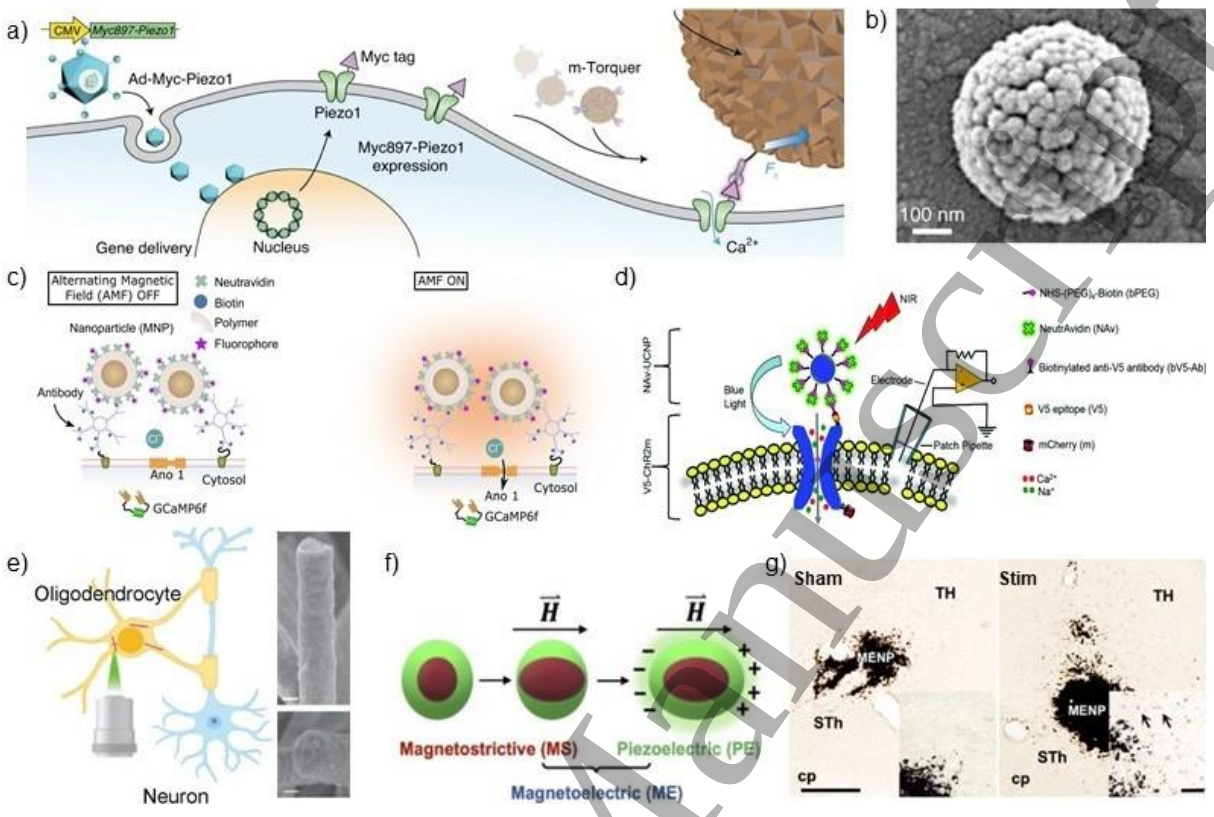

Figure 1: Mechanisms of nanoparticle-mediated neuronal stimulation. a) Schematic representation of the m-Torquer system, in which mechanosensitive channel Piezo1 is expressed in neurons. m-Torquer binds to Piezo1 and activates it after a magnetic field is applied. b) SEM image shows the m-Torquer consists of several spherically connected magnetic nanoparticles. c) Illustration of magnetothermal silencing. Nanoparticles bind to neurons via a biotin-avidin bond. When a magnetic field is applied, they heat up and open ion channel Ano1. d) UCNPs bind to neurons via ChR2. The UCNPs convert NIR to blue light, which activates ChR2. e) Left: shows stimulation of SiNWs in oligodendrocytes via light and transmission of the electrical signal to the DRGs. Right: shows SEM images of SiNWs. Scale bar: 100nm f) Schematic representation of magnetoelectricity, which can convert magnetic energy into electrical energy. g) shows staining of c-fos around the injection site of magnetoelectric nanoparticles (MENPs) without (left) and with (right) previous stimulation. After stimulation (right) shows more c-fos positive neurons. Reproduced with permission [6, 7, 9, 11, 14].

