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Nanoparticle-based drug delivery for treatment of traumatic brain injury

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Abstract

Introduction: Traumatic brain injuries (TBIs) impact the breadth of society and remain without any approved pharmacological treatments. Despite successful Phase II clinical trials, the failure of many Phase III clinical trials may be explained by insufficient drug targeting and retention, preventing the proper attainment of an observable dosage threshold. To address this challenge, nanoparticles can be functionalized to protect pharmacological payloads, improve targeted drug delivery to sites of injury, and can be combined with supportive scaffolding to improve secondary outcomes.

Areas covered: This review briefly covers the pathophysiology of TBIs and their subtypes, the current pre-clinical and clinical management strategies, explores the common models of focal, diffuse, and mixed traumatic brain injury employed in experimental animals, and surveys the existing literature on nanoparticles developed to treat TBIs.

Expert opinion: Nanoparticles are well suited to improve secondary outcomes as their multifunctionality and customizability enhance their potential for efficient targeted delivery, payload protection, increased brain penetration, low off-target toxicity, and biocompatibility in both acute and chronic timescales.

Keywords

TBI; nanoparticles; drug delivery; neural engineering

1. Introduction

Traumatic brain injuries (TBIs) are a leading cause of death and disability worldwide [1]. Without accounting for TBI cases that are left untreated, seen in outpatient care, and

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Declaration of interest

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in federal facilities [2], there are still 69 million people who reportedly experience TBIs each year globally [3]. The incidence of TBIs spans all ages and affects particularly racial and ethnic minorities [4], military and public service members [5], people experiencing homelessness [6], and victims of assault and motor vehicle crashes [7]. TBIs in extreme conditions like in space travel will also require attention in the future. In a recent longitudinal study, blood samples from cosmonauts who spent, on average, 169 days on the International Space Station, saw significant elevations of neurofilament light chain, a biomarker of TBI and other neurodegenerative disease [8, 9].

TBIs constitute both closed head injuries (CHI) and, less common, penetrating injuries. CHIs are caused by blunt trauma, rapid acceleration, or deceleration forces [10] whereas in penetrating injuries, an object perforates the skull, breaches the meninges, and injures the brain [11]. To categorize the severity of TBIs and triage patients, the Glasgow Coma Scale (GCS), a physiologic measure of injury for all types of acute medical and trauma patients, is often employed in the clinical setting [12]. Typically, GCS scores between 13-15 are classified as mild TBI, 9-12 for moderate TBI, and 3-8 for severe TBI. Though useful, the GCS is weakly correlated with survival and functional outcomes [13, 14], thus, physicians also use imaging tools such as computerized tomography (CT) scans [15, 16] and magnetic resonance imaging (MRI) to determine injury severity [17]. Evidence has shown that employing additional anatomical measurements in combination with the GCS can improve the correlation with Glasgow Outcome Scale Extended (GOS-E) scores, a common tool used to assess global disability and recovery after a TBI [18]. For example, combining the GCS with the Injury Severity Score (ISS) and the Abbreviated Injury Score (AIS) has shown to improve the correlation with GOS-E scores, $R=.335$, $p<.001$ and $R=.275$, $p<.001$ respectively [19]. Other studies point to measuring a combination of biomarkers of injury to improve outcome correlation, including neuroendocrine hormones, micro-RNA, and brain-specific proteins [20].

TBIs begin with a primary injury as a result of the immediate impact. This event is not treatable and can be characterized by intra- and extraparenchymal hemorrhage, focal contusions, cerebral edema, and focal and/or diffuse axonal injury (DAI), due to the biomechanical shearing, tearing, or stretching of white matter tracts [21]. Concurrently initiated with the primary injury, the secondary injury sequelae can evolve over minutes to days and even months [22], providing a window for intervention. During this period, cellular and neurochemical mechanisms can result in an inflammatory response characterized by ischemia, hypotension, hypoxia, increased intracranial pressure (ICP), decreased cerebral perfusion pressure, and edema [21]. Glutamate excitotoxicity, the generation of reactive oxygen species (ROS), mitochondrial dysfunction, DAI, apoptosis, and the eventual death of neuronal, endothelial, and glial cells may also occur [23, 24]. These delayed deleterious processes can lead to acute and chronic sensorimotor [25, 26] and cognitive impairments [27-29].

Clinical management to reduce secondary damage and improve the prognosis of motor and cognitive functioning remains a challenge as no pharmacologic agents that have succeeded in Phase III clinical trials to treat TBIs. For instance, progesterone initially showed promise in pre-clinical studies by reducing inflammatory cytokines [30], stimulating neurogenesis,

and synaptogenesis [31], and enhancing functional recovery of sensory, cognitive, and motor tasks [32-34]. Phase II clinical trials even proved moderately successful, but two Phase III trials, ProTECT III in the USA and SyNAPSe in China, failed to meet their primary outcome of improving scores on the GOS-E [35]. Though there may have been flaws in the experimental design [36], it is noteworthy that the mode of drug delivery and location of administration were problematic for efficient targeted treatment and determining an effective dosage. As another example, dexanabinol also failed to see a difference in GOS-E score at 6 months, despite being able to show improved ICP in Phase II trials [37]. Researchers of these trials noted that the distribution of drug volume may have varied in patients, necessitating a more targeted delivery mechanism.

2. Pathophysiology of TBI

The pathophysiology of a TBI is biphasic, beginning with the primary injury, a direct consequence of the mechanophysical forces responsible for the insult. This phase can not be treated and only preventative measures can be taken to avoid the incident [38]. Upon immediate impact, the resulting damage can be focally concentrated, diffuse, or a combination of both. Focal brain injuries stem from collision forces acting on the skull, resulting in epidural, subdural, and intraparenchymal hematomas, compression, and contusion of the underlying brain tissue at either the site of impact (coup), opposite the site of impact (contrecoup) or both [39]. Such physical changes restrict blood flow and often produce necrotic areas near the site(s) of impact. Diffuse injuries occur when rapid acceleration-deceleration or rotational forces shift the brain inside the skull causing dynamic shear, tension, and compressive forces that impact the brain. These processes elicit DAIs predominantly in subcortical and deep white matter tissue such as the brain stem and corpus callosum [40] and result in traumatic axonal damage, diffuse vascular injury, hypoxic-ischemic injury, and edema [41].

The secondary injury phase represents the body's attempt to limit the extent of damage, repair consequences of the primary injury, and restore the brain's structural and functional integrity. The physical, cellular, and biochemical processes that occur during the primary injury progress into secondary injury mechanisms on a time scale of hours to years, resulting in cerebral swelling, herniation phenomena, and neurodegeneration, which can lead to cognitive and motor impairment [42]. This secondary phase reveals the only window for pharmacological intervention to reduce injury progression and improve outcome severity. For example, delayed Wallerian degeneration, a phenomenon where severed axons maintain independent function for weeks after the initial impact event, has revealed a special window for TBI treatment and has been a topic of interest in studies aimed to improve functional recovery [43, 44].

The blood-brain barrier (BBB) is a selectively permeable microvasculature system that shields the brain from toxic substances in the blood, provides nutrients to brain tissue, and relinquishes harmful substances in the brain to the bloodstream. At the moment of impact, the BBB is often compromised, triggering the downregulation of the platelet-derived growth factor (PDGF)-B/ PDGF receptor- β signaling pathway, impairing pericyte-endothelium interactions and resulting in pericyte loss near the BBB, further compromising

the BBB's integrity (see Figure 1) [45]. This dysfunction of BBB dynamics is followed by an increased permeability to water, marked by a significant increase in aquaporin4 (AQP4) expression and possibly edema [45]. Also contributing to brain swelling, a ruptured BBB can encourage vasogenic edema, where protein-rich fluid from circulating blood enters the brain's interstitial fluid [46]. Cytotoxic edema can also form, where the dysregulation of homeostatic ion channels and pumps disrupt the ionic gradient leading to intracellular swelling of astrocytes and glial cells. Both cytotoxic and vasogenic edema can elevate ICP, worsening functional outcome [47].

Evidence has shown that prolonged neuroinflammation recruits macrophages, activates microglia, and promotes astrogliosis [48]. In response to the injury microenvironment, microglial polarization allows for a phenotypic response to surrounding biochemical cues which alter microglia function accordingly. Infiltrating microglia/macrophages can assume either two main terminally defined states: M1, which is pro-inflammatory/neurotoxic, or M2, which is anti-inflammatory/neuroprotective [49]. Research has shown that early after a TBI, both M1- and M2-like phenotypic markers are expressed followed by a transient up-regulation of the M2-like phenotype, which is soon replaced by a predominant M1-like phenotype associated with neurodegeneration up to 7 days post-injury [50]. During astrogliosis, activated astrocytes undergo hypertrophy and intermingle their processes with oligodendrocytes, meningeal cells, microglia, and fibroblasts to develop scar-like structures in an attempt to limit the injured area. Such mechanisms curtail the potential for axonal regeneration [51], though, some investigators believe that glial scarring provides perineuronal nets for both synaptic maturation and plasticity [52].

