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# Current advances in in vitro models of central nervous system trauma

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## Abstract

CNS trauma is a prominent cause of mortality and morbidity, and although much effort has focused on developing treatments for CNS trauma-related pathologies, little progress has been made. Pre-clinical models of TBI and SCI suffer from significant drawbacks, which result in substantial failures during clinical translation of promising pre-clinical therapies. Here, we review recent advances made in the development of *in vitro* models of CNS trauma, the promises and drawbacks of current *in vitro* CNS injury models, and the attributes necessary for future models to accurately mimic the trauma microenvironment and facilitate CNS trauma drug discovery. The goal is to provide insight for the development of future CNS injury models and to aid researchers in selecting effective models for pre-clinical research of trauma therapeutics.

### Keywords

CNS trauma; traumatic brain injury; spinal cord injury; *ex vivo* injury model; *in vitro* injury model

## 1. Introduction

Traumatic brain injury (TBI) and spinal cord injury (SCI) are major causes of death and disability in the world. Thus far, little substantial progress has been made in the development of effective therapies to treat TBI- and SCI-associated neuropathologies. Current models of TBI and SCI do not accurately recapitulate many of the neurophysiological and

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heterogenous aspects of CNS trauma, leading to a bottleneck in the research and development of novel therapeutics. Animal models, often utilizing mouse or rat, are currently the gold standard for pre-clinical CNS trauma drug development. However, the heterogeneity of types of injury in humans, lack of standardization across different animal models, and fundamental differences between murine and human physiology complicate clinical translation of discovered pharmaceuticals, many of which fail in Phase II and Phase III TBI clinical trials [1]. The use of higher order mammals in CNS trauma models mitigates some of these issues for translation, but these models are costly and significantly limit throughput of candidate drug studies. Nonetheless, animal models are critical for pre-clinical assessment of behavioral outcomes and systemic effects of drug administration and will remain a necessary component of pre-clinical drug development. In contrast, *in vitro* models provide researchers with several advantages over animal models, which include significantly decreased costs, higher throughput capabilities, and greater control over experimental conditions. Due to these advantages, *in vitro* models are ideal for drug discovery and mechanism of action studies. However, significant improvements can be made to facilitate drug development for CNS trauma through the development of *in vitro* CNS injury models, which accurately mimic the physiological structure and function of *in vivo* tissue, are reproducible, allow for precise control over injury and treatment, and are suited for high throughput candidate assessment. Here, we review recent advances made in *in vitro* TBI and SCI models, promises and drawbacks of current *in vitro* models, and necessary features of future models to facilitate drug discovery.

## 2. Applications for *in vitro* models of CNS trauma

The primary goal of *in vitro* trauma models is to recapitulate injury pathology in a controlled system and decrease the influence of confounding systemic effects, allowing for discernment of specific mechanistic injury cascades and effects of therapeutic interventions on injury parameters. Well-developed models promote basic science research into mechanistic sequalae of CNS injury, facilitate discovery and validation of new therapy targets, and promote discovery of novel therapeutics. Brain and spinal cord trauma are highly heterogenous, and although both types of trauma share similarities in pathogenesis, there are distinct differences between TBI and SCI in the mechanisms of secondary injury progression. Furthermore, barriers to therapeutic success and types of therapeutic strategies applied in research of potential treatments vary between TBI and SCI. Many models in use today either recreate aspects of mechanical injury directly or induce facets of secondary injury cascades by means of chemical intervention or co-culture of affected cell types. Due to fundamental differences in pathobiology between TBI and SCI, distinct *in vitro* models of injury are used to mimic the human condition to discern and understand specific elements of the injury cascade and to test the efficacy of potential therapeutic interventions in overcoming the barriers to successful therapy.

