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Nanomedicine for acute brain injuries: insight from decades of cancer nanomedicine

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Abstract

Acute brain injuries such as traumatic brain injury and stroke affect 85 million people a year worldwide, and many survivors suffer from long-term physical, cognitive, or psychosocial impairments. There are few FDA-approved therapies that are effective at preventing, halting, or ameliorating the state of disease in the brain after acute brain injury. To address this unmet need, one potential strategy is to leverage the unique physical and biological properties of nanomaterials. Decades of cancer nanomedicine research can serve as a blueprint for innovation in brain injury nanomedicines, both to emulate the successes, but also to avoid potential pitfalls. In this review, we discuss how shared disease physiology between cancer and acute brain injuries can inform the design of novel nanomedicines for acute brain injuries. These disease hallmarks include dysregulated vasculature, an altered microenvironment, and changes in the immune system. We discuss several nanomaterial strategies that can be engineered to exploit these disease hallmarks, for example passive accumulation, active targeting of disease-associated signals, bioresponsive designs that are ‘smart’, and immune interactions.

Keywords

Nanomedicine; traumatic brain injury; stroke; engineering design

Introduction

Acute brain injuries such as traumatic brain injury (TBI) and stroke are large contributors to declines in disability-adjusted life years (DALYs) worldwide and it is estimated that approximately half the world’s population will have at least one TBI in their lifetime. [1], [2] In the United States, TBI and stroke affect over 3 million Americans annually, at an estimated economic cost of 110.5 billion dollars. [3], [4] Although there are other causes of brain injuries such as anoxic brain injury or encephalitis, in this review we focus our discussion on TBI and stroke due to their prevalence. [5], [6] The predominant causes of TBI include blunt force trauma or a penetrating wound to the head after traffic accidents,

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falls, sporting activities, gunshot wounds, or assault. [5], [7] In stoke, blockage of an artery (ischemic stroke) or excessive bleeding from an artery in the brain (hemorrhagic stroke) causes an interruption of blood flow to the brain. [3], [5] In both TBI and stroke, initial causative injuries are followed by a secondary injury that can evolve over minutes to months, and is responsible for further deterioration of brain tissue through multiple mechanisms, including mitochondrial dysfunction, cell apoptosis, and inflammatory responses.

Although there are differences between TBI and stroke in disease initiation and the long-term pathophysiology of the subsequent secondary injury, there are a number of shared disease hallmarks. These disease hallmarks include neuronal excitotoxicity and apoptosis, vasculature dysregulation, upregulation of adhesion molecules, release of inflammatory mediators, and increased levels of reactive oxygen species (ROS) and proteases. [8]–[10] Current TBI treatments are merely palliative, and include life-sustaining interventions and surgery to minimize tissue damage. [11], [12] Neurological assessment with the Glasgow Coma Scale (GCS) and imaging of high-risk patients is used to gauge injury severity. [12] For patients identified to have large hematomas or contusions, rapid surgical intervention is crucial in order to restore blood flow and minimize tissue damage. Corrective surgery within 4 hours of clinic admittance demonstrated shorter hospital stays and a 50% lower mortality rate in TBI patients. [13] Similarly, in stroke there is a short time window for administration of reperfusion therapies in order to restore blood flow without increasing the risk of complications like intracerebral hemorrhagic conversion. [14] Thrombolytic therapy such as tissue plasminogen activator (tPA) injection must be delivered within ~4.5 hours after injury in order to be effective. [15] Additionally, endovascular thrombectomy of large-vessel clots is the standard of care for acute ischemic stroke and is typically performed 6-24 hours after stroke onset. [16], [17] While these interventions at acute time points are necessary to sustain life and mitigate tissue death in the brain, there is a need for new therapeutics that halt, attenuate, or ameliorate the pathophysiology of the secondary injury in the remaining tissue to improve the long-term disease management of acute brain injuries.

Despite the unmet clinical need, there has been a challenge to obtain approval for new therapeutics for the treatment of stroke and TBI by the food and drug administration (FDA). Although systemically administered therapeutics designed to address the secondary injury have improved biochemical and functional outcomes in preclinical animal models, they have failed to show efficacy in clinical trials. Potential factors that contribute to the failure of preclinical to clinical translation include the inability of a single drug to address the complex and heterogeneous disease sequelae that follows the primary injury, difficulty in recapitulating human disease using animal models, and the inadequate bioavailability of drug to the injured brain [18], [19]. In recent efforts, progesterone, a neuroprotective steroid that showed success in multiple preclinical TBI models, failed to demonstrate clinical endpoint improvement assessed by Glasgow Outcome Scale over placebo in both ProTECT III and SyNAPSe phase III clinical trials (2010-2013) for moderate-to-severe TBI patients. [20], [21] This latest trial is representative of the current landscape of clinical trials for brain injuries; 30 years of TBI clinical trials have yet to yield a single treatment that improves long-term brain health. [22] In stroke, a meta-analysis of therapeutics developed over a twenty year timespan from 1995-2015 established that only ~5% of drugs that entered the clinical trial pipeline reached the market in various countries when drugs in preclinical

testing were excluded from the analysis. [23] This success rate is considerably lower compared to the estimated 13.8% worldwide success rate (2000-2015) across all therapeutics made in another analysis [24], and is similar to the 3.4% success rate for oncology. Furthermore, the molecules under investigation predominantly target the clotting cascade (thrombolytic, anti-thrombotic or anti-platelet molecules) and therefore aim to prevent further damage and do not address the complex secondary injury brain pathophysiology. [23], [25], [26] The lack of clinical successes for both TBI and stroke highlights the need to innovate in therapeutic development for brain injuries.

Nanomedicines, therapeutic and/or diagnostic materials with dimensions on the nanometer length scale, have been actively developed for the treatment of cancer over the past two decades, and have yielded benefits such as decreasing off-target toxicity, improving drug distribution, and incorporating challenging cargoes such as hydrophobic small molecules and labile macromolecules. The first FDA-approved nanomedicine, Doxil®, is a polyethylene glycol (PEG)-modified liposome encapsulating the chemotherapeutic drug, doxorubicin. [27] Doxil® improves tumor accumulation due to its longer blood half-life and reduces off-target cardiotoxicity due to the altered organ biodistribution of nanometer-sized materials or ‘nanomaterials’. [28] In another example, FDA-approved Abraxane® uses albumin to formulate the hydrophobic molecule paclitaxel to improve solubility and biodistribution into target tumors. [29] The field of cancer nanomedicine has experienced significant research growth since its inception, supported by funding agencies like the National Cancer Institute’s Centers of Cancer Nanotechnology Excellence program. [30], [31] After the first FDA approval of Doxil® in 1995, five additional cancer nanomedicine technologies, including Abraxane®, have entered the clinical market in the United States and there are currently over thirty nanoparticle formulations are in the clinical trial pipeline [32]. Several of these nanomedicines innovate beyond passive tumor accumulation, employing technologies such as active targeting, gene delivery, and stimuli-responsive materials. [30], [32], [33] On the horizon, there are multiple promising nanomedicine technologies under development for application to cancer. For example, cancer vaccines require the delivery of multiple molecules to an antigen presenting cell that can educate the immune system; the supramolecular structure of nanomaterials provides technology to package cancer antigens and adjuvant into one entity and exploits the natural behavior of antigen presenting cells to phagocytose nanometer-sized materials. [34]

Nanomedicines are promising technologies to fulfill the desperate need for new therapeutics in the clinical management of acute brain injuries. Herein, we compare nanomaterial engineering as a discipline between cancer and acute brain injuries, and in particular nanomaterial design that leverages disease physiology. We also look for insight from two decades of largely empirical cancer nanomedicine research to inspire future innovation in nanomedicine design for brain injuries. Successful nanomedicine destined as a treatment for use in humans requires an understanding of the complex host biology and subsequent engineering of nanomaterials to interact with that biology. As such, in this review we elaborate on the perspective that cancer and acute brain injury share major disease pathologies that can be exploited by nanomedicine design (Figure 1, Table 1).

Common disease pathologies include dysregulation of the vasculature, increased access to cells and extracellular matrix in the tissue microenvironment, changes in the biochemistry of the interstitial fluid (e.g., ectopic ROS and protease activity), and an activated immune system. In both diseases, pathology causes vascular damage that activates a host response such as clotting or receptor expression which can be targeted by nanomaterials. Vascular damage also leads to permeability in vasculature that presents an opportunity for nanomaterials to passively accumulate into the parenchyma. [35]–[37] As a consequence, in disease, nanomaterials delivered in the systemic vasculature have increased access to the cells and microenvironment of the tissue, which in health is typically sequestered. [38] After brain injury, there is a wound bed that may have an improper healing response while cancer has been described as a “wound that never heals”. [39] The resulting wound microenvironment may have an extracellular matrix (ECM) composition that differs from physiologically healthy tissue, and an accumulation of disease-associated oxidative species and proteases. Nanomaterials can be engineered to respond to these changes in the microenvironment as bioresponsive materials, increasing the temporal and spatial specificity of therapeutic delivery. [40], [41] Lastly, both diseases have a dysregulated immune response that could be modulated by nanomaterials to either activate or suppress the immune system in cancer and acute brain injury respectively, or take advantage of endogenous immune cell homing to deliver therapeutics to the diseased tissue. [42]–[44] Although the goals for therapeutic outcomes between cancer and brain injuries may diverge (i.e. tumor cell killing vs. neuro-regenerative response), there are commonalities in the pathophysiology between diseases that can be leveraged for nanomaterial design.