Synchronous with the processes described, a TBI insult also signals the release of damage-associated molecular patterns (DAMPs) as a response to cellular stress from injured tissue and are recognized by macrophages and pattern recognition receptors such as toll-like receptors and inflammasomes of the innate immune system [53]. DAMPs trigger inflammatory responses through multiple pathways which create a positive feedback loop of DAMPs production and inflammation (see Figure 2). A pathway of popular research interest, the nuclear factor- κ B (NF- κ B) inflammatory pathway, is a central mediator in pro-inflammatory gene induction [54] and plays a large role in edema [55] and neuronal apoptosis [56].

DAMPs also guide the extravasation of activated leukocytes into the brain parenchyma which, in concert with microglia and astrocytes, produce ROS and inflammatory molecules including cytokines such as IL-1 β , IL-6, and TNF- α , and chemokines such as MIP- α , MCP-1, and IL-8, recruiting more leukocytes to the injury site [57, 58]. These inflammatory biomolecules that follow leukocyte invasion encourage the demyelination and degradation of axonal cytoskeletons, resulting in axonal swelling and the accumulation of transport proteins at the synaptic terminals, compromising neuronal activity [59, 60]. Neurotransmitters can also accumulate in the synaptic space due to release from sheared neurons, glutamate-induced aggravated release from pre-synaptic terminals, and impaired reuptake mechanisms of the ischemic brain and activate both ionotropic and metabotropic receptors on the post-synaptic membranes fostering an influx of calcium ions, inducing excitotoxicity [61]. Furthermore, the activation of mitogen-activated protein kinases (MAPK), protein

phosphatases, ROS, nitric oxide, calcineurin, calpain, and caspases follow, which induce apoptosis and increase ROS-mediated lipid peroxidation [62]. These processes impair mitochondrial function by depolarizing the membrane, reducing ATP production, and increasing oxidative stress, further exacerbating the secondary injury [61, 62].

3. Current management of TBI

Management of TBIs includes preventative measures to avoid TBIs, pre-hospital strategies, and clinical interventions to manage injuries. Primary prevention aims to circumvent brain trauma events by transforming social behavior through an array of mechanisms from public policy reforms [63], such as revising speed limits [64], improving road engineering [65], and enforcing helmet use [66], to altering cultural practices, such as creating programs to address alcohol abuse [67] and teaching athletes proper tackling techniques while playing rugby [68]. Secondary prevention focuses on minimizing the biological injury sequelae [63]. These efforts include addressing systemic issues through blood-pressure management, choice of fluid for resuscitation, temperature management, ICP management, monitoring cerebral oxygenation, and improving ventilation techniques [63]. As mentioned, clinical strategies to reduce secondary injury progression from a subcellular approach have not resulted in any successful Phase III clinical trials. Lastly, tertiary prevention, which is now often seen as a part of secondary prevention, aims to maximize the patient's functional abilities and restore their quality of daily life after injury [63]. Such efforts include neuro-rehabilitation and understanding the relationships between imaging, function, and the underlying pathology to administer the most effective treatment for all injury types. The Centers for Disease Control and Prevention has recently published guidelines on tertiary prevention techniques for mild TBI in children which include symptoms scales, proper sleep methods, and assistance with the time given to complete assignments, all of which can aid in recovery [69].

The clinical management of TBIs aims to categorize the severity of the injury, relieve cerebral swelling and mass effect through medical and surgical strategies, and provide treatment to improve cognitive and functional outcomes. After an impact, patients may experience physical symptoms such as headache, fatigue, anxiety, light sensitivity, and confusion for mild TBIs. With moderate to severe TBIs, patients can experience seizures, loss of consciousness, and significant cognitive, motor, and sensory deficits alongside the previously mentioned symptoms [70]. Clinicians commonly use the GCS or extended GCS [71] which measures impaired consciousness based on the eye-opening, motor, and verbal responsiveness to categorize injury extent as either mild, moderate, or severe and predict prognosis. As mentioned the correlation between outcome and GCS is weak therefore, clinicians often employ various imaging techniques to improve the accuracy of the prognosis [15-17].

Once injury severity is determined, clinicians focus on relieving the cerebral mass effect, a phenomenon in which a focal lesion, such as a blood clot or a contusion, compresses the surrounding brain tissue [72]. Understanding of the mass effect is, in part, driven by the Monro-Kellie doctrine, a hypothesis that posits that the sum of the volumes of brain tissue, cerebrospinal fluid, and intracranial blood is constant [73]. Therefore, alternations in one will elicit opposite fluctuations in one or both of the remaining two variables. This

behavior, in combination with principles of cerebral blood flow, accounts for many of the pathophysiological changes that occur after TBI. Depending on the extent of the acute focal mass effect, brain swelling, and increased ICP, physicians commonly administer mannitol and preferably hypertonic saline, since evidence has shown that this osmotic diuretic may increase pressure in the skull with excessive administration and present detrimental effects on mortality when compared to the latter [74]. In cases where herniation persists, surgeons are prompted to perform a decompressive craniectomy (DC) to relieve focal mass effect or high ICP. Early DC reduces mortality in patients with TBI but the clinical outcome compared to the standard medical management remains the same and determining the most optimal time frame for performing DCs still requires further investigation [75]. Unfortunately, there are still no proven treatment strategies to relieve the progressive secondary injury effects of TBI.

4. Modeling TBI

To investigate TBI treatment strategies and therapeutics, researchers are faced with the challenge of choosing an appropriate model organism and injury model to investigate the heterogeneous nature of TBIs in vivo. While larger mammals and non-human primates phylogenetically closer to humans may more accurately reflect the human biological response to TBIs, rodent models are often preferred due to their accessibility, comparatively lower cost, ease of handling, cheap post-surgical care, and existent standardized outcome measurements. For instance, to measure motor function in rodents there are many assessments including the rotarod test [76], beam walking [76], and cylinder test [77]. To assess cognition, there are also varied experimental apparatuses such as the Morris Water Maze [78] and Y maze [79]. Comparatively, large animals lack such a span of outcome measurements and models of delivering TBIs, though investigations are underway to bridge this gap [80, 81].

When choosing an appropriate TBI model, researchers must consider the type of injury that is to be modeled. There is no established guideline to measure injury severity in experimental TBI thus, investigators must tune mechanical parameters such as impact speed, depth, and dwell time [82]. Researchers can also note neurological changes, such as righting time [83], physiological changes, like weight loss [84], histological changes, including infarct volume and neuronal loss [85], and observe motor and cognitive performance [76-79]. It should be noted that there are limitations to using animal models. For example, although severe TBIs in rodents often reveal extensive tissue degeneration and motor dysfunction, these animals rarely mimic the state of coma often seen in diffuse TBI patients [86-88]. Furthermore, many TBI models require anesthesia prior to injury which can confer cerebral metabolic and physiological effects [89]. For instance, isoflurane, a commonly inhaled anesthetic used during surgical procedures, has been evidenced to attenuate functional deficits and elicit some neuroprotective action on the injured brain [90, 91]. Propofol, an anesthetic used in the clinic, has been associated with poor outcomes in rat focal TBI models [92]. For these reasons, it is important to have a sham animal group when designing TBI studies.

Broadly, animal models of TBI are classified as either mainly focal, diffuse, or mixed injuries. Mixed injuries represent a combination of focal and diffuse injuries, reflecting falls or sports-related injuries in humans. Details of commonly employed animal models to administer TBIs are discussed in Table 1. Ideally, when designing an experiment, the model should be tunable (commensurate with the desired injury severity), quantifiable, reproducible, and have some clinically relevant correlation. Common methods to mimic a focal brain injury include weight drop injury models (WDI), the open skull controlled cortical impact (CCI) model, and penetrating ballistic-like brain injury models. Diffuse injuries are commonly modeled using blast-wave models and mixed injuries can be modeled by some WDI, acceleration-deceleration models, and fluid percussion injuries. Though much of the current literature describes most injury models as being developed to represent a binary classification of TBI, mainly focal or diffuse, a large percentage of TBIs are of the mixed type. A recent MRI study revealed both focal lesions and diffuse injuries in 50% of patients studied with moderate and severe TBI [93].

In general, WDI models take advantage of the gravitational force elicited by a free-falling weight to generate the desired injury severity. The Feeney weight-drop model represents a focal injury model and generates reproducible graded cortical contusions using a 40 cm stainless steel tube attached to a circular footplate. A craniotomy is performed and the footplate is positioned above the exposed dura. The weight is then released, dislodging the footplate and impacting the region of interest [94]. This impact results in a cortical contusion characterized by edema [95], hemorrhaging [96], and damage to the BBB [97]. The subsequent inflammatory processes [94, 96] which activate microglia, astrocytes, the complement system [98], as well as the infiltration of neutrophils and macrophages, are commensurate with the weight falling height and consequentially, the injury severity [99]. The Shohami WDI model is a similar model except that the focal injury is created over one side of an intact skull using a flat silicone tip fixed at the end of an impacting rod [100]. The animal in both models is often fixed to a hard surface to minimize the dissipation of energy and avoid diffuse injury.