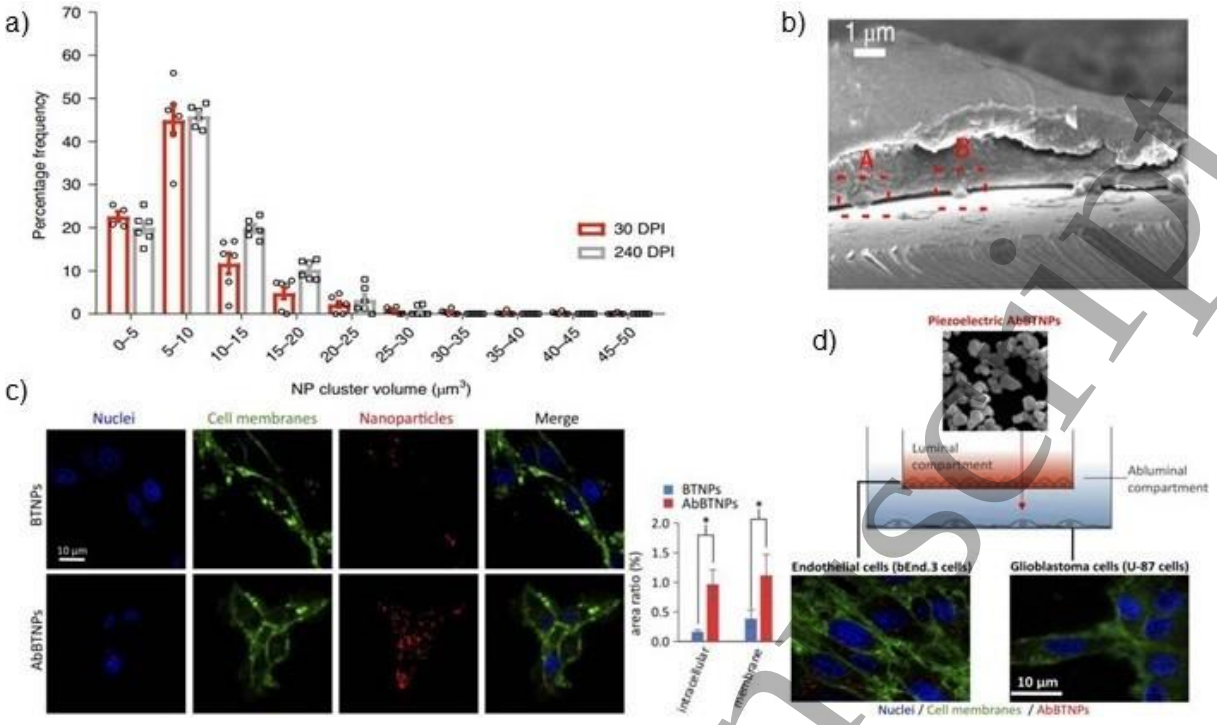

Figure 2: Advances in nanoparticle-mediated stimulation. a) Shows the distribution of P3HT NPs in the retina 30 and 240 days after injection. The nanoparticles are not cleared and are fully functional b) SEM images of P3HT-NPs with primary neurons shows close contact of the nanoparticles to the neurons without uptake. c) Left: Representative images of glioblastoma cells with nanoparticles after they have crossed a transwell BBB model. Top row shows barium titanate nanoparticles (BTNP) without antibody modification. Bottom row with antibody modification for specific binding to glioblastoma cells. Right: shows quantitative analysis of the images on the left. d) shows schematic representation of the BBB model. Nanoparticles migrated through a luminal compartment with endothelial cells into the abluminal compartment to glioblastoma cells. Reproduced with permission [10, 12]

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References

[1] J. D. Rolston, D. J. Englot, P. A. Starr, and P. S. Larson, "An unexpectedly high rate of revisions and removals in deep brain stimulation surgery: analysis of multiple databases," *Parkinsonism & related disorders,* vol. 33, pp. 72-77, 2016.

[2] M. Lange *et al.*, "Underutilization of deep brain stimulation for Parkinson's disease? A survey on possible clinical reasons," *Acta neurochirurgica,* vol. 159, no. 5, p. 771, 2017.

[3] M.-R. Kim *et al.*, "Patients' reluctance to undergo deep brain stimulation for Parkinson's disease," *Parkinsonism & Related Disorders,* vol. 23, pp. 91-94, 2016/02/01/ 2016, doi: https://doi.org/10.1016/j.parkreldis.2015.11.010.

[4] R. Chen, G. Romero, M. G. Christiansen, A. Mohr, and P. Anikeeva, "Wireless magnetothermal deep brain stimulation," *Science,* vol. 347, no. 6229, pp. 1477-1480, 2015, doi: doi:10.1126/science.1261821.

[5] S.-A. Hescham *et al.*, "Magnetothermal nanoparticle technology alleviates parkinsonian-like symptoms in mice," *Nature Communications,* vol. 12, no. 1, p. 5569, 2021/09/22 2021, doi: 10.1038/s41467-021-25837-4.

[6] R. Munshi, S. M. Qadri, and A. Pralle, "Transient Magnetothermal Neuronal Silencing Using the Chloride Channel Anoctamin 1 (TMEM16A)," (in English), *Frontiers in Neuroscience,* Original Research vol. 12, no. 560, 2018-August-14 2018, doi: 10.3389/fnins.2018.00560.

[7] J.-u. Lee *et al.*, "Non-contact long-range magnetic stimulation of mechanosensitive ion channels in freely moving animals," *Nature Materials,* vol. 20, no. 7, pp. 1029-1036, 2021/07/01 2021, doi: 10.1038/s41563-020-00896-y.

[8] D. Gregurec *et al.*, "Magnetic Vortex Nanodiscs Enable Remote Magnetomechanical Neural Stimulation," *ACS Nano,* vol. 14, no. 7, pp. 8036-8045, 2020/07/28 2020, doi: 10.1021/acsnano.0c00562.

[9] M. Y. Rotenberg *et al.*, "Silicon Nanowires for Intracellular Optical Interrogation with Subcellular Resolution," *Nano Letters,* vol. 20, no. 2, pp. 1226-1232, 2020/02/12 2020, doi: 10.1021/acs.nanolett.9b04624.

[10] J. F. Maya-Vetencourt *et al.*, "Subretinally injected semiconducting polymer nanoparticles rescue vision in a rat model of retinal dystrophy," *Nature Nanotechnology,* vol. 15, no. 8, pp. 698-708, 2020/08/01 2020, doi: 10.1038/s41565-020-0696-3.

[11] K. Yadav *et al.*, "Targeted and efficient activation of channelrhodopsins expressed in living cells via specifically-bound upconversion nanoparticles," *Nanoscale,* 10.1039/C7NR03246C vol. 9, no. 27, pp. 9457-9466, 2017, doi: 10.1039/C7NR03246C.

[12] A. Marino *et al.*, "Piezoelectric barium titanate nanostimulators for the treatment of glioblastoma multiforme," *Journal of Colloid and Interface Science,* vol. 538, pp. 449-461, 2019/03/07/ 2019, doi: https://doi.org/10.1016/j.jcis.2018.12.014.