The goal of this review is to highlight how nanomaterials can be engineered to interact with physiological changes that occur during acute brain injury; our discussion adopts a design-centered perspective and discussion of therapeutic payloads for nanoparticles is limited. For a detailed discussion on TBI therapeutic payloads that can be carried by nanomaterials, please refer to this excellent review. [45] Several routes of administration for nanomaterials to the brain are under investigation, such as intranasal, intrathecal, and convection-enhanced delivery (CED). [46]–[49] For example, intrathecal DepoCyt®, a liposomal formulation encapsulating the chemotherapeutic Cytarabine, was approved by the FDA in 2007 to treat lymphomatous meningitis caused by metastasis of non-Hodgkin lymphomas. [50] In patients with acute brain injuries, surgical intervention is often a necessary component of clinical care and creates an opportunity for direct access to the brain that circumvents many delivery barriers. While these routes of administration are promising, in this review we focus on intravenously administered nanomaterials as a minimally-invasive route of delivery commonly used in the clinical setting that is relevant in the treatment of both cancer and acute brain injury. Intravenous delivery can also be used as treatment regardless of whether patients receive surgical intervention.

Acute brain injury models

In order to discuss advances in acute brain injury nanomedicine, an overview of commonly utilized animal models is pertinent. Detailed discussions are available for TBI (Xiong [51]. Morales [52]. Wojnarowicz [53]) and stroke (Fluri [54]. Herson [55]. Jickling [56]). Common TBI animal models include controlled cortical impact (CCI), fluid percussion

injury (FPI), weight drop, penetrating brain injury (PBI) and blast injury. [51], [52] In CCI or FPI models, a craniotomy exposes the brain dura for direct focal injury via a controlled piston impact or fluid pressure pulse, respectively. In weight drop models, a guided weight falls on either an intact skull or exposed dura. CCI and FPI can be controlled and are highly reproducible but require craniotomy and therefore cannot reproduce disease physiology that includes the skull. PBI uses punctures instead of blunt trauma to simulate bullet or shrapnel wounds. Blast injuries simulate diffuse brain injury, such as injuries caused by the shock wave from military explosions, and is performed by placing the animal subject at the end of a shock tube that generates pressure waves. While maintaining the intact skull is more representative of human disease, reproducibility is difficult to control in closed skull injury models due to variability in how energy interacts with the skull. In these models, injury severity is modulated by varying injury velocity, depth, and size. [51] For ischemic stroke, the most widely used preclinical model is the middle cerebral artery occlusion model (MCAO) where a suture or filament is tied around or inserted into the middle cerebral artery. The occlusion can be permanent (pMCAO) or transient (tMCAO). The occlusion in tMCAO is typically maintained between 60-120 minutes followed by restoration of blood flow and is a model for ischemia/reperfusion injury in the clinic. [54], [57] Other models of stroke include photothrombosis and embolic stroke. In photothrombosis, light-responsive dye is injected into the brain and damages vascular structures when the skull is irradiated with light; this model is advantageous for the ability to spatially control lesion location. [58] Finally, the embolic stroke model, which best recapitulates human stroke etiology, initiates *in situ* clotting and ischemia by the injection of clotted blood, thrombin, or beads.

Dysregulated vasculature

Vascular dysregulation and dysfunction is pathophysiology shared between acute brain injury and cancer, and can be exploited by nanomaterials (Figure 2). In cancer, growing tumors generate new vasculature from nearby vessels networks through secretion of angiogenic factors, such as VEGF and angiopoietin. [59] The growth of new vessels in the tumor is rapid and poorly regulated, resulting in heterogeneous and dysfunctional vessels. Whereas normal vasculature involves the coordination of multiple cell types that have a well-regulated structure, tumor vasculature is disorganized, in particular the coordination of perivascular pericytes and smooth muscle cells around endothelial cells required to regulate oxygen and blood flow [35]. This disorganization leads to endothelial fenestrae, vesicles, transcellular holes, widened endothelial junctions, and a discontinuous basal membrane. [36] The defects in the vascular unit allows the passage of multiscale materials into the tumor that are typically excluded, including proteins, macromolecules, nanomaterials, and cells. [37]

The blood-brain barrier (BBB) describes the highly selective and regulated transport of multiscale materials (ions, molecules, proteins, etc.) from the blood into the brain parenchyma. The function of the BBB is created by the neurovascular unit, a precise spatial organization of multiple cell types, notably endothelial cells, pericytes, and astrocytes, although other cells are also observed to play a significant role. [38] In acute brain injury caused by ischemic stroke or TBI, BBB damage is a hallmark of disease and is imaged in the clinic for the purpose of diagnosis using medical imaging modalities such as MRI and CT. After acute brain injury, vasculature is severed by the physical force of the injury that

can result in endothelial cell death. In addition, weakened vascular structures can lead to the rupture of blood vessels and bleeding into the brain parenchyma, which present clinically as subdural hematomas and contusions. [60] Within the neurovascular unit, tight junction proteins between endothelial cells that are major barriers of paracellular transport become downregulated in response to injury, resulting in vascular permeability. The sized-based extravasation of multiscale molecules such as Evans blue dye, horseradish peroxidase, and dextran has been used to understand the extent of BBB permeability after the primary injury. [61]–[63] In addition, the morphologies and functions of pericytes and astrocytes can be altered after stroke and TBI. In both stroke and TBI, pericyte migration and the swelling of astrocytic end feet further compromise the integrity of the neurovascular unit. [64]–[66] In stroke, pericytes contract due to oxidative stress caused by the ischemic injury leading to constricted vasculature and reduced cerebral blood flow even after removal of the occlusion. [67] These changes in cellular morphology and function contribute to the secondary injury and further increase BBB dysfunction.

BBB permeability is dynamic. In TBI, expression of tight junction proteins contribute to BBB permeability; their expression increases within hours after the injury, peaks at 24 hours, and decreases back to baseline levels 5 to 7 days after the injury occurs. [68] There have also been observations of biphasic permeability, although the evidence is conflicting. Some studies show that the permeability is highest immediately after injury followed by a second opening occurring up to 3 days later. [69] The temporal study of BBB permeability after stroke also remains inconclusive. Some studies observe increased BBB permeability for up to 30 days after ischemic injury, while others show that BBB permeability is also biphasic, with maximum permeability at 3-5 hours after injury and then again at 48 hours. [70], [71] There is also evidence that vascular dysfunction after acute brain injury extends to chronic time points, weeks to months after injury. [72], [73] Conflicting accounts of permeability may be due to differences in animal models, strains of animals, and evaluation methods. Ischemic stroke also causes an increase in caveolae and vesicles in the endothelial cells of the BBB, indicating a potential increase in transcytosis after injury that could contribute to BBB permeability. [74], [75] These increases in the rates of endocytosis and transcytosis have been shown as early as 6 hours after tMCAO in adult male mice. [76] Similarly in cancer, recent studies have provided evidence to support the hypothesis that transcytosis is the major mode of entry of nanoparticles into tumors. [77] While further study is required to elucidate the mechanisms, increased BBB permeability has consistently been observed within hours after injury in multiple animal models of injury.