This practice is in contrast to the Marmarou weight drop model, often employed to mimic motor vehicle injuries and induce diffuse axonal injuries, where a rotational acceleration follows a linear acceleration injury [88, 101]. In this model, the animal is strapped to a spongy material, such as a foam-covered platform, which serves to decelerate the head after impact [102]. A segmented brass weight then free-falls through a Plexiglass tube striking a metal disk affixed to its skull which allows for widespread axonal damage while reducing the chances of cranial fracture and the appearance of focal injuries. In a graded manner, dependent upon the release height of the weight, characteristics of diffuse injury are apparent such as bilateral hemorrhaging, cell loss, DAI, activated microglia, and astrogliosis. While these WDI models are similar to the mechanism of human TBI, can undergo neuroscoring post-injury using the Neurological Severity Score (NSS) [103], and are easy, cheap, and convenient, these models have high mortality rates due to apnea and skull fractures, can sometimes produce rebound injuries, often have varied impact velocities, and thus present inaccuracies with brain tissue deformation and injury severity.

A more reproducible TBI model is the CCI model which generates a mainly focal TBI and has been used in many animals including rodents [82, 104-106], swine [107-110], and non-human primates [111]. A craniectomy is first performed, leaving the dura intact over the targeted area. Using an electromagnetic or pneumatic impactor, investigators can precisely control quantifiable mechanical parameters such as the velocity of impact, dwell time, and cortical depth penetration to achieve the desired severity of injury [104, 106]. While there is no consensus regarding the behavioral or histological criteria that constitute mild, moderate, or severe injuries as seen in patients, research is actively trying to standardize the CCI model injury severity. Recently, one study recommends injury severity to be determined by a multifactorial approach including the extent of tissue loss, NSS, and cognitive deficits in addition to the surgical parameters previously mentioned [82].

Similar to humans with injury-induced epilepsy, rodents with CCI-induced TBIs may experience post-traumatic seizure activity [112]. Furthermore, the CCI model may inflict cytotoxic and vasogenic brain edema, often seen in the clinical human pathophysiology of TBI [46, 105]. Dependent upon the severity, the neuropathology of rodent CCIs often includes a cortical contusion proximal to the impact site, subdural hematoma, BBB disruption, hypoperfusion, cavitation, and neurodegeneration. Upregulated inflammatory cascades lead to excitotoxicity, neuronal cell death, astrogliosis, microglial activation, axonal damage, and cortical spreading depressions [96, 107]. Weaknesses of this model include the lack of brainstem deformation resulting in minimal mortality, as well as the lack of post-injury neuroscoring though, implementation of an NSS could improve this. Other focal injury models such as cryogenic injury models [113-115] and penetrating injury models, the only one in use today is the balloon inflation technique [116], can mimic some aspects of human TBI pathology but their clinical relevance is limited. For instance, in the cryogenic models, the focal traumas often lack the contrecoup injury and DAI often associated with human cerebral injuries.

Blast-like injury models, apart from open field designs, represent another tunable and highly reproducible set of TBI models. The penetrating ballistic brain injury model represents the focal damage shrapnel from an explosion can cause where a cavity forms corresponding to the penetration location [117]. Most other blast injury models reflect mechanisms of diffuse and mixed injuries. Blast tube models employ the detonation of an explosive to simulate a shock wave and blast wind without reflected shock fronts from the ground and other surfaces. When designing these experiments researchers must consider accommodations for the model organism, intended placement of the organisms within or away from the tubes/subject standoff, and plan for specialized testing locations and personnel training for the safe use of explosives. Common models include the Parks tube, often used in swine models [118, 119], and the Clemenson tube [120] which can be used to study blast injury in rats [121, 122]. Shock-wave primary blast injury models are similar except that they use compressed gas instead of explosives, though the physics of gas-driven shock waves may differ from explosive shock waves and the resulting pathology may not accurately reflect that of the human condition [123].

Fluid percussion injury (FPI) models represent another mixed injury model where both cerebral and focal concussions are produced, similar to sports-related injuries such as

boxing. A pendulum first strikes a piston at the end of a tube filled with fluid and the fluid pressure impulse rapidly injects the exposed dura directly through a Luer-Lock surgically implanted onto the intact dural surface exposed via craniotomy which is usually made either centrally [124], over the sagittal suture midway between the bregma and lambda, or laterally over the parietal cortex [125]. The implant is connected to an FPI device fluid impulse outlet and fluid pressure detector which accurately measures the impact pressure. Injury severity is determined by adjusting the force of the fluid pressure pulse. Weaknesses of this model include the need for a craniectomy and like WDI and other models, FPI models demonstrate a high mortality rate due to apnea and lack immediate post-injury neuroscoring.

5. Nanoparticles address the challenges of treating TBI

The failure of many Phase III clinical trials [35-37, 126, 127] has revealed to investigators that to successfully improve TBI outcomes, novel pharmacological interventions must efficiently deliver therapeutics to the targeted injured area(s) and be retained in the brain long enough to confer its therapeutic effect while avoiding off-target toxicity. Successful therapies will also need to specifically address the dysfunction of the neurovascular unit (NVU) during a TBI, which includes the cellular and extracellular components of the brain and its vasculature such as neurons, perivascular astrocytes, microglia, pericytes, endothelial cells, and the basement membrane [128]. Drugs that can mediate the immune trauma response without needing to penetrate the cerebral tissue, such as a free radical scavenger, would not necessarily require NVU targeting, but those which must cross the BBB, such as ion channel blockers, calcium channel antagonists, trophic factors, and cell-specific targets should focus on increasing delivery efficiency when designing therapies [127]. Addressing these challenges will lower the barriers to establishing dosage thresholds, or the minimum amount of drug below which a biological effect does not occur, and enhance the potential of meeting the set endpoints of clinical trials.

NP-based drug delivery systems have the potential to meet the requirements that can increase the efficiency and efficacy of TBI therapies. Their customizable size, stealthy chemistry, and multifunctionality allow NPs to be optimized for stable targeted drug delivery systemically and to the brain [129, 130]. Modifications can also include functionalizing NPs with target-appropriate charges. To illustrate, in peptide-modified NPs that are delivered directly to the brain, positively charged peptides show restricted distribution compared to neutral, zwitterionic, or negatively charged particles, while the opposite is true for off-target organs [131]. Depending on their engineering, NPs can themselves elicit some therapeutic benefit in addition to shielding pharmacological payloads during targeted delivery.

The materials used in developing NP therapeutics are chosen based on some permutation of their ability to be functionalized with immune-evading, therapeutic, or targeting ligands, their capacity to protect their payload in the injury microenvironment, their chemistry to foster optimal timing for drug release, their improved biocompatibility, and low off-target accumulation and toxicity [130]. Poly(ethylene)glycol (PEG), a polyether compound, is regularly conjugated to NP polypeptides to prevent aggregation, improve solubility, and increase half-life in the circulatory system [132]. The PEG coating minimizes opsonization by avoiding plasma protein adsorption via steric hindrance and shield charging, thereby

abating interactions with phagocytotic cells and avoiding clearance [133, 134]. Poly(lactic-co-glycolic acid) (PLGA) and Tween[®] 80, an amphiphilic non-ionic polysorbate (PS) surfactant, are also commonly employed in NP synthesis to improve biodegradability and biocompatibility as well as foster delivery across the BBB through low-density lipoprotein-mediated endocytosis, respectively [135, 136]. Incorporation of metals, such as gold [137, 138] and cerium [139, 140], are also used when synthesizing NPs to target injured parenchyma since their native properties can be utilized for their enhanced drug release chemistry, enzymatic actions, and anti-inflammatory capabilities.

Across the literature, investigations which harness this multifunctional diversity and potential for developing NP therapeutics to treat TBIs center around different strategies to improve the secondary injury sequelae. Similar therapeutic applications of NPs have been achieved through employing different nanomaterials. Therefore, here, NPs are discussed based on their shared function or pharmacological effect. In general, the NPs studied to treat TBI, and their respective payloads, function to elicit some combination of immunomodulatory, hemostatic, neuroprotective, anti-oxidative, anti-inflammatory, and stem-cell integration assistance through either modified surface chemistry, peptide functionalizations on the NPs, encapsulating and delivering gene therapies, pharmaceutical agents, neuroprotective substances, or growth factors. A summary of the most recent NPs investigated to treat TBIs are categorized by their primary function listed in Table 2.

5.1 Immunomodulatory NPs

Immunomodulatory NPs include those nanoscale technologies which alter the immune system in a manner that improves TBI outcome. Investigators in this field have focused on designing NPs that mitigate upstream inflammatory players and sequester inflammation both in the vasculature and around the injured parenchyma sometimes solely through surface modifications, omitting a payload [141].

Immunomodulatory nanoparticles (IMPs), 500nm-diameter NPs made from Food and Drug Administration (FDA)-approved biodegradable biopolymer carboxylated PLGA, are shown to improve TBI in the acute phase by binding to macrophage receptors with collagenous structure (MARCO) positive hematogenous monocytes (hMos) [142]. These monocytes are then sequestered to the spleen (see Figure 3A for possible translation in humans), unable to home sites of inflammation evidenced by an overall reduction in infiltrating immune cells and glial scarring, mitigation of the inflammatory status of infiltrating cells exemplified by microglia shifting to a more M2-like profile, improved visual and motor function, and reduced edema in both CCI and WDI models [142]. Even at the chronic time point of 10 weeks, CCI animals saw a significant 44.7% reduction in lesion volume compared to the vehicle-treated animals.