[13] C. Rojas *et al.*, "Acoustic stimulation can induce a selective neural network response mediated by piezoelectric nanoparticles," *Journal of neural engineering,* vol. 15, no. 3, p. 036016, 2018. [Online]. Available: https://iopscience.iop.org/article/10.1088/1741-2552/aaa140/pdf.

[14] K. L. Kozielski *et al.*, "Nonresonant powering of injectable nanoelectrodes enables wireless deep brain stimulation in freely moving mice," *Science Advances,* vol. 7, no. 3, p. eabc4189, 2021, doi: doi:10.1126/sciadv.abc4189.

[15] Q.-B. Lu *et al.*, "Magnetic brain stimulation using iron oxide nanoparticle-mediated selective treatment of the left prelimbic cortex as a novel strategy to rapidly improve depressive-like symptoms in mice," (in eng), *Zool Res,* vol. 41, no. 4, pp. 381-394, 2020, doi: 10.24272/j.issn.2095-8137.2020.076.

Section 9 - Application of nanotechnologies in the treatment of neuroinflammation in neurodegenerative disorders

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Status

Neuroinflammation is critically involved in the central nervous system (CNS)-related diseases including neurodegenerative disorders like Alzheimer's (AD), Parkinson's (PD) and Huntington's (HD) diseases, Multiple sclerosis (MS) or Amyotrophic lateral sclerosis (ALS) [1]. The process endorses the involvement of the innate immune responses by glial resident cells (i.e. microglia and astrocytes) and the production of pro-inflammatory cytokines including IL1β, IL-6, IL18 and TNF-α, and chemokines such as C-C motif chemokine ligand 1 (CCL1), CCL5 and C-X-C motif chemokine ligand 1 (CXCL1), but also small-molecule messengers, including nitric oxide (NO), and reactive oxygen species (ROS) [1]. Neuroinflammation may also involve during disease path the transfer of antigen-experienced peripheral immune cells into the CNS ensuring adaptive immune responses (Figure 1). The specificity of the CNS is the existence of protective walls (i.e. blood-brain barrier: BBB, and the blood-cerebrospinal fluid barrier) that prevent neurons from external insults. However, these barriers likely impede the access of therapeutic agents to the brain. Advances in drug delivery design have focused on nanotechnology-based approaches with the aim not only to improve drug targeting to the brain, but also to increase the bioavailability and pharmacokinetics relative to free drugs. To date, several nanomaterials have been employed including organic (e.g. liposomes, dendrimers, polymeric NPs, micelles, nanogels, extracellular vesicles, and red blood cell membranes,), inorganic (e.g. metal nanostructures, magnetic nanoparticles, and quantum dots), and carbon-based (e.g. graphene and carbon nanotubes) materials [2].

Noticeably, nanoparticles (NPs) size, type and hydrophobicity will determine their biological fate, toxicity, distribution and targeting ability [2]. Several NPs have been used as carriers for drugs (i.e. curcumin, okadaic acid, quercetin, anthocyanin, and levodopa), increasing their bioavailability in the brain and thereby modulating neuroinflammatory responses and the release of pro-inflammatory cytokines. Moreover, inorganic NPs themselves have shown therapeutic benefit eliciting an anti-inflammatory phenotype of microglia. Besides, recent studies have highlighted the use of chimeric small extracellular vesicles (EVs), conjugated to nanoparticles, biomolecules and drugs, both as biomarkers and for therapeutic purposes in diseases. Among various therapeutic strategies, nanotechnologies targeting neuroinflammation represents a promising approach to improve neurodegenerative diseases conditions. Although, precautions have to be made in data interpretation obtained with NPs in experimental cellular and animal study models before their application in clinical studies.

Current and Future Challenges

Nanomaterials have been largely employed to alleviate neuroinflammation in CNS diseases (Figure 1). Lipid-based NPs used as carrier of curcumin attenuates neuroinflammatory and reactive gliosis in astroglia cells and organotypic brain slices. Interestingly, curcumin- and sesamol-loaded solid lipid nanoparticles provide anti-inflammatory, neuroprotective and behavioural outcomes in symptomatic AD-like mice [3-5]. Polymer-based NPs have also shown some beneficial effects towards neuroinflammation in cellular study models and in a PD-like mice model that occurs through a downregulation the NF-κB signaling pathway and the inhibition of lipid peroxidation [6]. Particularly, a novel synthetic nanoparticle, poly (lactide-co-glycolide)-block-poly (ethylene glycol) provided efficient brain targeting and beneficial learning and memory abilities in an AD mice model when

conjugated with B6 peptide and loaded with curcumin [7]. Synthetic nanoparticles have also been applied in epilepsy and cerebral ischemic reperfusion injury (IR) injury mouse models showing a reduction of pro-inflammatory markers and gliosis [8, 9]. In addition, some studies have reported the potential of NP dendrimers-mediated drug delivery to alleviate neuroinflammation *in vitro* and in cerebral palsy and in AD *in vivo* study models [10, 11]. However, the benefit-cost of dendrimers is high compared to linear polymers and their toxicity has been raised in some studies. Several evidences reported that inorganic NPs alone or modified drugs provides anti-inflammatory effects *in vitro* and *in vivo* models of neurodegenerative diseases. *Ex vivo* experiments have shown that L-DOPA conjugated inorganic NPs increases their transport across brain endothelial monolayers and are readily taken up by brain macrophages without inflammatory effects. At most interest, antocyanine carried PEG coated inorganic NPs reduced the levels of amyloid beta (Aβ) and BACE-1 in $A\beta_{1-42}$-injected mice brains and treated microglia cells *in vitro*. Moreover, they stimulated p-GSK-$3\beta^{Ser9}$/p-CDK5 signaling, reduced the microgliosis and astroglyosis and the level of pro-inflammatory markers including p-NF-*k*B, iNOS, TNFα, and IL-1β *in vivo* [12]. These observations were further supported by another study demonstrating that inorganic NPs may increase the level of anti-inflammatory IL- 4 in a drug-induced AD mice model [13]. Besides neurodegenerative diseases, the combination of organic NPs and n-acetylcysteine (NAC) also decreased pro-inflammatory cytokines production *in* sepsis-induced brain dysfunction in rats [14]. Recently developed NPs based on graphene quantum dots are considered a promising therapeutic approach to alleviate neuroinflammation as demonstrated in experimental autoimmune encephalomyelitis via the activation of MAPK/Akt signaling, and in PD and AD study models through the disaggregation of α-synuclein and $A\beta_{1-42}$ peptides respectively [2].