The tortuous and leaky vasculature present in tumors is the basis of passive transport of systemically administered nanometer-sized materials across multiple cancer types. [78] Once nanomaterials enter the tumor through the leaky vasculature, they are retained due to inadequate lymphatic drainage in the tumor microenvironment. This phenomenon was first described by Maeda et al. in 1986 who coined the “enhanced permeation and retention” (EPR) effect. Although the heterogeneity of the EPR effect in human tumors have recently been challenged [79], studies have correlated the presence of EPR (as measured by accumulation of nanoparticle MRI contrast agents) with the efficacy of nanoparticle-formulated drugs in human tumors [80], [81] These recent observations support a precision medicine approach to implementing EPR effect, in which patient EPR may be measured

prior to administration of nanoformulated therapeutic. [82] Nevertheless, the EPR effect is the conceptual basis of multiple FDA-approved liposome-based cancer treatments (e.g., Doxil®, DaunoXome®, Marqibo®, and Onivyde®) to increase the therapeutic window of toxic chemotherapeutics. Because passive accumulation of nanomaterials into the tumor is a function of time, these nanoformulated therapeutics benefit from increased blood circulation half-life. Accordingly, two of these formulations, Doxil® and Onivyde, are surface-modified with PEG to increase their blood half-life by avoiding clearance by the reticuloendothelial system (RES). [83]

An EPR-like effect has been observed for nanomaterials in acute brain injury; the vascular damage caused by the injury allows nanomaterials to passively accumulate in the injured tissue. In a one-hour tMCAO model, when PEG modified liposomes were delivered intravenously between 0 and 24 hours after reperfusion, they maximally accumulated in the injured tissue when administered up to 6 hours after reperfusion. [84] Similarly, in a CCI model of TBI, passive accumulation of liposomes after vascular delivery was highest when administered at 3 or 6 hours after injury compared to uninjured controls. [85] This transient access before 6 hours was also observed when electrostatically complexed nanoparticles were applied in a penetrating brain injury model. [86] Importantly, these studies demonstrate that the accumulation of vascular delivered nanomaterials in the context of acute brain injury is local to the site of injury and not widespread throughout the brain, and therefore provides a mode to passively target the injured tissue. Another major observation is that in brain injuries, the passive targeting of vascularly delivered nanomaterials into injured brain tissue is transient. In addition, passive accumulation of nanomaterials is size-dependent in TBI, with smaller nanoparticles up to 100 nm in diameter having greater distribution into the brain, while accumulation of nanoparticles 500 nm in diameter decreases by several orders of magnitude in comparison [72], mirroring the well-established observations of size-dependent nanoparticle accumulation in cancer. [72], [87], [88]

The passive accumulation of nanomaterials into the injured tissue can be exploited to improve treatment efficacies in models of brain injury. Cerebrolysin, a mixture of peptide growth factors including brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), is used to treat neurological disorders, such as dementia and Alzheimer’s disease, but its therapeutic utility is limited by a short *in vivo* half-life. Cerebrolysin was formulated into polylactic-co-glycolide (PLGA) nanoparticles to protect the labile peptide cargo, and intravenous administration between 30 minutes to 4 hours after injury to a rat model of TBI led to reduced brain edema and BBB breakdown compared to free drug. [89] In another example, conjugates of squalenoyl lipid and the neurological therapeutic adenosine yielded an amphiphilic prodrug that can form ~120 nm nanoparticles through nanoprecipitation. [90] These nanoparticles extended the circulation half-life compared to free drug and interacted with cells of the neurovascular unit. Administration into a pMCAO mouse model 2 hours after the induction of ischemia led to decreased infarct volume by an impressive 64% compared to vehicle control and a concomitant reduction in caspase-3 activation when tissues were analyzed at 24 hours.

Beyond the initial damage of the vasculature that allows the passive accumulation of nanomaterials from the blood, the clotting cascade is initiated for hemostatic regulation of

bleeding within minutes to hours after injury. A major challenge in the treatment of TBI is the clinical management of clotting; excessive clotting can lead to stroke whereas inadequate clotting can lead to brain hemorrhage. [91] A critical aspect is to manage clotting locally to the brain, an organ that is particularly vulnerable to changes in blood flow. The clotting cascade is initiated by damage to the endothelium that exposes basement membrane collagen and allows binding of the glycoprotein von Willebrand factor (vWF) present in the circulating blood. [92] The bound vWF at the damaged endothelium in turn binds and activates platelets that upregulate GPIIb/IIIa receptors (integrin $\alpha_{IIb}\beta_3$), leading to further platelet accumulation and the formation of a plug to halt bleeding at the site of injury. [92] In addition to platelet hemostasis, activation of both the intrinsic and extrinsic clotting cascades lead to processing of soluble blood fibrinogen monomers into an insoluble fibrin network surrounding the platelet thrombus. [92] Due to the rapid and robust formation of clots in response to vascular damage, the clot can be used as a beacon to recruit nanomaterials. In order to take advantage of the innate ability of platelets to incorporate into clots, iron oxide nanoparticles have been coated with platelet membranes and applied in a photothrombotic stroke model. [93] Combination of the natural platelet homing with application of a magnetic field led to maximal accumulation of materials at 6 hours post-injection and this strategy was used to deliver L-arginine for *in situ* production of nitric oxide in order to increase blood reperfusion of the ischemic tissue. In another strategy to use clots as a way to target nanomaterials to the injury site, PLGA nanoparticles modified with RGD peptide was used to target glycoprotein receptors on the surface of platelets in a rodent blast injury model with polytrauma. [94] When rodents were treated with these nanoparticles encapsulating the anti-inflammatory dexamethasone, there was increased survival due to decreased internal lung hemorrhaging, improved BBB integrity, and reduced astrogliosis within the amygdala of the brain. Apart from targeting the clotting cascade to achieve greater accumulation, nanoparticles can also be engineered to interact with the clotting cascade itself to create hemostatic materials. This strategy has been pursued in peripheral injuries, and include strengthening the clot with synthetic polymers modified with clot-binding peptides [95] or homeostatic nanomaterials that release drug in response thrombin activity. [96] In the future, hemostatic technologies can be developed in the context of the specific challenges presented in acute brain injuries. [97]

The neurovascular unit itself is dysregulated hours up to months after injury and can upregulate molecules that can be targeted by nanomaterials to deliver therapeutics to the site of injury. [98] Receptors for glutathione, apolipoproteins, and low-density lipoproteins (LDL) are upregulated on the endothelium after injury, and create a potential “vascular zip code” that can be targeted by materials delivered into the blood. [99] In order to interact with LDL receptor related protein (LRP1) on the surface of the BBB for the delivery of BDNF, poloxamer 188 and BDNF were adsorbed onto the surface of 200 nm PLGA nanoparticles. [100] When these nanomaterials were injected into the weight drop model of TBI three hours after injury, the addition of poloxamer 188 significantly increased the levels of BDNF in the brain compared with nanoparticles without poloxamer 188 and free BDNF in both injured and sham injured mice. Mice treated with PLGA nanoparticles modified with BDNF and poloxamer 188 showed significant improvements in behavioral testing seven days after injury (motor, reflex, balance, and cognitive function) compared to control groups treated

with nanoparticles and BDNF without poloxamer 188. Similarly, chitosan modified with the temperature-sensitive N-Isopropylacrylamide (NIPAAm) was used to encapsulate the hydrophobic neuroprotective molecule riluzole, followed by surface coating with the surfactant Tween 80 to create 50 nm nanoparticles. [101] Intraperitoneal delivery immediately upon reperfusion after a one-hour ligation in a rat tMCAO model led to decreased infarct volume, decreased lipid peroxidation, and increased antioxidant markers. Peptides have also been used to interact with the BBB after injury. In a dual targeting strategy to sequentially cross the BBB and interact with ischemic tissue, Zhao et al. used two peptides: T7 peptide (HAIYPRH) is a ligand of the transferrin receptor on brain endothelium and SHp (CLEVSRKNC) was identified by phage display to preferentially target ischemic tissue in stroke. [102] When administered immediately after reperfusion in a two-hour tMCAO model, dual targeted ~100 nm liposomes modified with both peptides and encapsulating the small molecule inhibitor ZL006 had an increased accumulation in the ischemic injury at 6 and 24 hours after administration when compared with untargeted or singly targeted liposomes. Increased accumulation of dual targeted liposomes was consistent with decreased infarct volume and decreased neurological severity scores over free drug.

Microenvironment

The cellular and non-cellular components of the microenvironment are unique to a given tissue and the homeostasis of the microenvironment is perturbed in disease. [103] In the tumor microenvironment, there is ectopic expression of receptors on stroma and tumors cells, changes in the ECM composition, and accumulation of metabolic molecules. Due to the need to penetrate deep into solid tumors for effective treatment, investigators have engineered nanomedicines that capitalize on the changes in the tumor microenvironment to increase treatment efficacy. For example, antibody-targeted liposomes or “immunoliposomes” that target cancer cells or tumor stromal cells have been extensively developed to increase anti-tumor efficacy and decrease off-target activity of chemotherapeutic treatments. [104] Doxorubicin liposomes modified with antibodies against epidermal growth factor receptor (EGFR) are currently in phase II clinical trials for treatment of triple negative EGFR-positive breast cancer (ClinicalTrials.gov identifier: NCT02833766) based on solid tumor preclinical and phase I clinical trials. [105], [106] There are also major changes to the ECM in cancer [103], creating a reservoir of disease-specific substrates for therapeutic binding. For example, polylactic acid (PLA)-PEG nanoparticles have been modified with CLT1, a peptide discovered by phage display, to bind to enriched fibronectin in gliomas, leading to increased accumulation after intravenous delivery and improvement of survival of animals when used to deliver paclitaxel. [107] In recent work, Ishihara et al. created protein engineered fusions of the anti-tumor cytokine interleukin-2 with a collagen-binding domain to bind to the collagen-rich stroma of tumors and temper the well-documented toxicity of interleukin-2. [108] In this approach, they showed reduced interleukin-2 in the blood due to enhanced tumor accumulation, attenuated tumor growth in three different tumor models, and increased populations of cytotoxic T cells over regulatory T cells.