Biomimetic nanoparticles can also be employed as they provide the stealth of evading clearance by the mononuclear phagocyte system (MPS), reduce elimination by Kupffer cells in the liver, and glomerular filtration in the kidneys, while still being able to maintain the delivery capabilities of a synthetic NP [141]. Leukocyte-based biomimetic NPs (Leuko NPs) are <200 nm and are fabricated using liposomes and membrane proteins extracted from cultivated leukocytes. These NPs target activated endothelia through CD11b targeting and

evade MPS uptake through CD45 present on their surface [143]. Using the In Vivo Imaging System, Leuko NPs were shown to accumulate in the brain through paracellular diffusion and breaks in the BBB (see Figure 3B) as well as in peripheral organs such as the liver, spleen, kidneys, and lungs where no abnormal or pathological morphology was found as a result of this treatment. A significant increase in Iba-1 positive cells 24 hours post-injury was observed and can be linked to an increase in M2 microglia, decreased lesion size, and a reduction in F4/80 positive cells which are usually highly expressed on infiltrating macrophages. On a systemic level, Leuko NPs also attenuated peripheral inflammation as those organs which saw NP accumulation are speculated to aid in blocking the inflammatory response, thereby contributing to the reduction in infiltrating macrophages observed (see Figure 3A) [143]. In line with immunomodulating NPs, interleukin-4 protein-loaded liposomes were evidenced to facilitate oligodendrocyte precursor cell differentiation and oligodendrogenesis through peroxisome proliferation-activated receptor- γ signaling, thereby reducing oxidative stress, promoting mitochondrial function, and improving sensorimotor neurological recovery [144].

Also in the arsenal to modulate the immune response are NPs which deliver gene therapies. Short hairpin RNA-loaded shCCL20-CCR6 nanodetriplexes, made of polyamidoamine, target chemokine (C-C motif) ligand 20 via receptor chemokine receptor 6 to attenuate inflammation after repeated TBI [145]. This reduction of the inflammatory microenvironment improves the efficacy of human mesenchymal stem cell transplantation, resulting in reduced pro-inflammatory cytokine IL-6, neurodegeneration, microgliosis, and astrogliosis, and increased brain-derived neurotrophic factor (BDNF) expression in the cerebral cortex indicating possible neurogenesis in mice. MicroRNAs (miRNAs), small non-coding RNA molecules that regulate post-transcriptional gene expression, have also been employed in gene therapy strategies toward thwarting the deleterious immune response post-TBI. Upon observing that certain miRNAs in the mitochondria, compared to the cytosol, display a compartmental re-distribution post-injury, one study developed melittin-derived cell-permeable peptide (p5RHH)+miR-146a NPs which have shown to significantly reduce the expression of tumor necrosis factor (TNF) receptor-associated factor 6 and interleukin-1 receptor-associated kinase 1, two important modulators of the NF- κ B pro-inflammatory pathway after injury [146]. Future studies in this field must continue to consider how inflammatory cells and immunological signaling regulate the post-TBI recovery mechanisms, keeping in mind the timing of pro- and anti-inflammatory processes when developing immunomodulatory NPs [147].

5.2 Hemostatic NPs

Though all TBIs are susceptible to hemorrhaging, severe blast-induced injuries can especially elicit internal bleeding in multiple organs. Immediate intervention is among the most effective solutions to minimizing mortality in cases of severe polytrauma yet, the only available treatments are pressure dressings and absorbent materials which are only effective for superficially exposed wounds and are unable to ameliorate internal injuries. To find more viable solutions to reduce the chance of coagulopathy and mortality in severe trauma, many investigators are developing hemostatic NPs which aim to stop bleeding and reduce the progression of inflammatory processes. Commonly functionalized to hemostatic NPs, the

synthetic peptide Gly-Arg-Gly-Asp-Ser (GRGDS) contains the amino acid sequence Arg-Gly-Asp (RGD) which mimics a recognition site in the interaction between extracellular matrix molecules and cell membrane receptors. When platelets are activated, they expose glycoprotein IIb/IIa receptor. RGD can block the receptors of these glycoproteins, inhibiting fibrinogen binding and modulating hemostasis through anti-thrombotic actions [148, 149].

In one study 500 nm PLGA-poly(ϵ -cbz-L-lysine)-PEG-GRGDS NPs (hNPs) were synthesized to encapsulate dexamethasone (hDNPs), an anti-inflammatory glucocorticoid loaded at a final concentration of $22 \pm 1 \mu\text{g}$ of dexamethasone/ mg of NP, to stop bleeding and mitigate pro-inflammatory processes in a blast TBI model [150]. Results revealed an elevation in survival with these NPs regardless of whether dexamethasone is encapsulated or not. Interestingly, hDNPs were able to confer a decrease in apoptosis and BBB disruption in the amygdala which correlated with a significant reduction in anxiety-like behavior when tested in an open-field tracking system. Importantly, hNPs significantly mitigated hemorrhaging and hDNPs conferred a significant reduction in astrogliosis, a restoration of microglia levels, an alleviation of vascular endothelial growth factor expression, which at high levels can indicate BBB permeability, and increased expression of SM1-71, a recognized antibody against rat endothelial barrier antigen that indicates the functionality of the BBB [150].

In an earlier study, PLGA-poly(ϵ -cbz-L-lysine)-PEG-GRGDS NPs encapsulated poly(acrylic acid) (PAA) [151], a flocculating agent implicated as an anticoagulant due to its antithrombin-activating properties [152]. Results from these hemostatic NP studies showed a significant increase to 95% survival following a 20-psi blast compared to 60% in the control and there were no complications or toxicity observed in the experimental groups. In a more recent study, carboxyl-functionalized biodegradable polyurethane (PU) NPs have been delivered with SpongostanTM as a hemostatic agent and has shown that PU NP-contained gelatin attenuated brain edema, suppressed expression of M1 biomarkers such as IL-1 β , elevated M2 biomarkers, reduced the activation of inflammatory cells near the implant site, and increased BDNF by almost 3-folds [153].

5.3 Neuroprotective NPs

Neuroprotectants are those agents which restrict injury to the brain parenchyma and attempt to salvage or regenerate neuronal cell structure and function [154]. Though in general, NPs which elicit some immunomodulatory, hemostatic, anti-inflammatory, and anti-oxidant effects can result in neuroprotection downstream, discussed here are those NPs that contain agents in which their primary function is to elicit a direct neuroprotective or neuronal-related interaction.

Recently, many studies have utilized NP encapsulation of short interfering or silencing RNA (siRNA) to deliver gene therapies across the BBB and improve neuronal pathology post-TBI, since their efficiency and specificity avoid off-target effects that can be seen with some small-molecule drugs. Neuron-targeted nanocomplex (TP-RVG) NPs were developed to encapsulate siRNA against caspase 3, a lysosomal enzyme involved in apoptosis. Researchers synthesized this NP using a tandem peptide consisting of a targeting peptide found in the rabies virus, RVG, and intracellular trafficking molecule transportan (TP) [155].

Data revealed that after treatment with these TP-RVG NPs 5 minutes post-TBI, there was a significant accumulation of NPs in neurons. By day 3 there was an ~80% decrease in caspase 3 in the injured hemisphere, with no knockdown observed in the contralateral hemisphere. These results show the potential for TV-RVG NPs to target injured neurons and deliver siRNA to reduce neuronal apoptosis. Another group utilized 60 nm high-density Tau siRNA-loaded-PLGA-PS 80-coated NPs (PS 80 (H)-NPs) to penetrate an intact BBB and accumulate in the brain at a level 3-folds higher than conventional PEGylated NPs in mice with WDI modeled TBI [135]. Tau pathology is strongly linked to TBI-caused neurodegeneration and brain dysfunction [156]. This study was the first to show that PS 80 (H)-NPs could reduce tau expression in cultured primary neural cells and also achieved 40-50% tau silencing in TBI mice regardless of being administered within or outside the window of the physically breached BBB.

In a recent study, NP-wrapped siRNA-Fyn and siRNA-c-Src were shown to knockdown Fyn and c-Src messenger RNA, members of the Src family kinases activated by transmembrane receptors implicated in pathways leading to the release of toxic molecules post-TBI [157]. Researchers in this study support that Src kinases activate neurotoxic downstream signaling including the Rho-associated protein kinase (ROCK) pathway which suppresses central nervous system regeneration, leading to neuronal loss and cognitive decline post-injury. Data from this study revealed that combined NP-wrapped siRNA-Fyn and siRNA-c-Src delivery demonstrated significantly reduced CA2/3 NeuN⁺ neuronal cell loss in the hippocampus post-TBI and improved cognitive function 12-16 days post-injury. Another group recently looked to target the RhoA/Rock pathway using poly (lactide-co-glycolide)-graft-polyethylenimine (Pgp) as a NP to encapsulate and deliver siRNA targeting RhoA (siRhoA) [158]. Data showed that in rats with CCI modeled TBIs, the Pgp/siRhoA NPs significantly reduced RhoA expression, lesion volume, neuroinflammation, apoptosis, and increased neuronal survival. This team recently used Pgp NPs to deliver Rolipram which restored cyclic adenosine monophosphate (cAMP) levels in the injured brain close to the sham level and reduced brain lesion volume, neuroinflammation, and apoptosis at 7 days post-TBI [159].