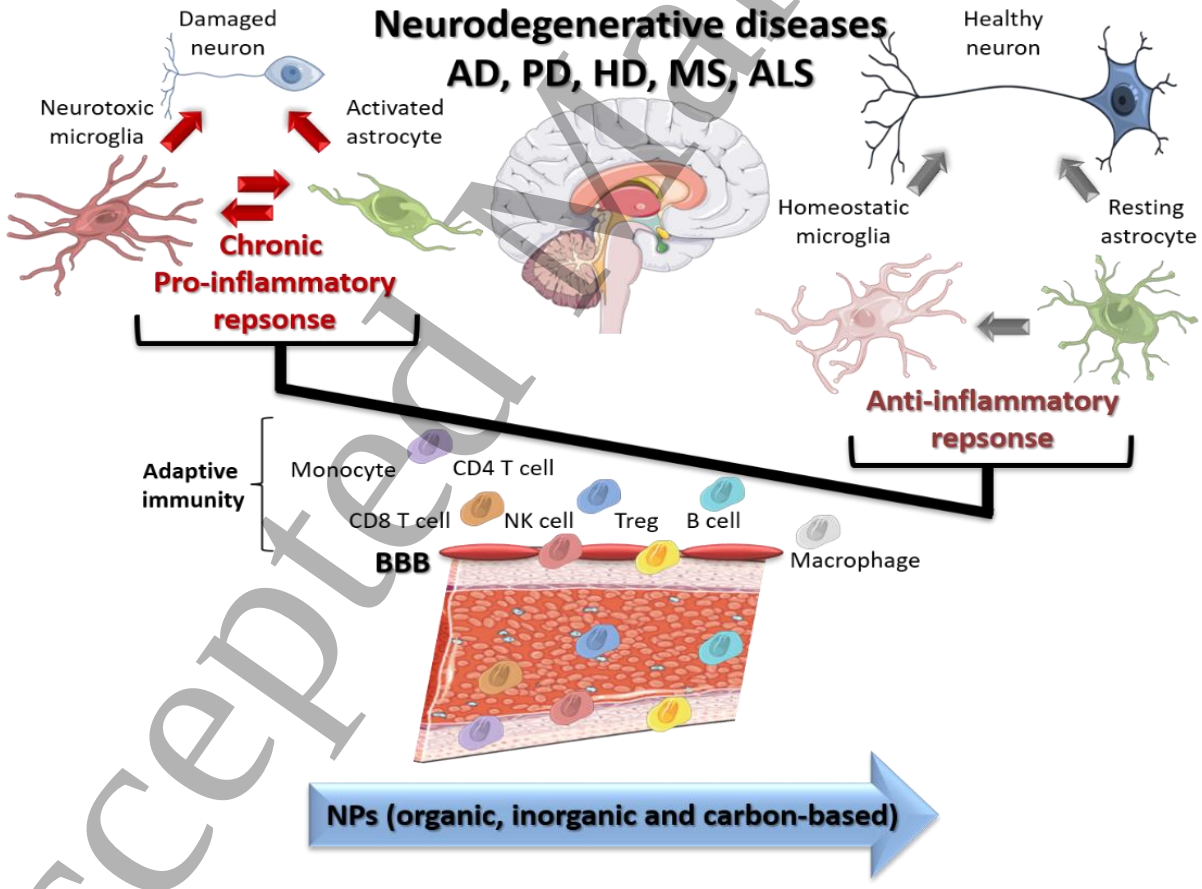

Figure 1. Scheme summarizing innate and adaptive immune response in neurodegenerative diseases. NPs facilitate drug delivery to the brain modulating the function of central neuroinflammatory cells and preserving neuronal health.

Advances in Science and Technology to Meet Challenges

Besides being involved in several neurodegenerative diseases, neuroinflammation is common to several psychiatric disorders including major depression, anxiety, post-traumatic stress, and bipolar disorders. Thus, advances in drugs targeting brain inflammation remain a scientific challenge that will benefit to a large set of CNS diseases. NPs are increasingly recognized as a valuable therapeutic approach to alleviate deleterious chronic neuroinflammation in animal CNS disease study models. However, many NPs have been reported to exhibit neurotoxicity and pro-inflammatory responses. Therefore, it is imperative to develop better nanotechnologies, and to have a better mechanistic understanding of nanomaterials. Conjugating NPs with specific antigens/peptides may also be envisioned to target a specific set of CNS inflammatory cells (i.e. microglia or astrocytes). Similarly, loading NPs with existing drugs/small molecules is another avenue to pursuing and gaining better pharmacokinetic properties (i.e. a long half-life, better target specificity, high lipophilicity) and BBB penetrability. Recent studies reported the potential use of EVs for treating brain cancer. In fact, EVs have the advantage being biocompatible and may be considered immunologically inactive and able to cross the BBB. However, it is still somewhat premature to predict the therapeutic applications by modified-EVs as drug delivery systems for CNS diseases. Recent studies have highlighted that peripheral myeloid cells may play a fundamental role in CNS diseases development. Data obtained in animal models have demonstrated the beneficial effects in targeting peripheral myeloid cells in AD, PD and MS. This includes blockade of their migration to the CNS, modulation of their biological functions, immunological activity and cytokine production. Thus, NPs represent an exciting new tool for regulating myeloid cell functions, likely impacting pro-inflammatory response in the CNS.