Similarly, nanomaterials can be tailored to the specific microenvironment created after acute brain injuries. We will discuss two major modes in which nanomedicines can interact with

the microenvironment of acute brain injury. The first mode is based on access; typically the brain parenchyma is sequestered from the periphery by the selective and highly regulated BBB. In brain injuries, the passive accumulation of nanomaterials through disrupted vasculature allows access to the unique microenvironment of the brain, including cells and ECM. The second mode is based on changes that occur in the microenvironment after acute brain injuries; the disease progression during the secondary injury creates changes in the ECM composition of the brain, leads to ectopic protease activity, and generates the metabolites such as reactive oxygen species. [45], [109]

Targeting nanomaterials to cells and molecules in the microenvironment

Cells and molecules of the brain are sequestered by the intact BBB in the healthy brain and become transiently accessible to intravenously delivered nanomaterials after acute brain injury, as discussed above. The goal of numerous therapeutic strategies is to protect neurons, identified as the major cell type responsible for functional deficits seen in patients of brain injury, but also neurodegenerative diseases. As such, nanoparticles have been modified with peptide to target neurons once they have passively accumulated into the brain. Electrostatic peptide-siRNA complexes incorporating RVG, a peptide sequence from a coat protein of rabies virus used to target neurons [110], accumulated with 80% specificity in neurons over other cells of the brain parenchyma in a model of penetrating brain injury. [86] These nanomaterials formulated with siRNA against caspase 3, an important protein in the apoptotic cascade, were able to downregulate caspase 3 protein by ~80% in the injured brain when administered immediately after injury. These same siRNA and peptide components were able to accumulate into the injured brain and mediate silencing when they were encapsulated into the pores of an inorganic porous silicon nanoparticle using a non-covalent calcium silicate trapping chemistry. [111]

In addition to targeting cells, the ECM also presents a potential strategy to increase the retention of nanoparticles in the injured tissue due to a potential large extracellular surface area available for binding. The ECM in the healthy brain is largely composed of the glycosaminoglycan hyaluronic acid and chondroitin sulphate proteoglycans, heparin sulfate proteoglycans, and glycoproteins, with relatively low levels of collagen, fibronectin, and laminin compared to other tissues. [112], [113] The relative levels and spatial localization of ECM constituents is thought to change in brain pathology. [109], [114] In the injury response, cells release cytokines and matrix metalloproteases to remodel the ECM. In the injured brain, ECM components including chondroitin sulfate proteoglycans (neurocan, aggrecan, NG2), heparin sulfate proteoglycans (syndecans, perlecan, agrin), fibronectin, tenascins, and laminin are deposited around the lesion site. [109], [115]

In order to take advantage of this change in the ECM after acute brain injury, the Ruoslahti group used *in vivo* phage display to identify the peptide motif CAQK to bind to the injured brain tissue six hours after initiation of a penetrating brain injury model. [115] They identified the receptors of CAQK as veriscan and tenascin-R, proteoglycans known to be upregulated after injury. [109], [116], [117] Modification of nanomaterials with CAQK led to increased accumulation into the injured brain tissue, and CAQK-modified porous silicon nanoparticles carrying siRNA achieved efficient gene silencing. In support of the potential

therapeutic translation to humans, CAQK peptide conjugated to silver nanoparticles also bound to cortical brain sections of a human TBI patient ex vivo. A separate group used CAQK in a self-assembled coiled-coil protein nanoparticle sensitive to thrombin cleavage to deliver a neuroprotective peptide, Tat-NR2B9c. [118] In the CCI model of TBI, CAQK-modified protein nanomaterials had a greater than 2-fold accumulation in the injured brain compared to untargeted nanomaterials when administered immediately after injury, and treatment led to reduced lesion volumes and improvements in behavioral testing, indicating this peptide ligand could be applied across nanoparticle platforms and to both penetrating and non-penetrating TBI. Future work remains to fully delineate the unique composition of brain ECM in health and disease, including its spatial localization near the injury and vasculature in order to fully harness its potential as a target for nanomaterials.

Bioresponsive Nanomaterials

Bioresponsive or ‘smart’ nanomaterials are designed to autonomously react to stimuli without external triggers. Bioresponsive nanomaterials have been engineered to respond to stimuli in the tumor microenvironment, including acidic pH, increased reactive oxygen species (ROS), hypoxia, or increased proteolytic activity, as reviewed elsewhere. [119], [120] These stimuli can lead to physical changes in the nanomaterials such as swelling, disassembly, degradation, or precipitation that actuate a response, such as the release of therapeutic molecules or generation of a diagnostic signal. Because the presence of the stimuli is typically restricted spatially to the microenvironment of the diseased tissue and manifests temporally with disease progression, therapeutics designed as bioresponsive nanomaterials can have increased specificity. In a diagnostic technology, Lumicell Inc. has developed a nanoscale polymer-peptide fluorescent imaging agent that is cathepsin-activated, LUM015 (clinicaltrials.gov NCT01626066), to aid resection of residual breast cancer during lumpectomy that has completed Phase 2 clinical trials. [121], [122] In acute brain injuries, there are changes in the microenvironment that could be used to activate bioresponsive materials; we will focus our discussion on materials that respond to ROS and enzymes as technologies that have been the furthest developed at this point in time.

In acute brain injuries, cell death, neurovascular inflammation, and mitochondrial dysfunction initiated by the primary injury cause an increase in the concentration of ROS, including superoxide anion, hydrogen peroxide and nitric oxide. [123] Excessive ROS levels quickly deplete endogenous antioxidants within the brain microenvironment, increasing the peroxidation of lipid membranes and the oxidation of proteins that initiate apoptotic pathways in surrounding cells through inhibition of the electron transport system in mitochondria. [124] This identifies ROS as a potential therapeutic target to mitigate further damage in brain injuries and also a microenvironmental stimulus for bioresponsive nanomaterials. Core-cross-linked nanoparticles made from polysorbate 80 created a thioether core that scavenges excess ROS in the injury microenvironment. [125] These materials accumulated in the damaged brain in a CCI model of brain injury after systemic administration immediately after injury, reduced neuroinflammation in the hippocampus, and improved behavioral outcomes. Another ROS scavenging nanomaterial was created with poly(propylene sulfide) nanoparticles. These materials decreased neuroinflammation in 17-minute tMCAO mice when administered 3 hours after reperfusion, including decreased

microglial activation and reduced neutrophil infiltration, and led to reduced infarct volume compared to vehicle controls. [126] Hydrophilic ~50 nm carbon clusters have been developed as a ROS scavenging nanomaterial after acute brain injury. [127] The graphitic domains of the carbon cluster sequester ROS through covalent bonding. When carbon clusters were administered 80 minutes after injury, cerebral blood flow was restored to baseline pre-injury measurements in a rat CCI model. On a molecular level, carbon clusters were found to reduce vascular superoxide levels and normalize nitric oxide levels when measured 6 hours after treatment. These ROS-scavenging technologies have also been applied to cancer. [128] Lastly, nanomaterials can be engineered to release therapeutic in response to elevated ROS in injury. Lv et al. created a nanomaterial with a polymeric core modified with ROS-responsive boronic ester that encapsulated the neuroprotective peptide NR2B9C. [129] These cores were wrapped with a red blood cell shell modified with the stroke homing peptide SHp to form ~200 nm nanomaterials. In response to high concentration of ROS, such as found intracellularly in ischemic neurons, the boronic acid core hydrolyzes to release therapeutic cargo with an in vitro half-life of >4 hours. Application of this ROS-responsive nanomaterial immediately after reperfusion in a rat model of two-hour tMCAO model led to a significant decrease in infarct area and improvement in neurological score compared to free drug.