The use of peptides in NPs systems have also been a popular target of neuroprotective studies on improving TBI outcome. A couple of studies have explored the use of NPs for sustained release of cerebrolysin (CBL), a neuroprotective agent characterized by a peptide mixture able to ameliorate TBI symptomology with factors including BDNF, glial cell line-derived neurotrophic factor, nerve growth factor, and ciliary neurotrophic factor [160, 161]. CBL-loaded PLGA NPs, ranging 250-300nm, showed superior neuroprotective effects following a CHI compared to free CBL [161]. BDNF, in particular, is known to regulate neuronal plasticity, neuronal growth, proliferation, cell survival, and long-term memory but its short half-life and low BBB permeability create a barrier to effective therapeutic delivery [162, 163]. BDNF-loaded-PLGA NPs coated in poloxamer 188 have also circumvented this obstacle by demonstrating that they can increase BDNF levels in both sham and WDI mice in both ipsi- and contralateral hemispheres and also markedly improved NSS [164].

Another peptide, NR2B9c, has also been studied for its neuroprotective properties. N-methyl-d-aspartate receptor (NMDAR)-dependent excitotoxicity, induced by postsynaptic

density protein-95 (PSD-95) binding, is the primary mechanism of neuronal injury following ischemic injury [165]. Activatable protein nanoparticles (APNPs) are made of 3 independent polypeptides with enzyme-responsive sequences located between therapeutic peptides. APNPs home to target tissues and when activated by proteases rich in the injury microenvironment, release their payload. In one study Tat-NR2B9c, a post-synaptic PSD-95 inhibitor, was encapsulated with APNPs conjugated with PEG (TN-APNPs). When TN-APNPs were delivered to the injured ischemic brain, there was a significant reduction in infarct size, which was on average 22.6% larger than the contralateral side (compared with 58.8% and 38.7% in rats treated with PBS and free Tat-NR2B9c, respectively), and significant improvement in neurological function [166]. Tat-NR2B9c colocalized with PSD-95 only in the ipsilateral hemisphere of the ischemic injury, suggesting that the improved biological outcomes are due to the release of Tat-NR2B9c from TN-APNPs. In a more recent study, researchers enhanced the efficiency of TN-APNPs to deliver Tat-NR2B9c to TBI brain tissue by conjugating the NPs with peptides CAQK, which has a high affinity for extracellular matrix at the site of the injured brain, and CCAQK which contains an extra cysteine since the N-terminal cysteine could be important for the targeting effect of CAQK. In mice that underwent CCI and were treated with both peptide conjugated TN-APNPs, both showed improved brain penetrability and CCAQK-TN-APNPs demonstrated higher brain targeting efficiency, reduced injury size, and improved psychological outcomes [167]. CAQK has been implicated in many other TBI NP studies [168, 169] including the use of CAQK-conjugated silver NPs for targeting chondroitin sulfate proteoglycans-rich scars on mature oligodendrocytes of the injured region which would address a major barrier to regeneration [170].

Neuroprotectants can also be derived from natural and unconventional sources. For instance, curcumin, the principal secondary metabolite of turmeric or *Curcuma longa*, has been explored as a neuroprotectant in many studies [171-173]. Angiopep-2 functionalized and manganese-doped eumelanin NPs encapsulate curcumin (AMEC) as a neuroprotectant to influence anti-oxidation and anti-neuroinflammation in TBI parenchyma [171]. According to results from this study, after CCI in mice and subsequent intravenous administration of AMEC, these specialized ~160 nm nanoparticles can penetrate the brain through angiopep-2 binding low-density lipoprotein receptor-related protein-1 on the BBB enhancing drug accumulation, reduce inflammation and oxidative stress as exemplified by a lower M1/M2 ratio through ROS scavenging, and promote neuronal regeneration and functional recovery through the synergistic effects of eumelanin and curcumin [171]. In another study, curcumin-loaded niosome NPs were orally administered, after transplantation of human neural stem/progenitor cells, for 10 days. This combinatorial treatment showed a significant improvement in brain edema after TBI, increased locomotor activity, a decrease in astrogliosis, and an overall decrease in TLR4-, NF- κ B, and TNF- α -positive cells [172]. Another recent study evaluated curcumin-loaded AmyloLipid nanovesicles (ALNs) administered intranasally and showed evidence for its potential to increase its bioavailability to treat CNS disorders. ALNs are NPs based on a cross-linked starch enveloping a solid lipid core which liquifies at body temperature exposing the payload [173]. Multi-lamellar vesicle NPs have also shown great potential as a carrier for therapeutic agents to the injured

brain considering they are non-toxic, biocompatible, biodegradable, can cross the BBB, and maintain stability in the blood [174].

Nanocoffee NPs have also been investigated to exploit the neuroprotective potential of caffeine to prevent neuronal death. Caffeine can increase protein kinase A, suppressing β -secretase by reducing the Raf-1/ NF- κ B inflammatory pathway, and reducing γ -secretase, through lowering GSK-3 α , which decreases brain amyloid-beta, a marker of neurodegenerative disease [175]. Data from CHI animals treated intraperitoneally with nanocoffee NPs showed an enhancing effect on recognition memory, reduced anxiety, displayed an increase in T-type spines associated with learning, mitigated the increase in poly(ADP-ribose) polymerase (PARP) expressions seen after TBI which has been linked to microglia activation, and demonstrated a decrease in Erk and other MAPK signaling pathway members which mediate secondary injury [175]. Another NP encapsulating a neuroprotectant is poly(n-butyl-2-cyanoacrylate) (PBCA) coated in Tween[®] 80 which can cross the BBB via low-density lipoprotein receptor-mediated transcytosis and was recently studied for its potential to deliver β -nerve growth factor (β -NGF), a neurotrophin essential to maintaining the survival and normal functioning of neurons [176]. After a TBI, intravenous administration of PBCA- β -NGF-Tween[®] 80 NPs revealed a dramatic elevation in β -NGF concentration within 1 day which kept increasing significantly to day 7 compared to treatment with free β -NGF. The NP-treated group saw reduced mortality in vivo and increased neurite regeneration in vitro in PC12 cells through PBCA-delivered β -NGF enhanced MAPK kinase activity [176].

Recombinant human erythropoietin (rh-EPO) has shown pre-clinical evidence in protecting nerves, resisting apoptosis, promoting vascular regeneration, and reducing cerebral edema [177]. In efforts to increase neuroprotectant rh-EPO penetrability and accumulation in the brain, rh-EPO loaded Tween[®] 80 modified albumin nanoparticles (~438 nm) administered after TBI was shown to be non-toxic and significantly enhanced the distribution of rh-EPO (5000 IU/kg), reduced the expression of activated astroglia, and recovered AQP4 levels, relieving brain edema. As demonstrated the efficacy of neuroprotectants has been greatly augmented by the use of NP encapsulation technology for efficient targeted delivery. Relevant to previously failed Phase III clinical trials, progesterone has been studied for efficient encapsulation using Flash NanoPrecipitation to produce 300 nm progesterone-loaded polymeric NPs [178]. Work continues in this field to more effectively harness the neuroprotective potential of progesterone and other agents to improve secondary outcomes of TBI.

5.4 Anti-inflammatory and anti-oxidative NPs

The pro-inflammatory and oxidative pathways described in the Pathophysiology of TBI section contribute to the neurodegenerative and other deleterious effects exacerbated by positive feedback loops, such as those prompted by DAMPs, in secondary injury processes. Described here are those NPs which have been employed in brain injury studies to directly confer either anti-oxidative, anti-inflammatory, or both, effect(s).

Thioether core-crosslinked NPs (NP1) were shown to enter the brain parenchyma through a break in the BBB to scavenge and inactivate ROS such as hydrogen peroxide and superoxide

[179]. NP1, made from PS 80, is evidenced to reduce ROS in the ipsilateral hemisphere 1-day post-CCI and reduce acrolein, a product of lipid peroxidation, up to 1-month post-CCI [180]. Furthermore, NP1 conferred a significant reduction in activated microglia in the contralateral CA1 region and neuron-astrocyte-microglia triad formation in the contralateral CA1, ipsilateral CA2/3, and bilateral DG region 1 month post-CCI [180].

Many studies including the development of cerium oxide nanoparticles (CeONPs) [139] and redox-reactive nitroxide radical containing NPs [181] have also elicited antioxidative effects that resulted in improved TBI outcomes. The crystal lattice of cerium oxide has multiple valence states that confer its high redox capacity. After FPI, the higher concentration and dosing paradigm of CeONPs were shown to increase neuronal survival, preserve endogenous calcium anti-oxidant activity, reduce oxidative stress in part through improved superoxide dismutase activity, and restored reduced glutathione (GSH)/oxidized glutathione (GSSG) ratios [139]. Interestingly the shape of cerium oxide nanoparticles affects its efficiency. Ceria nanorods, ~130nm, were shown to exhibit better antioxidant activity and cytotoxicity than Ceria nanospheres, ~3.5 nm, due to nanorods displaying more high-energy surfaces with more active sites [140]. Other metal-containing NPs developed to treat TBIs include Au₂₄Cd₁ and Au₂₄Cu₁ clusters [137] which utilize the enzymatic activity of gold while taking advantage of its negligible toxicity and high renal clearance, ability to safely target hydrogen evolution reactions (HER), oxygen evolution reactions (OER), and reduce O₂. Gold nanoclusters have also been explored with dihydrolipoic acid (DHLA) functionalization, an anti-oxidant with demonstrated neuroprotective potential that suppresses pro-inflammatory processes by inducing M2 polarization in macrophage/microglia associated with a reduction of NF- κ B signaling, a decrease in ROS, and improved cell survival [182]. This study also highlighted the role of autophagy as a potential target for immunomodulation in regenerative medicine.