Concluding Remarks

Neurodegenerative disorders still pose several therapeutic challenges. The development of nanotechnologies has improved the targeting of therapeutic agents to brain forcing the brain barriers, enhancing the bioavailability and pharmacokinetics of drugs. Moreover, nanotechnologies, able to recognize specific brain receptors or transporters, favour the selective release of drugs to the target site, reducing side effects and systemic exposure. NPs guided delivery of therapeutic agents has demonstrated benefits alleviate chronic neuroinflammation in preclinical study models. Looking forward, the regulatory process for nanotechnology should consider both the risk and the reward in a balanced manner, to enable translation of nanomedicine from bench to bedside in the CNS disorders application. The short-term objective will be to prove the efficiency of nanotechnologies in pre-clinical experimental models with pathophysiological similarities closer to human inflammatory disease.

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References

[1] M. T. Heneka, M. P. Kummer, and E. Latz, "Innate immune activation in neurodegenerative disease," *Nat Rev Immunol,* vol. 14, no. 7, pp. 463-77, Jul 2014.

[2] D. Kim, K. Shin, S. G. Kwon, and T. Hyeon, "Synthesis and Biomedical Applications of Multifunctional Nanoparticles," *Adv Mater,* vol. 30, no. 49, p. e1802309, Dec 2018.

[3] P. Maiti, L. Paladugu, and G. L. Dunbar, "Solid lipid curcumin particles provide greater anti-amyloid, anti-inflammatory and neuroprotective effects than curcumin in the 5xFAD mouse model of Alzheimer's disease," *BMC Neurosci,* vol. 19, no. 1, p. 7, Feb 23 2018.

[4] A. K. Sachdeva, S. Misra, I. Pal Kaur, and K. Chopra, "Neuroprotective potential of sesamol and its loaded solid lipid nanoparticles in ICV-STZ-induced cognitive deficits: behavioral and biochemical evidence," *Eur J Pharmacol,* vol. 747, pp. 132-40, Jan 15 2015.

[5] M. Abdul-Ghani, P. Y. Gougeon, D. C. Prosser, L. F. Da-Silva, and J. K. Ngsee, "PRA isoforms are targeted to distinct membrane compartments," (in eng), *J Biol Chem,* vol. 276, no. 9, pp. 6225-33, Mar 2 2001.

[6] N. Nehal *et al.*, "Chitosan coated synergistically engineered nanoemulsion of Ropinirole and nigella oil in the management of Parkinson's disease: Formulation perspective and In vitro and In vivo assessment," *Int J Biol Macromol,* vol. 167, pp. 605-619, Jan 15 2021.

[7] S. Fan *et al.*, "Curcumin-loaded PLGA-PEG nanoparticles conjugated with B6 peptide for potential use in Alzheimer's disease," *Drug Deliv,* vol. 25, no. 1, pp. 1091-1102, Nov 2018.

[8] A. Cano *et al.*, "Epigallocatechin-3-gallate loaded PEGylated-PLGA nanoparticles: A new anti-seizure strategy for temporal lobe epilepsy," *Nanomedicine,* vol. 14, no. 4, pp. 1073-1085, Jun 2018.

[9] X. Yun, V. D. Maximov, J. Yu, H. Zhu, A. A. Vertegel, and M. S. Kindy, "Nanoparticles for targeted delivery of antioxidant enzymes to the brain after cerebral ischemia and reperfusion injury," *J Cereb Blood Flow Metab,* vol. 33, no. 4, pp. 583-92, Apr 2013.

[10] Z. Zhang *et al.*, "Systemic dendrimer-drug nanomedicines for long-term treatment of mild-moderate cerebral palsy in a rabbit model," *J Neuroinflammation,* vol. 25, no. 17 (1), p. 319, 2020.

[11] A. Singh *et al.*, "Formulation development of tocopherol polyethylene glycol nanoengineered polyamidoamine dendrimer for neuroprotection and treatment of Alzheimer disease," *J Drug Target,* vol. 30, no. 7, pp. 777-791, 2022.

[12] M. J. Kim, S. U. Rehman, F. U. Amin, and M. O. Kim, "Enhanced neuroprotection of anthocyanin-loaded PEG-gold nanoparticles against Abeta1-42-induced neuroinflammation and neurodegeneration via the NF-KB /JNK/GSK3beta signaling pathway," *Nanomedicine,* vol. 13, no. 8, pp. 2533-2544, Nov 2017.

[13] N. Dos Santos Tramontin *et al.*, "Gold Nanoparticles Treatment Reverses Brain Damage in Alzheimer's Disease Model," *Mol Neurobiol,* vol. 57, no. 2, pp. 926-936, Feb 2020.

[14] F. Petronilho *et al.*, "Gold nanoparticles potentiates N-acetylcysteine effects on neurochemicals alterations in rats after polymicrobial sepsis," *J Drug Target,* vol. 28, no. 4, pp. 428-436, Apr 2020.