Proteases are important regulators of the microenvironment, and have important roles in the progression of cancer and during the immediate and chronic response after brain injuries. [130]–[132] In brain injuries, thrombin and the family of matrix metalloproteases (MMPs) in particular are important for regulating neuroinflammation, degradation of ECM components, and function of the BBB. [133], [134] The disease-specific expression of proteases can be used to mitigate the toxic side effects common for drugs. For example, glyburide is an FDA-approved diabetes medication that blocks SUR1-TRMP4, an ion channel that is upregulated in the neurovascular unit after stroke implicated in edema and hemorrhage, but is dose restricted due to the risk of hypoglycemia. [135] In order to increase disease-specific release in stroke, glyburide was encapsulated in micelles formed from poly-(e-caprolactone) and PEG block copolymers; the polymer blocks were linked with either a thrombin- or MMP-9-cleavable peptide. [136] The design was such that cleavage by proteases released PEG from the outer shell of the micelle, leading to a smaller diameter nanoparticle that could distribute further into the brain parenchyma. Nanomaterials made with thrombin-sensitive polymers had a 5.5-fold higher accumulation into the infarct of a 90-minute tMCAO mouse model compared to nonresponsive nanomaterials, and the delivery of glyburide using this bioresponsive shrinking nanoparticle decreased infarct size and improved neurological scores compared to the vehicle and free glyburide controls with multiple administrations of 0, 24, and 48 hours after surgery. Thrombin-reactive nanoparticles can also be used for site-specific dissociation of clots in order to prevent vessel occlusion. A sequential biomimetic approach for the dual delivery of neuroprotective and thrombolytic drugs was engineered by coating a polymeric core carrying the small molecule neuroprotective drug ZL006e with a platelet membrane that naturally accumulates at the site of clots. [137] The surface of the platelet was modified with the cell penetrating peptide Tat sterically shielded by the recombinant tissue plasminogen activator (rtPA) linked to the membrane with a thrombin-sensitive linker. In the presence of thrombin activity, the

thrombolytic rtPA was released to dissociate the clot and also revealed Tat peptide on the nanomaterial surface to increase transport into the brain. When intravenously administered in a two-hour tMCAO model, this dual delivery, protease-sensitive nanomaterial decreased the ischemic area an impressive ~60% compared to no treatment and ~40% compared to animals treated with free drug or nanomaterial with linker insensitive to thrombin cleavage. Protease activity can also be used in diagnostic nanomedicines for acute brain injuries. Similar to the fluorescent cancer diagnostic material LUM015 developed by Lumicell Inc., [121], [122] a diagnostic nanomaterial responsive to TBI-associated proteases was engineered. [138] In this work, a fluorogenic substrate sensitive to calpain-1 cleavage was attached to a polymeric nanomaterial carrier in order to increase its passive accumulation into injured tissue. When applied to a CCI model of TBI, fluorescence signal from this nanosensor was detected in the injured tissue compared to uninjured brains, indicating an increase in calpain-1 activity. Future work in bioresponsive materials for acute brain injuries can couple disease stimuli, such as ROS or protease activity, and bioresponsive therapeutic delivery for more precise treatments.

Nanomaterial penetration in the brain microenvironment

The abnormal vasculature, poorly developed lymphatics, high interstitial fluid pressure, dense ECM, and necrotic regions in the tumor core are obstacles to nanomaterial access throughout the entire tumor. [139] For example, liposomes 90 nm in diameter are observed to predominately accumulate in the tumor periphery near vasculature. [140] While nanomedicines such as Doxil® and Abraxane® have longer blood half-lives and reduced off-target effects, they do not exhibit significant improvements in therapeutic efficacy over free drug due to poor penetration into the tumor microenvironment. [140], [141] Similarly in acute brain injury, there is a necrotic injury core surrounded by tissue that is damaged but not yet dead, called the traumatic or ischemic penumbra. Attenuating, halting, or ameliorating the secondary injury in the penumbra is the goal of many therapeutics in order to mitigate widespread tissue damage after acute brain injury. Due to BBB dysfunction that is common in secondary injury, the vasculature in the penumbra has increased permeability [142], [143], presenting a possible entry point for nanomaterials into the injured tissue. Although there have been observations that systemically delivered nanomaterials can reach the core of the injury in stroke models, [144] tissue access is likely influenced by multiple factors, including severity of injury, size of nanomaterials, and also the time delay between the injury and nanomaterial administration. [72], [84]–[86] Based on the context of the injury and when materials are administered, the penetration of nanomaterials throughout the injured brain tissue is one potential obstacle to effective nanomedicines for acute brain injury.

One approach to increase distribution of nanomaterials throughout the tissue microenvironment is through engineering their physicochemical properties and surface chemistries. In our discussion of passive delivery above, we have already discussed how nanomaterial infiltration can be size-dependent. Nance et. al. reported that nanoparticles modified with a dense PEG surface 100 nanometers and smaller can distribute widely when applied ex vivo to healthy rat and human brain samples and in vivo through direct brain injection. Through analysis of nanoparticle diffusion ex vivo, they estimated that pore sizes

in the brain extracellular space are as large as 200 nm and that more than one-quarter of pores are at least 100 nm in size. [145] In this study, surface properties also affected nanoparticle diffusion within the brain; nanoparticles with a dense PEG surface exhibited significantly increased diffusion compared to the same nanoparticle cores with carboxyl surfaces. The Saltzman group observed that poly(lactic acid) nanoparticles modified with PEG or hyperbranched glycerol surfaces have decreased cellular uptake compared to nanoparticles modified with 'bio-adhesive' aldehyde surfaces when administered via convection enhanced delivery (CED) to healthy brains or glioblastoma tumors. [146] These papers establish groundwork for an early understanding of how nanomaterial properties may affect distribution and cellular interactions within the brain microenvironment, however more studies are warranted to establish systematic design rules.

Tumor-penetrating peptides are a biological approach to increase distribution of nanomaterials throughout tumors. The Ruoslahti group identified a tumor penetrating C-end-rule (CendR) sequence, consisting of a C-terminal R/KXXR/K motif, through in vivo phage display, a method for screening a large diversity of peptides in a living organism. [147], [148] One tumor-penetrating CendR peptide, iRGD, combines the RGD integrin binding motif with the CendR motif. [148] After RGD binding with the primary integrin receptor, proteolytic cleavage exposes the CendR motif that can bind to the secondary receptor neuropilin-1, which mediates transcellular transport and penetration into the tissue. [148] Importantly, iRGD peptide could increase the penetration of nanomaterial both when covalently modified to the surface of nanomaterials, or when co-delivered with nanomaterials. In a model of prostate cancer that develops a thick tumor stroma, co-injection of iRGD with liposomal chemotherapeutics led to ~40% tumor shrinkage compared to co-injection with non-penetrating RGD control. [149] These penetrating peptides have yet to be explored for nanomaterial delivery to acute brain injuries, although we note that one primary receptor for iRGD, $\alpha_v\beta_3$ integrin, is known to be expressed in wound beds and the secondary receptor, neuropilin-1, is known to be upregulated in hypoxic tissues. [150]

Immunomodulatory nanomaterials in brain injury

Engineered nanomaterials are attractive technologies to interface with the immune system and there has been rapid development of these materials for applications in cancer. Nanomaterials are supramolecular assemblies and therefore they can deliver antigens and adjuvants (e.g., CpG oligonucleotides, imidazoquinoline, monophosphoryl lipid A, or plant virus proteins) to the same cell for the purpose of vaccination [34]. In addition, nanometer scale materials carrying diagnostic or therapeutic payloads are naturally phagocytosed by antigen presenting cells (e.g., dendritic cells and tumor-associated macrophages (TAMs). For example, maleimide-modified PEG nanoparticles have been used to capture circulating tumor antigens released after radiotherapy and subsequently be phagocytosed by dendritic cells to enhance the abscopal effect of anti-tumor immunity. [151] Nanoparticles can also mimic immune cell behavior, such as target binding, T cell activation, and tissue infiltration. Polymeric PLGA nanoparticles wrapped with neutrophil membranes used the endogenous affinity of neutrophils to bind circulating cancer cells to deliver the proteasome inhibitor carfilzomib after intravenous delivery. [152] Metal-organic framework nanoparticles, utilized for *in vivo* fluorescence imaging, were coated with a fusion of dendritic and cancer

cell membranes to simultaneously mimic a dendritic antigen presenting cell and present tumor antigens to provide efficient T cell co-stimulation as a single nanomaterial. [153] In addition to direct stimulation of T cells, this material could also be phagocytosed by dendritic cells for indirect stimulation of T cells. This nanomaterial, termed a cytomembrane nanovaccine, homed to the lymph node *in vivo* after subcutaneous injection and successfully immunized 60% of mice against a tumor challenge. Lastly, immune cells can be used to increase the delivery of nanomaterials into solid tumors. Nanoparticles carrying chemotherapeutics have been attached *ex vivo* to T cells to exploit the innate infiltration of T cells for delivery of adjuvant (IL-15 super-agonist) and chemotherapy (topoisomerase I poison SN-35) in several tumor models. [154]–[156] Advantages of cell-mediated therapeutic delivery include improvement in the therapeutic window due to increased biodistribution to the tumor.

The immune system in acute brain injury after TBI and stroke plays an integral role in disease progression and resolution. [43], [157] Like TAMs, resident microglia and macrophages are aberrantly activated after injury. The therapeutic goal in the brain is regeneration, as opposed to cancer killing, and therefore the polarization of macrophages and microglia between pro- to anti-inflammatory states requires precise regulation during the multiple phases of injury resolution. For a detailed discussion of the immune response in stroke and TBI, see excellent reviews by Loane and Kumar [158], Gyoneva and Ransohoff [159], and Kawabori and Yenari. [157] In summary, the elevation of danger associated molecular patterns (DAMPs) released by apoptotic neurons bind to toll-like Receptors (TLRs) on microglia and tissue-resident macrophages. [158] TLR activation leads to transcriptional upregulation of inflammatory genes, such as interleukins and TNF-α [43], [157] to polarize immune cells to an inflammatory “M1” phenotype. Inflammatory immune cells generate arachidonic acid metabolites, nitric oxide (NO), ROS, and MMPs that perpetuate the inflammatory response. [157], [158] Simultaneously, astrocytes, microglia, and oligodendrocyte progenitor cells become reactive, leading to gliosis as a hypothesized mechanism to sequester tissue damage from healthy tissue. [160] Adhesion molecules, including selectins, IG, and integrins, upregulated on damaged endothelium leads to the recruitment of leukocytes from the blood. [157] The infiltrating leukocytes, mainly neutrophils and monocytes, release cytokines, ROS, and MMPs that perpetuate the inflammatory microenvironment leading to tissue damage and cell death.