Other studies that focus on reducing oxidative and inflammatory processes include redox-active nitroxide radical-containing NPs which encapsulate antioxidant 4-amino-2,2,6,6-Tetramethylpiperidine-1-oxyl to scavenge free radicals and improve lesion volume and cognitive behavior while greatly lowering the toxicity of its payload [181]. In another study using sinomenine, an anti-inflammatory/anti-oxidant drug, conjugated to hydroxyl-terminated generation-4 poly(amidoamine) dendrimers (D-Sino), these NPs were able to target activated microglia/macrophages without conjugation of a targeting ligand [183]. D-Sino NPs were synthesized using enzyme-sensitive covalent linkages via a highly efficient and robust copper (I) catalyzed alkyne-azide click reaction to attach sinomenine to dendrimers. Results demonstrate that D-Sino NPs specifically target activated microglia/macrophages via endocytotic activity and can inhibit NF- κ B, a key regulator of atherosclerotic pathogenesis, proving its potential in suppressing acute inflammation post-TBI [183]. Another dendrimer NP, Triphenyl-phosphonium-Dendrimer-N-acetyl cysteine encapsulated NAC (TPP-D-NAC), an antioxidant and anti-inflammatory agent, for mitochondrial targeting under oxidative stress. Results showed that TTP-D-NAC specifically co-localized with mitochondria in activated microglia after systemic administration, confirming promise in BBB penetrability and targeting injured glia [184].

Betulinic acid (BA) NPs derived from the herb *E. ulmoides* have been shown to penetrate the BBB and elicit an antioxidative effect, improving functional recovery after a middle cerebral artery occlusion injury. BA NPs can cross the BBB via cannabinoid receptor 1-mediated transcytosis (see Figure 3B) [185]. Recently, researchers converted BA to betulinic amine (BAM). BAM accelerates drug release in the acidic conditions of the ischemic environment after a stroke and when surface conjugated with AMD3100, which interacts with CXCR4 abundant in ischemic tissue, enhances brain penetration [186]. In this study, investigators delivered NA1, a peptide designed to protect neurons against NMDA receptor-mediated excitotoxicity. These NA1-AMD3100-BAM NPs significantly increased survival, reduced infarct volumes by 69.8%, and enhanced neurological scores [186]. Acidic conditions also characterize injured tissue post-TBI [187], thus acid-responsive NPs such as these which can cross the BBB and quickly deliver a payload would be advantageous to treating TBI in the acute phase. There are many other ROS scavengers considered in NP therapeutics for TBI treatment including nimodipine [168], PEG conjugated hydrophilic carbon clusters [188], and surfactant poloxamer 188- N-acetylcysteine-loaded PLGA NPs [189].

5.5 NPs enhance stem-cell integration for improved TBI outcome

In the past decade, nanotherapeutics began to focus on enhancing stem cell transplantation and recruitment to repair the secondary injury-induced lesion cavity and promote tissue regeneration usually through some NP-complex [138, 145, 172, 190-192]. Mesenchymal stem cells (MSCs) are of special interest to cell therapy approaches as their autologous origins evade any immune response, display inherent pluripotency, and demonstrate reparative properties at sites of injury [193, 194]. In an in-vitro study, gold NPs surface-modified with a zwitterionic pentapeptide designed from Bax inhibiting peptide (Ku70) to enhance cellular uptake and a linearized expression vector to induce expression of BDNF in rat-derived MSCs have shown promise in engineering for efficient transplantation of MSCs post-TBI in the future [138]. This technique demonstrated higher transfection efficiency than more complicated viral gene transfer technology as MSCs exposed to a single transfection by the gold NP-gene construct showed ~80% cell transfection and exhibited successive expression of BDNF/mCherry fusion [138].

In vivo, transfected human fetal tissue-derived neural stem cells (hNSCs) with neurogenin-2-loaded poly(β -amino ester)-based NPs produced a larger number of neuronal cells compared to non-transfected cells. Delivery of these hNSCs with a hyaluronic acid hydrogel promoted vascular formation at the lesion site at 4 weeks following injection in a CCI injury model [190]. In a different study, another hydrogel model was investigated using imidazole groups-modified gelatin methacrylate (GelMA-imid) loaded with polydopamine NPs used as a carrier for stromal-derived factor-1 (SDF-1 α). Human amniotic mesenchymal stromal cells (hAMSCs) were injected into the damaged area of cryogenic TBI in rats and SDF-1 α promoted the migration of hAMSCs and their differentiation into nerve cells [191]. More recently, SDF-1 α -loaded NPs were shown to slowly release SDF-1 α and induce NSC/neuroblasts to migrate to injured areas and potentially improve the lesion volume [192]. Though the work in this field is few and in the early stages, results show promise in recovering lesion volume through efficient NP complexing.

6. Conclusion

The multifunctionality and customizability of NPs make this versatile technology best suited to address the challenges that have previously impeded potential therapeutics from meeting their clinical endpoints. In this review, we have presented an overview of the major pathways that constitute the complex pathophysiology of the continuum of focal to diffuse TBIs, described the current pre-clinical and clinical management of TBI, discussed the logistical considerations and clinical relevance of popular animal models of TBI, and reported on the existing literature centered on the use of NPs for TBI treatment. NP technology shows promise in safely and specifically delivering pharmacological payloads across the BBB while potentially eliciting therapeutic benefits through surface chemistry and functionalizations, improving secondary outcomes.

7. Expert opinion

The prospect of an approved TBI therapy is contingent upon the specific delivery of a therapeutic which confers minimal off-target effects while maintaining a predictable release profile. TBIs present on a spectrum of focal to diffuse types and therapeutics must be able to penetrate both the intact and compromised BBB not only to address this injury continuum but also to be used in both acute and chronic therapies. Nanoparticle technology is the best fit to meet these demands as their modifiable chemistry, size, charge, and customizable multifunctionality harness the potential to foster payload protection and improve therapeutic efficacy, avoiding off-target toxicity and biodegradation. NPs can also complex with other materials like hydrogels to improve therapies to regain structural and cognitive functioning of TBI patients, in combination with existing post-injury clinical treatment strategies. To facilitate the translation of the use of NPs to treat TBIs, NPs should be simple to formulate, stable at clinically relevant temperatures, and easily stored and transported for use in both acute and long-term treatment.

Though in general, research in this field is in the nascent phase of clinical translation, its potential to address multiple facets of downstream injury cascades is noteworthy. Stem cell therapies alone have shown great promise in treating TBI [195-199], and their efficacy can be improved by NP complexing as demonstrated in section 5.5. For instance, in the 2021 STEMTRA Phase II trial, intracranial implantation of allogenic modified bone marrow-derived mesenchymal stromal cells in patients with chronic motor deficits secondary to TBI showed significant improvement from baseline Fugl-Meyer Motor Scale scores at 6 months but failed to meet the secondary efficacy endpoint at statistically significant levels [200].

Future studies should investigate the effects of combining multiple NP therapeutics on TBI outcomes, consider how the dosage volume and frequency can be optimized for human applications, and study how practical these approaches are in a clinical setting or even pre-hospital environments, like in the case where, for example, hemostatic NPs could potentially improve acute survival on-site. Investigators must also be diligent about measuring off-target NP accumulation in other organs, measuring for toxicity, and having quantifiable evidence that the NPs improve physiological, functional, and cognitive outcomes. Future studies should also focus on designing more assessments in these areas for animals phylogenetically

closer to humans as well as continue to improve the injury model designs to better simulate human TBI outcomes. Bearing in mind these considerations, NP technology has the capacity to greatly improve the chances of achieving an approved therapeutic for TBI treatment.

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Article highlights

- The pathophysiology of TBIs is biphasic and pharmacological intervention is only possible in the secondary injury phase. Determining prognosis can be complex and is accomplished through a combination of interpreting imaging data such as MRI and CT scans in addition to employing the Glasgow Coma Scale and assessing other motor and cognitive symptomology.
- Many Phase III clinical trials fail mainly due to poor targeting efficiency, experimental design, and low retention of the drug in the injured brain.
- When using animal models to understand the pathophysiology and cognitive and functional outcomes of TBI, researchers must choose between models that elicit either focal, diffuse, or mixed injuries, tune the parameters of the injury setup, when possible, to reflect the target injury severity, and use sham animal groups to account for cerebral metabolic and physiological effects that are a result of the surgical environment.
- Nanoparticles can increase the efficiency and efficacy of therapeutics as their customizable size, stealthy chemistry, and multifunctionality allow NPs to cross the BBB, target the injured brain, and protect and deliver its pharmacological payload all while avoiding off-target effects.
- Nanoparticles designed to treat TBIs broadly include NPs with hemostatic, immunomodulating, anti-inflammatory, anti-oxidative, and gene-editing properties to enhance trauma recovery.