Section 10 - Nanoparticle-mediated immune therapy for the central nervous system

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10.1 Status

The difficulty to safely and precisely release drugs in the diseased central nervous system (CNS) limits the outcome of the current treatments. CNS immunotherapy aims at modulating the activity of the immune system to recognize and treat diseased tissues and neuropathological conditions. In this scenario, smart nanomaterials can be designed to target specific cell types, cross biological barriers, and modulate the pathologic microenvironment. The passive immune nanomedicines expose or release traditional immunotherapy agents (*e.g.*, antigens and cytokines) while the active ones (*e.g.*, iron oxide nanoparticles and aAPC nanoconjugates) are designed to directly boost the host immunoresponse [1]. Nano-enabled CNS immunotherapy primarily finds application in counteracting brain cancer but also in treating neurodegenerative conditions, such as Alzheimer's disease (AD), and spinal cord injuries [2].

In brain cancer, immune nanomedicines are formulated to teach the innate immune system to target and attack cancer cells. The approach involves the activation of the cytotoxic T cells by the dendritic cells (DC). Scarcely immunogenic brain tumors like glioblastoma multiforme (GBM) show multiple protection systems to resist the immune system attacks (*e.g.*, the immune checkpoint –ICP- ligands). Different therapeutic nanomedicines are currently focused to overcome this challenge [3].

Nano-enabled immunotherapy has been also recently applied to trigger an antibody response against the amyloid-beta (Aβ; **Figure 1**) [4]. In the brain of AD patients, abnormal deposition of this peptide and its accumulation in the amyloid plaques are observed. Researchers proposed innovative nanomaterials able to recruit microglia and promote Aβ phagocytosis in APPswe/PS1dE9 mice, therefore improving neuronal functionality, decreasing inflammation, and rescuing memory. This pioneering approach of "Aβ cleaning", despite is still at an early stage of development, represents an innovative strategy for AD therapy. In the future, alternative immune nanomedicines can be developed to reduce the neural toxicity associated with the accumulation of the tau protein in AD and α-synuclein in Parkinson's disease.

10.2 Current and Future Challenges

In neuro-oncology, the treatment of glioblastoma multiforme (GBM) with immunotherapy remains a huge challenge due to its elevated grade of heterogeneity and scarce immunogenicity.

GBM is defined as an immunologically "cold" tumor microenvironment (TME) characterized by a reduced T cell activity and infiltration capability. Likely other "cold" tumors, the outcomes of the immunotherapy against GBM with antibodies targeting ICPs are poor in most cases [5]. This represents a relevant therapeutic challenge. Nanomaterials can be specifically designed to address this obstacle, finely modulating the TME and promoting the expansion/engagement of T cells [6]. Furthermore, the multifunctionality of smart nanomedicines allows to efficiently cross the blood-brain tumor barrier (BBTB), promote the release of immunomodulators or chemotherapy drugs, remotely stimulate the tumor tissue, and absorb tumor antigens upon tumor damage for priming immune response.

Another fundamental challenge is associated with the elevated interindividual heterogeneity in immunoresponse. The high variability in therapeutic outcome derives not only from the specific molecular characteristics of the tumor (*e.g.*, the levels of the tumor mutational burden and the pattern of the neoantigen expression) but also from extrinsic interindividual differences. In this regard, an

important role is mediated by the microbiota diversity, hormonal-related sex-specific peculiarities, and the aging of the immune system [7]. The use of nanoparticles for immunotherapy increases the level of complexity of the therapy approach, amplifying the variability of the treatment responses. As an example, female patients show a faster plasma clearance rate when treated with doxorubicin-loaded liposomes compared to males. Sex-related differences in nanoparticle-cell interaction may be attributed to hormonal levels. In this regard, recent studies are showing as hormonal concentrations affect the nanoparticle internalization in cancer cells [8].

Finally, the adverse effects of the adopted nanomedicines on the immune cells should be carefully evaluated. The active or passive modulation of the immune system can indeed change the cytotoxicity response to a specific nanomaterial [7]. Also, the nano-enabled immunotherapy can induce the release of inflammatory cytokines and, as a consequence, induce side effects to distal organs[9].

10.3 Advances in Science and Technology to Meet Challenges

Advances in science revealing in detail the interactions between nanoagents and the immune cells will be required. Future studies will have to take into account and highlight the differences in immunotherapeutic response due to sex, age, hormone levels, and specific molecular characteristics of the disease (*e.g.*, brain cancer and AD). The experimental design will have to adequately consider the abovementioned aspects [7]. Furthermore, the neurotoxicity of immune nanomedicines will have to be thoroughly evaluated, preferring *in vitro* the adoption of highly biomimetic systems, such as the 3D brain-on-chip models incorporating immune cells. Basic research evaluating the effects of nanomaterial morphology, size, and composition on immune cell behavior still requires elucidations.

Improvements in the targeting specificity of the nanomaterials toward selected types of immune cells, cytokine receptor-expressing populations, and cancer cells are highly demanded to improve the therapy outcomes and reduce side effects. In addition to the multi-ligand functionalization strategies, a new trend is the nanomaterial surface modification with patient-derived cell membranes [9]. In this regard, nanoparticles camouflaged with cancer cell membranes display a dual function to selectively target cancer cells owing to homotypic membrane-membrane interactions [10] but also to expose tumor antigens and elicit the systemic anticancer immune response. First experimental proofs are revealing the positive role of this coating strategy in promoting the BBB crossing. Plasma membranes from different cell types, such as those from leukocytes, red blood cells, platelets, dendritic cells, and natural killer cells, have been exploited as a coating strategy for imparting specific functionalities to the nanoparticles. Improved performances and multifunctionality can be provided by exploiting hybrid membranes (*i.e.*, obtained by mixing membranes from different cell types) and genetically engineered cell membranes (**Figure 2**) [12]. The long-term safety, biodistribution, and excretion of these innovative nanomedicines should be carefully examined since the literature data in this regard are very limited.

Finally, to complement the function of immune nanomedicines in “cold” immunosuppressive TME, the development of new strategies promoting tumor recognition and infiltration by immune cells will be required.