Based on the destruction a sustained proinflammatory response can cause after acute injury, there has been significant effort to improve the delivery of anti-inflammatories, such as glucocorticoids, NSAIDs, TNF-α inhibitors, IL-1β inhibitors, and drugs with pleiotropic mechanisms such as statins. [161] Due to the endogenous phagocytic activity of microglia, a common goal for nanomedicines is to deliver immunomodulatory molecules to polarize microglia to an anti-inflammatory state. The ability for nanomaterials to accumulate in microglia has been demonstrated with poly(amidoamine) (PAMAM) dendrimers after intravenous delivery in several models of brain inflammation. [162], [163] In a pediatric rabbit CCI model of TBI, PAMAM dendrimer modified with a triphenylphosphonium (TPP) mitochondrial targeting moiety demonstrated accumulation in the mitochondria of microglia when delivered 6 hours post-TBI. [164]

In a different approach, nanoparticles can be used to deliver cytotoxic agents to deplete infiltrating neutrophils and monocytes in order to reduce the tissue damage, since these cells are known to exacerbate the destructive injury response. [165] In one example, albumin nanoparticles linked with cytotoxic doxorubicin through an acid-labile hydrazine bond accumulated in activated phagocytic neutrophils in the blood to induce apoptosis and prevent their infiltration into inflamed tissue. [166] The hydrazine bond was designed to be stable in circulation but degrade in the acidic endosome of neutrophils after phagocytosis. Systemic administration of these nanoparticles one hour after reperfusion in a tMCAO model depleted blood neutrophils and reduced neuroinflammation, as measured by brain levels of TNF-α, IL1β and IL-6. Depletion of monocyte infiltration into brain tissue represents another immunomodulation strategy. Highly anionic 500nm polymeric nanoparticles injected 2, 24, and 48 hours post-CCI bound to blood monocytes *in situ* via the macrophage receptor with collagenous structure (MARCO), leading to a 84.5% decrease in monocyte infiltration into the brain 3 days post-injury compared to vehicle control. [167] This decrease was concomitant with decreased lesion volume and ventricular volume, and improvements in functional behavior up to 6 months post-treatment. A similar functional outcome was reproduced when these same nanoparticles were applied to a closed head injury (CHI) model. In another study, prophylactic delivery of clondronate liposomes in a CCI model showed both beneficial (reduced ventricle enlargement) and detrimental (increased BBB permeability) outcomes with no effect on lesion volume and edema, illustrating the complicated role immune cells may have in acute injury. [168], [169] Although prophylactic treatment of TBI is a clinically unrealistic treatment strategy, these preclinical data may provide clues for future research. While using nanomaterials to deplete phagocytic immune cells is a promising strategy, further research is needed to illuminate the role of these cells in brain injury pathophysiology.

Based on the natural infiltration of innate immune cells into the brain after injury, mimicry of these cells can increase nanomaterial accumulation into the brain, where the BBB is a formidable barrier to access. In one approach, ~190 nm nanovesicles were made from a neutrophil cell line HL-60 to mimic neutrophil infiltration into the injured brain. [170] Nanovesicles loaded with the therapeutic molecule resolvin D2 maintained major surface proteins found on neutrophils (integrin β2, PECAM-1, PSGL-1, and VLA-4) and trafficked to the injured brain endothelium after intravenous injection one hour post-reperfusion in a tMCAO mouse model. The therapeutic mechanism of resolvin D2 is to reduce leukocyte-endothelium interactions, and treatment of the tMCAO model with resolvin D2 loaded neutrophil nanovesicles led to decreased neutrophil infiltration and reduced levels of inflammatory cytokines TNF-α, IL1β and IL-6 in the injured brain compared to no treatment controls.

Nanomaterials have also been used to “hitchhike” onto infiltrating immune cells, wherein nanomaterials can modify leukocytes in the blood and translocate into the brain based on the endogenous chemotaxis of these cells. For example, liposomes modified with circularized RGD targeting peptide targeted overexpressed αvβ3 integrin receptors on activated blood monocytes and neutrophils after tMCAO and become phagocytosed. [171] Liposomes labeled with dye accumulated in neutrophils and monocytes in the bloodstream, co-migrated across the BBB into the injured lesion, and transferred to neurons and microglia in the

microenvironment. In related studies in models of lipopolysaccharide (LPS) induced brain inflammation, 30 nm superparamagnetic iron oxide nanoparticles with oleic acid and amphiliphilic polymer coating (SHP-30, Ocean NanoTech) were attached *ex vivo* to bone marrow-derived monocytes and intravenously injected. [172] These adoptively transferred cells carrying nanoparticles could be detected by flow cytometry in the inflamed brain 14 hours after injection. Additionally, macrophages modified with a layer-by-layer disc-shaped polymer patches 7 μm in diameter and 600 nm in height via an anti-CD11b antibody were able to infiltrate into brains with LPS-induced inflammation. [173] These studies demonstrate nanomaterials can modulate the natural activity of the immune system or exploit the natural trafficking of immune cells for therapeutic benefit in acute brain injuries.

Path to clinical translation

We have discussed preclinical research on nanomaterial designs that can intelligently interact with host biology for application to acute brain injuries. Before successful translation of these nanomaterials from preclinical studies into humans, there remain several obstacles. Many of the obstacles are attributed to the translation of therapeutics for acute brain injuries in general, for example inadequate understanding and control of the pharmacokinetics and pharmacodynamics of therapeutics (biodistribution, half-life, dose-response curve, etc.), limitations in preclinical models to represent the heterogeneity of disease phenotype, understudied biological variables such as age and sex, and lack of specific biomarkers that can predict long-term outcomes. While nanomaterials are a potential solution to these hurdles, such as improvements in the pharmacokinetic and pharmacodynamics, they also pose their own complications. For example, there are challenges associated with the scale-up and manufacture of nanomaterials and their path for FDA-approval is complicated due to their multiple components. We will focus our discussion on the obstacles to translation as they relate to nanomaterials. For a comprehensive discussion of clinical translation of therapeutics for brain injuries please see the excellent reviews by Stein [22], Loane and Faden [19], and Bosetti [174]. The translation of nanomaterials are discussed in reviews by Hua and Storm [175] and Anselmo and Mitragotri [176].

One major hurdle to clinical translation of therapeutics for brain injuries is to achieve desirable pharmacokinetic profiles, most notably increased accumulation and retention in the brain and reduced accumulation in off-target organs. Formulation of drugs as nanomaterials offer potential solutions. For example, Doxil ® is chemotherapeutic formulated in PEG-modified ~100 nm liposomes; the large size reduces off-target organ accumulation to mitigate toxicity and the PEG reduces interaction with phagocytes to increase circulation times [176]. Besides passive targeting, active targeting via surface modification with peptides (RGD, RVG, CAQK) or antibodies is one avenue to increase nanomaterial selectivity to cell types and retention in brain tissue, although active targeting has yet to be implemented in clinically-approved nanomaterials. [32], [176] In addition to regulating organ-level biodistribution, increasing nanoparticle penetration through biological barriers, such as the BBB and brain tissue, is another delivery challenge. [176] Further investigation is needed into the effects of nanoparticle size, charge, and other physiochemical properties on pharmacokinetics. [145], [146]

Accurate quantification of therapeutic bioavailability in target organs and scaling preclinical dosage levels to humans is necessary for translation; ambiguity in effective progesterone dose and schedule has been speculated to be one reason for lack of efficacy observed in the ProTECT III and SyNAPSe III clinical trials. [22] The accurate quantification of nanomaterials which do not have intrinsic imaging properties *in vivo* is one difficulty because traditional organ collection and histology is not applicable in a clinical setting. [176] Bioresponsive nanomaterials that only release drug in the presence of stimuli are one potential solution that would alleviate the need for precise dosing, since therapeutics could be engineered to release only in the presence of disease and could therefore increase the therapeutic window. For example, self-titrating hemostatic nanoparticles are engineered to release heparin only in the presence of thrombin activity. [177]