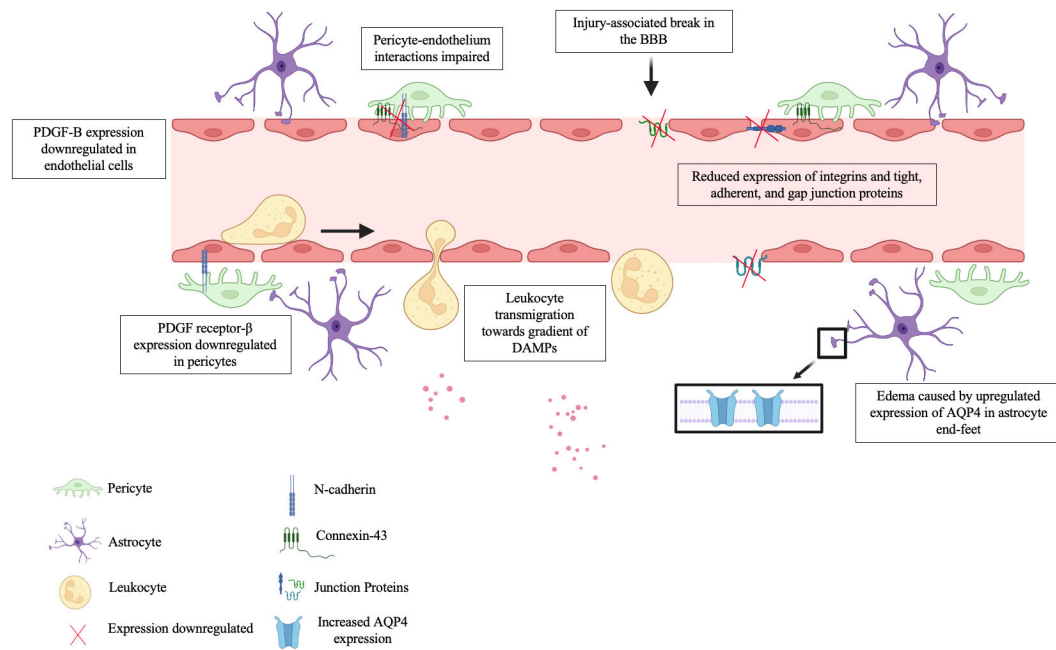


Figure 1.

BBB-level cellular and molecular response after a TBI. Leukocytes including neutrophils, macrophages, mast cells, eosinophils, natural killer cells, and dendritic cells transmigrate between and through endothelial cells, as well as through breaks in the BBB following the gradient of DAMPs released into the parenchyma after a TBI. Concurrently, PDGF-B/PDGF receptor- β expression is downregulated resulting in impaired pericyte-endothelium interactions evidenced by a decreased in the expression of gap junction proteins like Connexin-43, adherent junction proteins like N-cadherin, which connect endothelium and pericytes, and tight junction proteins, increasing BBB permeability. Vasogenic edema can also be seen by the increased expression of AQP4 towards the astrocyte end-feet surrounding the perivascular region. Created with [BioRender.com](https://www.biorender.com).

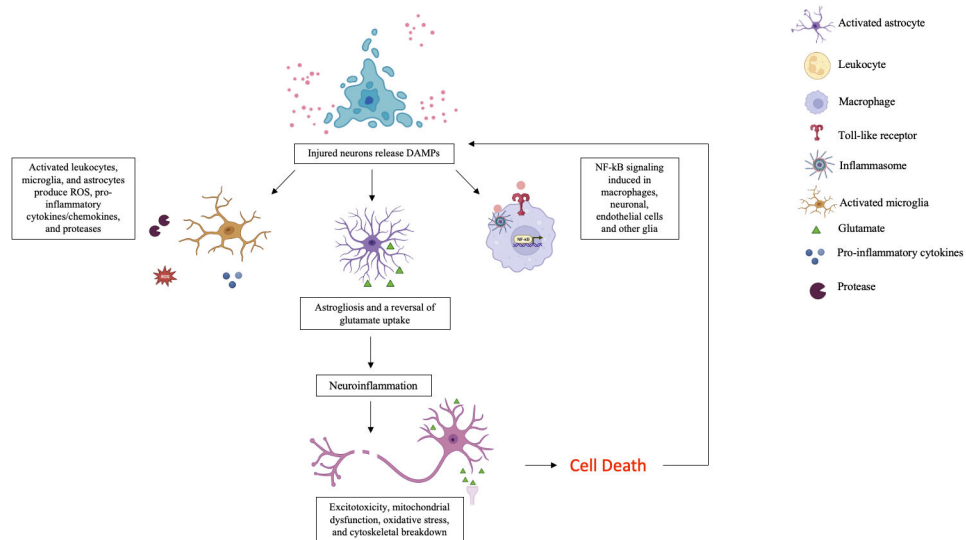


Figure 2.

Brain tissue-level response to TBI. After the BBB is compromised, DAMPs are released from injured cells and are recognized by pattern recognition receptors. In response to the DAMPs signals, an inflammatory response is elicited by leukocytes, microglia, and astrocytes which release pro-inflammatory molecules in a positive feedback loop. Prolonged inflammation promotes astrogliosis resulting in a scar-like formation around the injury perimeter and a reversal of glutamate uptake mechanisms which can contribute to persistent activation of N-methyl-d-aspartate acid (NMDA), α -amino-3-hydroxy-5-methylisoxazole propionic acid (AMPA) receptors, and voltage-gated ion channels, resulting in a lethal influx of calcium and subsequent neuronal excitotoxicity. Macrophages, microglia, and other cell types also respond to DAMPs signals by activating pro-inflammatory pathways such as the NF- κ B inflammatory pathway which contributes to edema and neuronal apoptosis. The pro-inflammatory mechanisms and molecules described, along with glutamate accumulation from sheared neurons, impaired reuptake mechanisms, and neighboring glutamate-induced aggravated release from pre-synaptic terminals, exacerbate excitotoxic mechanisms, reduce ATP production leading to mitochondrial dysfunction, increase oxidative stress, and eventually cell death, triggering the further release of DAMPs. Created with [BioRender.com](https://www.biorender.com).

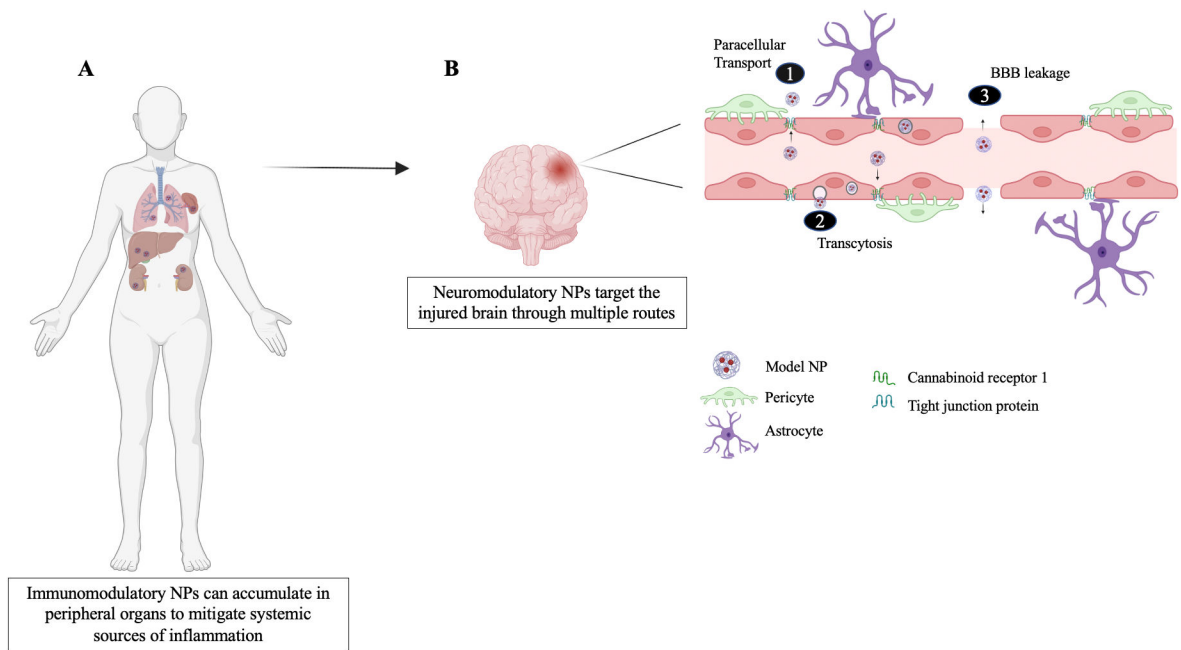


Figure 3.

Nanoparticles can accumulate in the brain and peripheral organs to improve secondary injury outcomes. (A) Immunomodulatory NPs attenuate peripheral inflammation by accumulating in the spleen, liver, kidneys, and lungs to block inflammatory processes and render immune cells unable to home sites of inflammation. (B) Neuromodulatory nanoparticles enter the brain to elicit, hemostatic, immunomodulatory, and neuroprotective functions as well as deliver targeted therapeutics into the injured parenchyma. Depending on its chemistry, size, and charge NPs can enter the injured brain through breaks in the BBB, mechanisms of transcytosis such as cannabinoid receptor 1-mediated transcytosis and low-density lipoprotein receptor mediated transcytosis (not shown), and to a lesser extent paracellular transport which allows permeation of selective substances regulated by tight junction proteins between endothelia of the BBB. Created with [BioRender.com](https://www.biorender.com).