10.4 Concluding Remarks

Immunotherapy, despite being primarily applied for cancer treatment, shows high potential also for AD, PD, and spinal cord injury. Nanomaterials represent a potential solution for most of the challenges of immunotherapy since they can be specifically designed to cross BBB/BBTB, target the diseased cells/microenvironment, and trigger a specific immune cell response. On the other hand, the use of nanomedicines increases the complexity of the therapeutic approach, amplifying the interindividual variability of the therapeutic response and limiting the clinical applicability in the short-term period. More sophisticated preclinical investigations on nanomedicine safety and future advancement in BBB-crossing and targeting technologies will pave the way for the clinical exploitation of different immune

nanomedicines. Although clinical studies on nano-enabled immunotherapy have not been applied to the treatment of CNS diseases yet, the approach is being tested in clinical trials for the treatment of other non-CNS tumors by using different nanoplatforms. Examples of such therapeutic nanoplatforms are the poly(lactic-co-glycolic acid) (PLGA) nanoparticles loaded with the invariant natural killer T cell activator threitolceramide-6 (ThrCer6, IMM60) plus the New York esophageal squamous cell carcinoma-1 (NY-ESO-1) cancer-testis antigen peptides (NCT04751786) and the high-density-lipoprotein-methylene diphosphonate nanoparticles (NCT05280379). Moreover, the first clinical study (phase I) with autologous total tumor mRNA-loaded liposomes for immunotherapy of specific types of newly diagnosed GBM is currently in the recruiting stage (NCT04751786). Results from these clinical trials will provide crucial information regarding the safety and efficacy of nano-enabled immunotherapy in humans, hopefully paving the way for successful clinical exploitation of this approach in the near future.

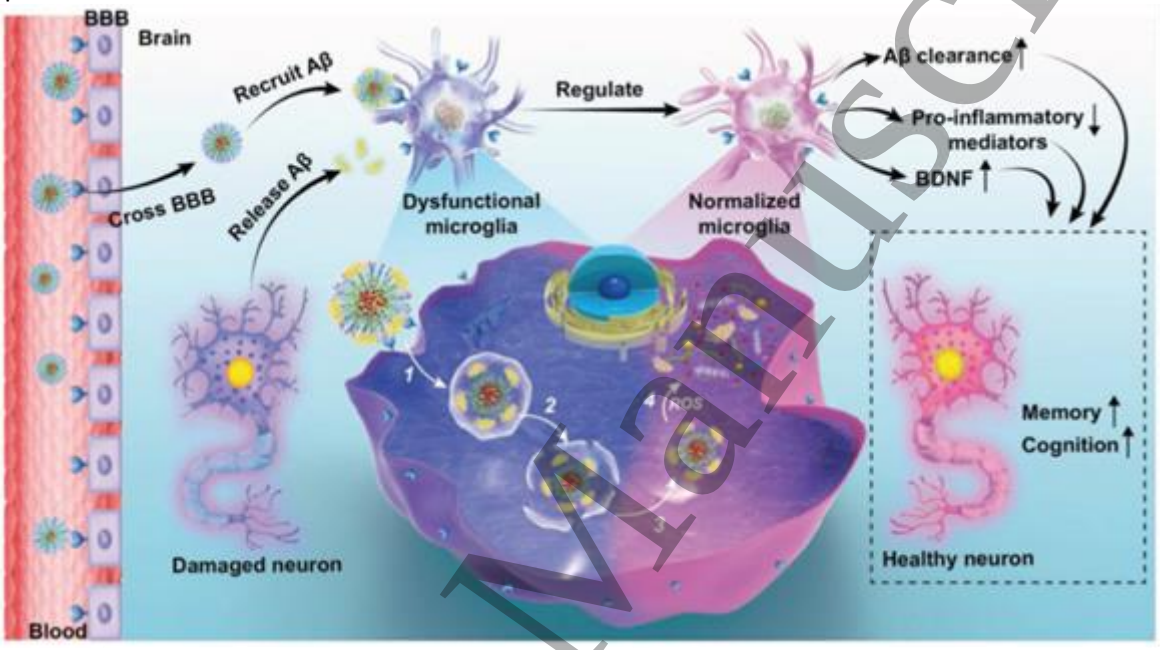

Figure 1 - Amyloid-β (Aβ) cleaning through nanoparticle-mediated modulation of microglia function in the APPswe/PS1dE9 mouse model of Alzheimer's disease (AD). Adapted from R. Liu et al. [4].