Testing promising therapeutic candidates in preclinical animal models of brain injury is a mainstay of research and is a requirement for the FDA to support clinical trial feasibility. The utility of animal modeling is indisputable given the scientific precedence, availability, low cost, and ability to screen a multitude of therapeutic agents. [53] Using Bayesian analysis, Goodman et al. estimated that preclinical testing increases the likelihood of clinical success by several orders of magnitude. [178] Despite the value of preclinical testing for therapeutic translation and the availability of different types of injury models as discussed above, the limitation of animal models to recapitulate human biology in response to an acute brain injury must be considered. [51], [53], [55], [56], [174] For one, rodents have a different brain geometry and white-to-gray matter ratio, among other anatomical differences. [51], [52] Several variations in innate and adaptive immune response also exist, including percentage of neutrophil populations in blood and chemokine receptor expression. [179] These biological discrepancies can result in differences in acute brain injury sequelae between humans and rodents. For example, gene expression patterns can differ in mice and humans after ischemic stroke. [56], [180]–[185] Additional factors including the use of anesthesia and post-operative analgesia during induction of injury in animal models in compliance with animal welfare guidelines [186]. Finally, the patient population enrolled in clinical trials is typically heterogeneous and represent different ages, genders, pre-morbid conditions, injury origins and disease severity. Preclinical studies often fail to match the heterogeneous nature of the clinical patient population because the majority of experiments employ generally one optimized injury model in healthy adult rodents of the same strain and gender; rarely are geriatric, pediatric, or female mice represented in studies. More research is needed to delineate the role age plays in both TBI pathology and therapeutic response [187] given the risk of TBI is higher among children, young adults, and those over 75 years of age. [188] Variations in rodent strain, [189] age, [190], [191] and sex [192] may influence measured outcomes and potentially confound results. For example, the sex hormones estrogen and progesterone may be neuroprotective after TBI in female mice compared to their male counterparts. [192], [193] While biological differences between rodents and humans are intrinsic to preclinical testing, increasing awareness of the factors that are within experimental control and rigorously defining aspects of models that accurately predict disease phenotype in humans is important in future design of clinical trials based on preclinical research. [53]

A final hurdle for clinical translation stems from the complexity of acute brain injury pathology in the clinic. The most commonly utilized injury assessment metrics in the clinic, Glasgow Coma Scale (GSC) or Glasgow Outcome Scale-Extended (GOS-E) for TBI and modified Ranking Scale (mRS) for stroke, involve a series of questions to qualitatively assess neurological deficits and quality of life in patients. [5], [15] However, analysis of neurological surveys like GSC often lack sensitivity in distinguishing injury severity and stage which make monitoring patient outcomes difficult. [194] The questionnaire format of GSC, described as a “blunt instrument”, can potentially miss small improvements in patient outcome. [22], [174] The subjective nature of GSC, GOS-E and mRS contrast with preclinical testing which typically uses multiple quantitative and functional endpoints to assess therapeutic efficacy. [22] In addition to selection of appropriate endpoints, the complex continuum of acute and chronic secondary injury clouds determination of appropriate timelines for therapeutic intervention. [19] Matching therapeutic dose schedules gleamed from preclinical studies to timepoints in the clinic can be difficult or unrealistic. [195] For example, a significant number of preclinical studies employ prophylactic or short timepoints post-injury for therapeutic delivery, whereas it can take up to 6 hours for patients to present with TBI, provide informed consent, enroll in a clinical trial, and receive therapy. [19] In the ProTECT III and SyNAPSe III clinical trials, treatment was administered within 4 hours and 8 hours of injury, respectively. [22]

Whereas preclinical studies have provided the biological and mechanistic basis for past clinical trials, future efforts to improve the predictive accuracy of preclinical studies is a critical step to achieve success in clinical trials. Health experts aim to develop research guidelines like Stroke Therapy Academic Industry Roundtable (STAIR) recommendations to improve clinical translation. [174], [196] The Federal Interagency Traumatic Brain Injury Research (FITBIR) is an informatics system built to share TBI related research across the research field. For the development of nanomaterials to treat acute brain injuries, future efforts include accurate measurement of nanomaterial pharmacokinetics in living organisms, careful selection of animal models that recapitulate specific human pathology relevant to nanomaterial design and payload, designing experiments that consider multiple biological variables, and development of technology to quantitatively measure biomarkers that can accurately predict outcomes in humans. [174], [197] PAMAM dendrimers are one example of nanomaterials that were tested across species and in multiple models of brain injury. [163] Based on comprehensive preclinical studies, PAMAM dendrimer nanomaterial drug OP-101 manufactured by Orpheris is currently undergoing Phase I clinical trials of safety, tolerability, and pharmacokinetics (clinicaltrials.gov NCT03500627).

Conclusion

There is an urgent need for new therapeutics to treat acute brain injuries, and engineering novel nanomedicine is one potential avenue for innovation. We drew from two decades of cancer nanomedicine in order to gain insight and inspiration to apply to the burgeoning field of nanomedicine for acute brain injuries. In particular, we focused on nanomaterials that are engineered based on disease-specific changes that occur in the vasculature, microenvironment, and the immune system. Within the current field of nanomaterials for acute brain injuries, there remain significant opportunities to innovate within the topics

discussed, as well as opportunities in directions that have been largely unexplored as of yet. For example, the secondary injury after an acute brain injury is multi-factorial and the failure of single drug therapies have initiated investigations into combination therapies. While there are promising ongoing studies of combination therapies [198], maintaining multiple drug molecules with disparate pharmacokinetics in their therapeutic windows may be a challenge. As supramolecular structures, nanomaterials can package multiple drugs for simultaneous delivery to the same tissue region or cell. In addition, nanomaterials can deliver challenging cargoes such as nucleic acids and proteins; the first FDA-approved siRNA therapy, ONPATTRO®, is a lipid nanoparticle that gained approval in 2018. Nanomaterial-mediated delivery of proteins and nucleic acids has the potential to directly translate basic biological findings from research labs into the clinic without the time-consuming development of small molecules. Lastly, many drugs, especially those designed for the brain, have poorly tolerated side effects. Future nanomaterials can be implemented as a precision medicine approach; for example, a system composed of a nanomaterial sensor that measures disease-causative protease activity paired with a homeostatic nanomaterial therapeutic that releases protease inhibitors only in response to that protease's activity.

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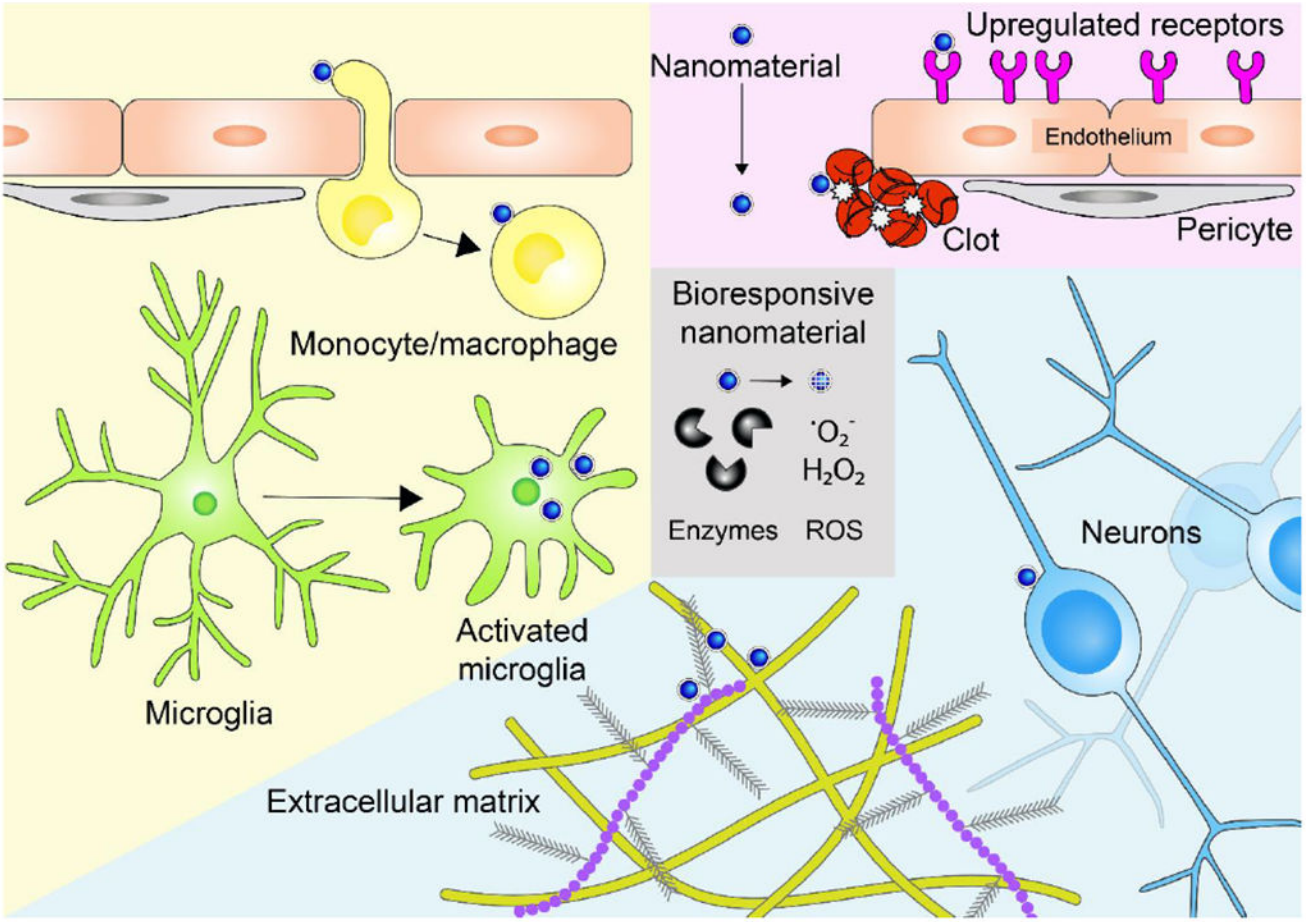

Figure 1. Nanomaterial paradigms in acute brain injury.