Table 1

Common traumatic brain injury models. Created with BioRender.com.

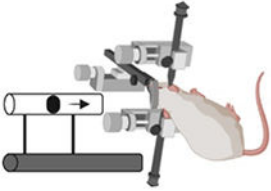
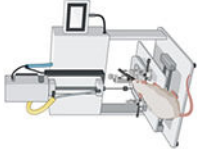

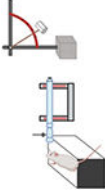
Model	Setup	Examples of Injury Model	Advantages	Disadvantages
Weight Drop Injury Models		Feeney WDI model <i>Focal injury</i> Shohami WDI model <i>Focal injury</i> Marmarou WDI model <i>Mainly diffuse injury</i>	<ul style="list-style-type: none"> • Reproducible graded cortical contusions (Feeney) • Biomechanics of injury similar to those in human TBI • Neuroscoring possible (e.g. NSS) • Cheap and convenient 	<ul style="list-style-type: none"> • Chance of rebound injury with varied velocity • Risk of apnea and skull fractures • High mortality rate compared to other models
Controlled Cortical Impact Models		<i>Focal injury</i>	<ul style="list-style-type: none"> • Highly reproducible • Low mortality • Highly tunable 	<ul style="list-style-type: none"> • Need for craniectomy • No established injury severity parameters • No brainstem deformation
Blast-like Injury Models		Penetrating ballistic injury model <i>Mainly focal injury</i> Parks Tube model <i>Diffuse/mixed injury</i> Clemmedson Tube model <i>Diffuse/mixed injury</i>	<ul style="list-style-type: none"> • Tunable • Reproducible • Biomechanics of injury mechanism similar to those seen in human TBI 	<ul style="list-style-type: none"> • Needs standardization • May require specialized testing locations • May require experienced personnel to handle explosives
Fluid Percussion Injury Models		Midline FPI model <i>Diffuse injury</i> Lateral FPI model <i>Mixed injury model</i>	<ul style="list-style-type: none"> • Highly reproducible • Tunable 	<ul style="list-style-type: none"> • Need for craniectomy • Lack of immediate post-injury neuroscoring • High mortality due to apnea

Table 2

List of NPs developed to treat TBIs categorized by function.

Targeted Effect	Relevant NPs Developed	Payload	Target	Reference
Immunomodulation	Immunomodulatory NP (PLGA-COOH)	N/A	MARCO ⁺ hMos	[142]
	Leuko NPs (Leukocyte-Based Biomimetic NP)	N/A	Activated endothelial cells through CD11b	[143]
	Interleukin-4 protein-loaded-liposome	IL-4	Peroxisome proliferator-activated receptor gamma	[144]
	shCCL20-CCR6 nanodendriplexes	shRNA (CCL20 or CCR6)	Chemokine (C-C motif) ligand 20 via receptor chemokine receptor 6	[145]
	p5RHH+miR-146a NPs	miR-146a	Nuclear factor-kappaB inflammatory modulators TRAF6 and IRAK1	[146]
Hemostasis	PLGA-PLL-PEG-GRGDS NPs	Dexamethasone Or Poly(acrylic) acid (PAA)	Glycoprotein IIb/IIIa receptors & Glucocorticoid receptor	[150]
	Polyurethane NPs in Gelatin (Spongostan™)	Polyurethane NPs	Antithrombin mechanisms	[152]
			M1/M2 mRNA expression / Nuclear factor-kappaB pathway	[153]
Neuroprotection	Neuron-targeted nanocomplex NP (TP-RVG NP)	Caspase 3 siRNA	Receptor for rabies virus (e.g. nicotinic acetylcholine receptor) on neurons and caspase 3 expression	[155]
	Tau siRNA-loaded-PS 80 (High Density) (PS 80 (H)-NPs)	Tau siRNA	<i>PS 80</i> : Endogenous apolipoproteins such as lipoprotein receptor-related protein 1 to promote BBB penetration <i>Tau expression</i> : Pathology linked to neurodegeneration and brain dysfunction in TBI	[135]
	NP-wrapped siRNAs-Fyn/siRNA-c-Src	Fyn and c-Src siRNA	Fyn & cSrc messenger RNA (to inhibit ROCK)	[157]
	Poly(lactide-co-glycolide)-graft-polyethylenimine	RhoA siRNA or Rolipram	RhoA expression Restore cAMP	[158] [159]
	Cerebrolysin-loaded PLGA NPs	Cerebrolysin	Injured neuronal tissue	[160, 161]
	BDNF-loaded-PLGA NPs coated with poloxamer 188 (NP-BDNF-PX)	BDNF	Penetrate CNS to deliver BDNF	[164]
	Tat-NR2B9c-loaded Activatable protein nanoparticle (TN-APNPs) *conjugated with CAQK or CCAQK for TBI	NR2B9c	Disrupt interaction between NMDARs & PSD-95	[166, 167]
	Angiopep-2 functionalized and manganese doped eumelanin NPs (ANG-MnEMNPs-Cur/AMEC)	Curcumin	<i>Angiopep-2</i> : low-density lipoprotein receptor-related protein-1 for transcytosis across BBB <i>Eumelanin</i> : Reactive oxygen species <i>Curcumin</i> : Inflammatory cytokines	[171]
	Curcumin-loaded niosome NPs (CM-NPs)	Curcumin	TLR4/NF-κB and inflammatory cytokines	[172]
	Curcumin-loaded AmyloLipid nanovesicles (ALNs)	Curcumin	Amyloid-β-protein, β-secretase, acetylcholinesterase, and anti-inflammatory cytokines such as IL-4 and IL-10	[173]

Targeted Effect	Relevant NPs Developed	Payload	Target	Reference
	Nanocoffee particles	N/A	Amyloid- β -protein, β - & γ -secretase Poly adenosine diphosphate-ribose polymerase (PARP), p-Erk, Erk2, phosphorylated GSK3	[175]
	Nerve Growth Factor (β -NGF) bound to Poly(n-butyl-2-cyanoacrylate) (PBCA) NPs	β -NGF	Mitogen-activated protein kinase kinase (MAPKK)	[176]
	Recombinant human erythropoietin-loaded Tween 80 modified albumin NPs (rh-EPO-Tw-ABNPs)	rh-EPO	AQP4 and pro-inflammatory processes (e.g. reactive astroglia)	[177]
Anti-inflammatory/ Anti-oxidative action	Thioether core-cross-linked NP	Sulfoxides and sulfones via thioether cores	Hydrogen peroxide & Superoxide	[179, 180]
	Cerium Oxide NP (CeONPs)	N/A	Radical species (e.g. superoxide, hydroxyl, and nitroxyl radicals)	[139]
	Au ₂₄ Cd ₁ , Au ₂₄ Cu ₁ Clusters	N/A	HER, OER, O ₂ , H ₂ O ₂	[137]
	Gold nanoclusters - dihydrolipoic acid (DHLA-AuNCs)	DHLA	Reactive oxygen species, NF- κ B pathway	[182]
	Redox-active nitroxide radical-containing NPs (RNP)	4-amino-2,2,6,6-Tetramethylpiperide-1-oxyl	Reactive oxygen species	[181]
	Sinomenine conjugated to hydroxyl-terminated generation-4 Poly(amidoamine) dendrimer (D-Sino)	Sinomenine	NF- κ B nuclear translocation	[183]
	Triphenyl-phosphonium-Dendrimer-N-acetyl cysteine (TPP-D-NAC)	NAC	Reactive oxygen species, glutathione	[184]
	Glyburide-Loaded Betulinic Acid NP	Glyburide	<i>Betulinic Acid NPs</i> : cross BBB via CB1-mediated transcytosis <i>Glyburide</i> : antagonist to the sulfonylurea receptor - transient receptor potential melastatin 4 channel	[185, 186]
	Nimodipine (CL-PPS/Np) NPs Surface material : lecithin, CAQK-DSPE-PEG2000, and DSPE-PEG2000 Core material: poly(propylene sulfide) ₆₀ (PPS ₆₀)	Nimodipine	<i>PPS₆₀</i> : Reactive oxygen species <i>Nimodipine</i> : Ca ²⁺ channels	[168]
	Poly(ethylene)glycol conjugated hydrophilic carbon clusters (PEG-HCCs)	N/A	Reactive oxygen species (e.g. superoxide anions & hydroxyl radicals)	[188]
	Surfactant poloxamer 188-N-acetylcysteine-loaded poly(lactic-co-glycolic acid) (P188-NAC-loaded PLGA) NPs	NAC	<i>P-selectin glycoprotein ligand-1</i> : E-selectin, P-selectin, L-selectin <i>P188 & NAC</i> : Reactive oxygen species	[189]
Stem cell therapy	Gold NP-Bax inhibiting pentapeptide-linker-modified(gene) (Au-NP-Ku70)	Ku70 Plasmid	Mesenchymal stem cell to secrete BDNF	[138]
	Neurogenin-2-loaded poly(β -amino ester)-based NPs	Neurogenin-2	Induce differentiation of hNSCs to neurons	[190]
	Stromal-cell derived factor-1 loaded Polydopamine NPs (PDA@SDF-1 α)	SDF-1 α	Receptor CXCR4/Guide stem-cell homing/ proliferate neuroblast	[191, 192]