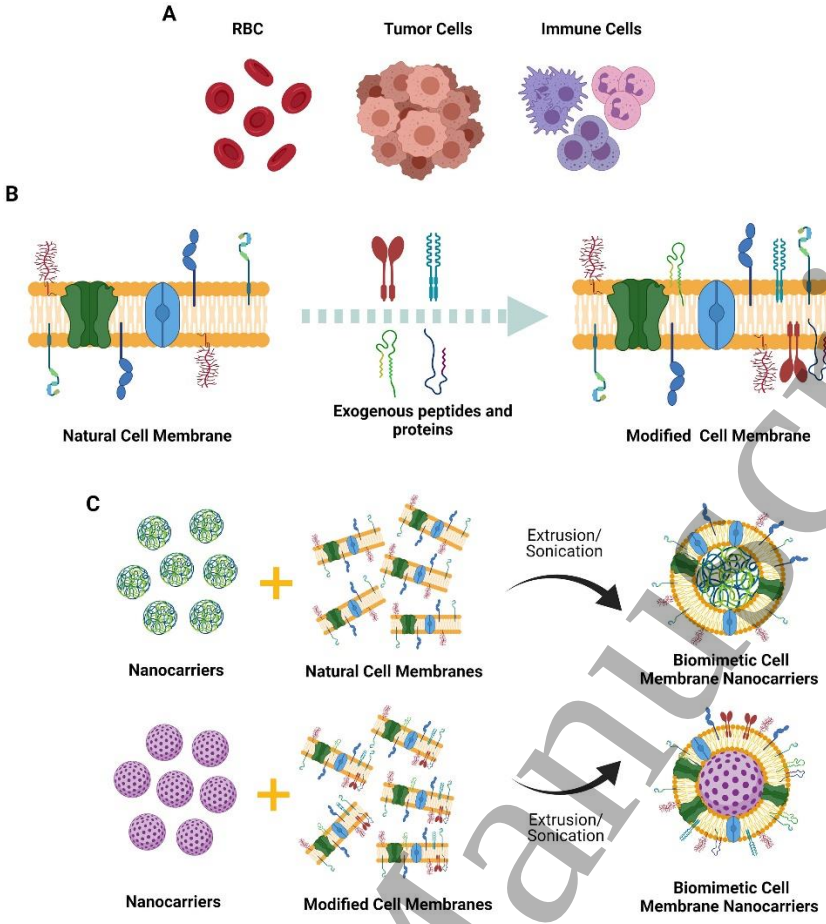

Figure 2 – Wild-type and modified cell membrane camouflaged nanoparticles. Adapted from Mendanha *et al.* [12].

10.5 References

[1] Z. Liu, W. Jiang, J. Nam, J. J. Moon, and B. Y. S. Kim, "Immunomodulating Nanomedicine for Cancer Therapy," *Nano Lett.*, vol. 18, no. 11, pp. 6655–6659, Nov. 2018, doi: 10.1021/ACS.NANOLETT.8B02340.

[2] S. Hanif, P. Muhammad, R. Chesworth, F.U. Rehman, R. jun Qian, M. Zheng, B. yang Shi, "Nanomedicine-based immunotherapy for central nervous system disorders," *Acta Pharmacol. Sin. 2020 417*, vol. 41, no. 7, pp. 936–953, May 2020, doi: 10.1038/S41401-020-0429-Z.

[3] H. Kinoh, S. Quader, H. Shibasaki, X. Liu, A. Maity, T. Yamasoba, H. Cabral, K. Kataoka, "Translational Nanomedicine Boosts Anti-PD1 Therapy to Eradicate Orthotopic PTEN-Negative Glioblastoma," *ACS Nano*, vol. 14, no. 8, pp. 10127–10140, Aug. 2020, doi: 10.1021/ACSNANO.0C03386.

[4] R. Liu, J. Yang, L. Liu, Z. Lu, Z. Shi, W. Ji, J. Shen, X. Zhang, "An 'Amyloid-β Cleaner' for the Treatment of Alzheimer's Disease by Normalizing Microglial Dysfunction," *Adv. Sci.*, vol. 7, no. 2, Jan. 2020, doi: 10.1002/ADVS.201901555.

[5] P. Bonaventura, T. Shekarian, V. Alcazer, J. Valladeau-Guilemond, S. Valsesia-Wittmann, S. Amigorena, C. Caux, S. Depil, "Cold tumors: A therapeutic challenge for immunotherapy," *Front. Immunol.*, vol. 10, no. FEB, p. 168, 2019, doi: 10.3389/FIMMU.2019.00168/BIBTEX.

[6] N. Gong, N. C. Sheppard, M. M. Billingsley, C. H. June, and M. J. Mitchell, "Nanomaterials for T-cell cancer immunotherapy," *Nat. Nanotechnol. 2021 161*, vol. 16, no. 1, pp. 25–36, 2021, doi: 10.1038/s41565-020-00822-y.

[7] W. Jiang, Y. Wang, J. A. Wargo, F. F. Lang, and B. Y. S. Kim, "Considerations for designing preclinical cancer immune nanomedicine studies," *Nat. Nanotechnol. 2020 161*, vol. 16, no. 1, pp. 6–15, 2020, doi: 10.1038/s41565-020-00817-9.

[8] C. Lara-Cruz, J.E. Jiménez-Salazar, M. Arteaga, M. Arredondo, E. Ramón-Gallegos, N. Batina, P. Damián-Matsumura, "Gold nanoparticle uptake is enhanced by estradiol in MCF-7 breast cancer cells," *Int. J. Nanomedicine*, vol. 14, p. 2705, 2019, doi: 10.2147/IJN.S196683.

[9] S.M. Amos, C.P.M. Duong, J.A. Westwood, D.S. Ritchie, R.P. Junghans, P.K. Darcy, M.H. Kershaw, Autoimmunity associated with immunotherapy of cancer, *Blood*. 118 (2011) 499–509. https://doi.org/10.1182/BLOOD-2011-01-325266.

[10] Z. Zeng and K. Pu, "Improving Cancer Immunotherapy by Cell Membrane-Camouflaged Nanoparticles," *Adv. Funct. Mater.*, vol. 30, no. 43, p. 2004397, Oct. 2020, doi: 10.1002/ADFM.202004397.

[11] D. De Pasquale, A. Marino, C. Tapeinos, C. Pucci, S. Rocchiccioli, E. Michelucci, F. Finamore, L. McDonnell, A. Scarpellini, S. Lauciello, M. Prato, A. Larrañaga, F. Drago, G. Ciofani, "Homotypic targeting and drug delivery in glioblastoma cells through cell membrane-coated boron nitride nanotubes," *Mater. Des.*, vol. 192, Jul. 2020, doi: 10.1016/j.matdes.2020.108742.

[12] D. Mendanha, J. Vieira de Castro, H. Ferreira, N.M. Neves, Biomimetic and cell-based nanocarriers – New strategies for brain tumor targeting, J. Control. Release. 337 (2021) 482–493. https://doi.org/10.1016/J.JCONREL.2021.07.047.