Nanomaterials can be engineered to respond to disease physiology in acute brain injuries, including dysregulated vasculature (pink), an altered microenvironment (gray and blue), and changes in the immune system (yellow). Specific examples for each nanomaterial design can be found in Table 1, color-coded by quadrant.

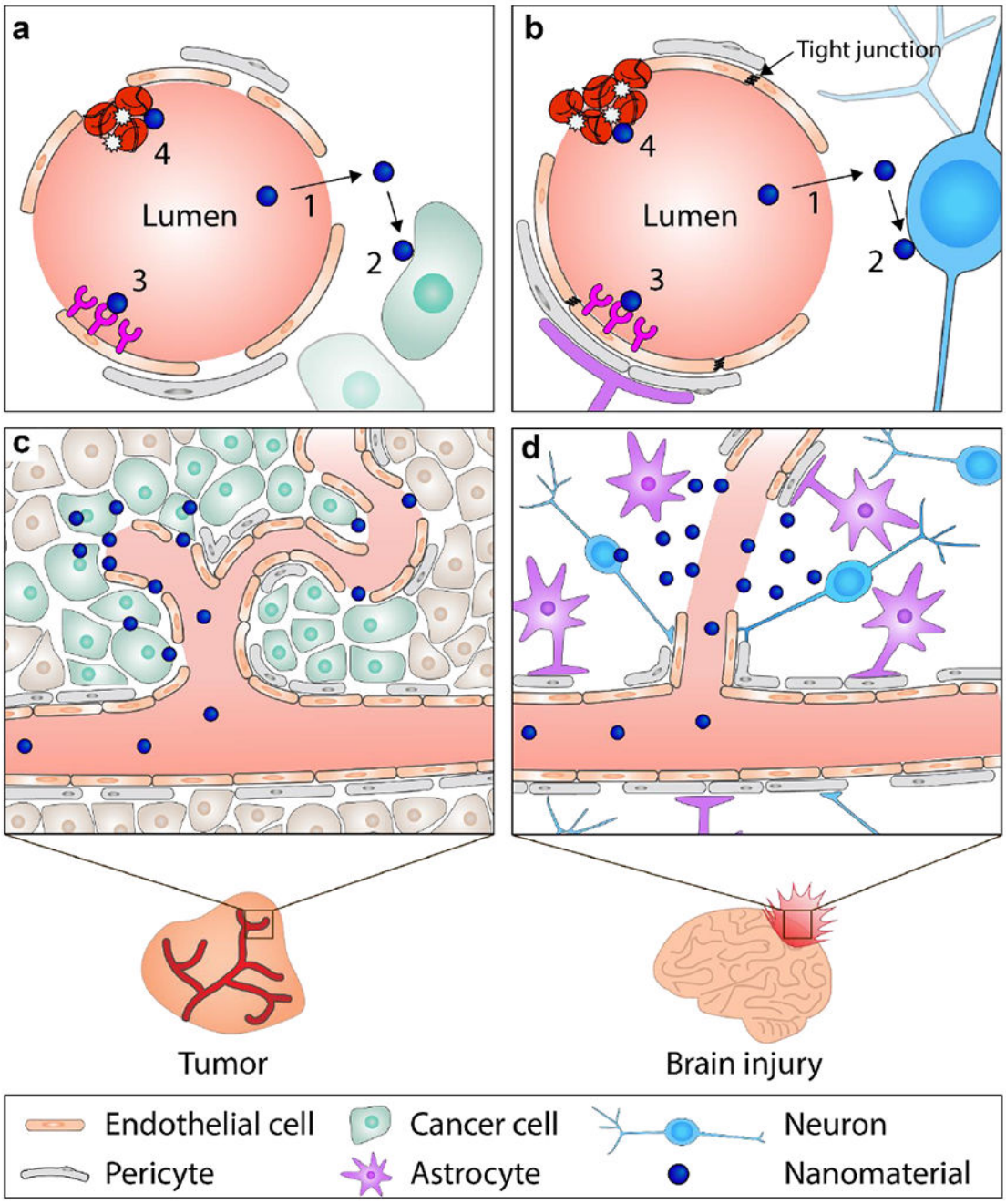

Figure 2. Nanomaterials that interact with vasculature in cancer and acute brain injury.

a, b. In cancer and acute brain injury, nanomaterials can (1) passively accumulate into adjacent tissue, (2) actively target cells in the tissue, (3) target upregulated receptors on endothelium, and (4) target clots. c, d. Dysregulated vasculature in cancer and acute brain injury allow for passive accumulation of nanomaterials in the diseased tissue.

Table 1.

Specific examples of nanomaterial design based on pathology found in cancer and acute brain injury.

		Cancer	Acute brain injury	References
Vasculature	**Permeable vasculature**			
	Pathology	Heterogeneous, tortuous, and leaky vasculature with disorganized and reduced pericyte and smooth muscle coverage; Poorly developed lymphatic structures	Transient disruption of BBB due to injury; Increased para-and transcellular transport, pericyte migration, swelling of astrocytic endfeet.	[84], [85], [89], [90]
	Design	EPR effect enables passive accumulation of nanomaterials into tumors	"EPR-like" effect enables passive accumulation of nanomaterials across transiently compromised BBB	
	Coagulation			
	Pathology	Fibrin deposition in tumor stromal	Clotting cascade activation leads to fibrin deposition and clot formation	[93], [94]
	Design	Fibrin targeting nanomaterials	Platelet mimicking and targeting nanomaterials	
	Ectopic receptor expression			
	Pathology	Upregulation of cell adhesion molecules, such as integrins and vascular cellular adhesion molecule 1 (VCAM1)	Upregulation of receptors on the BBB such as glutathione receptor, apolipoprotein receptor, and LDLR	[100], [101], [102]
	Design	Active targeting of nanomaterials to upregulated cell adhesion molecules	Active targeting of nanomaterials to upregulated receptors	
Microenvironment	**Active targeting: Cell targeting**			
	Pathology	Cancer cells and stromal cells are new targets not available in healthy state	Neurons sequestered from blood-borne agents in health are transiently accessible through injured BBB	[86], [111]
	Design	Active targeting of nanomaterials to cancer cells and TAMs	Active targeting of nanomaterials to neurons	
	Active Targeting: ECM targeting			
	Pathology	Upregulation of ECM (collagen, proteoglycans, and glycoproteins)	Deposition of ECM in glial scar (proteoglycans, HA, fibronectin, tenascins, and laminin) around injury site	[115], [118]
	Design	Active targeting of nanomaterials to tumor ECM via ligands and antibodies, such as collagen binding domains	Active targeting of nanomaterials to brain ECM, such as CAQK peptide	
	Bioresponsive materials: Reactive oxygen species			
	Pathology	Increased metabolic activity and mitochondrial dysregulation	Mitochondrial dysregulation, cell death, and neurovascular inflammation	[125], [126], [129], [127]
	Design	ROS-responsive and ROS-scavenging nanomaterials	ROS-responsive and ROS-scavenging nanomaterials	
	Bioresponsive materials: Protease activity			
	Pathology	Protease dysregulation (matrix metalloproteinases, peptidases, cathepsins)	Protease upregulation (matrix metalloproteinases, thrombin)	[136], [137], [138]
	Design	Protease-responsive therapeutic and diagnostic nanomaterials	Protease-responsive therapeutic and diagnostic nanomaterials	
Immune System	**Inflammatory state**			

		Cancer	Acute brain injury	References
	Pathology	Generally an immunosuppressive environment (TAM polarization to “M2-like” state, myeloid-derived suppressor cells)	Generally pro-inflammatory environment (upregulated inflammatory genes, reactive astrocytes, infiltration of bloodborne leukocytes)	[164], [166], [168], [169], [167], [170], [171], [172], [173]
	Design	Nanomaterial accumulation in TAMs, TAM repolarization, nanovaccines, and T cell backpacking	Nanomaterial accumulation in phagocytic microglia, depletion of infiltrating leukocytes, and biomimicry of immune cells for trafficking	