## 3. Aspects of TBI and SCI pathogenesis modeled *in vitro*

CNS trauma results from mechanical loading onto CNS tissue, propagating a series of molecular events that over time, lead to neuronal cell death in regions proximal and distal to the injury site. Injuries can be subdivided into focal and diffuse, depending on the mechanics

of the forces acting on the tissue (Fig. 1a,b). Focal injury occurs as a result of direct impact to tissue, and depending on injury severity, can mediate formation of a primary lesion. Diffuse injury results from rapid linear and/or rotational acceleration/deceleration of respective tissue that induces shear, compression, and tension stress in multifocal regions of affected brain or spinal cord [2]. While focal injury is common in both SCI and TBI, diffuse injury and resulting axonal stretch damage are more prevalent in TBI [2]. Thus, while penetrating brain injury and SCI share common pathology, there are distinct differences between mild TBI or concussion and SCI [2,3]. Additionally, direct damage to axonal tracts projecting through the spinal cord is common in SCI, while acceleration-induced axonal injury in TBI is diffuse and rarely involves axonal transection [3]. This is reflected in the clinical treatment strategies currently applied to SCI and TBI, as many SCI treatments aim to promote axonal regeneration while therapies for TBI commonly aim to decrease neuronal cell death resulting from glutamate excitotoxicity and excessive generation of reactive oxidative species [2–4].

During the mechanical phase of TBI and SCI, direct impact to or acceleration of the skull or the spinal column, respectively, can result in contusion, compression, hemorrhage, or direct damage to brain or spinal cord tissue. In the case of SCI, deformation of the spinal cord results in the formation of cystic cavitations, which are filled with extracellular fluid, bands of connective tissue, and macrophages, which ultimately develop into a lesion [5]. Following TBI or SCI, there is physical damage to neurons and glial cells, disruption of the blood-brain barrier (BBB), and damage to cerebral and spinal cord vasculature. Disruptions in microvasculature and breakdown of the BBB can induce ischemic conditions and inflammation. Furthermore, following injury, glial cells release proinflammatory cytokines, and macrophages and neutrophils may infiltrate the spinal cord or brain. Thus, neural cell viability (particularly for neurons), ischemic conditions, and the inflammatory microenvironment represent important components of SCI and TBI for researchers to study [3,5].

After SCI, heightened astrocyte activation and proliferation proximal to the lesion site during the subacute phase also result in the formation of a perilesional cellular barrier. Extracellular matrix proteins secreted from microglia, macrophage, and astrocyte-mediated signaling during the acute phase associate with this cellular barrier to form the glial scar and prevent axonal regeneration during earlier stages after mechanical injury. Although glial scar formation is generally associated with adverse effects following injury, recent research indicates that glial scars may also play a beneficial role during recovery, thus complicating current treatment strategies [3,4]. The glial scar plays an important role in restricting inflammatory cells to the injury site, thereby preserving healthy surrounding neural tissue, and recent data support the idea that glial scar borders promote axonal regeneration during later stages post-injury [3,4]. Thus, modeling astrogliosis and glial scar formation is important for more comprehensive characterization of SCI pathophysiology [5].

During the intermediate and chronic phases of SCI and TBI, some level of endogenous recovery, in the form of neuron remyelination, axonal outgrowth, and neural progenitor cell proliferation and differentiation, may occur. However, the extent of recovery is largely dependent upon injury severity. In the case of a complete injury, the spinal cord is severed,

and little to no recovery of tissue integrity and function below the injury site occurs (Fig. 1c). Patients with injuries that involve only a partial transection or less severe compression or contusion may have a greater chance of recovery [5] (Fig. 1d). It is important for investigators to choose appropriate *in vitro* models of injury respective to the severity of injury that is being studied. Thus, developing accurate models of injury that can reliably recapitulate the effects of varying severities of SCI and TBI is essential for advancements in the field and the development of new therapies to more effectively address the unmet needs of these patient populations.

## 4. Mechanical *in vitro* CNS injury models

To mimic impact trauma *in vitro*, models of TBI and SCI apply many of the same mechanical types of injuries as those used in the past [6], including mechanical stretch, transection or scratch, blunt impact, and compression (Fig. 2). Mechanical injury models aim to mimic the biophysical phenomena that occur in clinical TBI or SCI to recapitulate the pathobiological mechanisms and microenvironment of secondary injury. These models are used to elucidate and characterize the biomechanics and pathology of trauma, to test the efficacy of potential therapeutic interventions, and to facilitate drug discovery. In contrast with animal trauma models, mechanical *in vitro* models allow for precise control of applied injury biomechanics, observation of live cell injury response, and acquisition of high-content data from large sets of experimental conditions. Thus, these types of models are used to further characterize the cellular and molecular response to injury and the biomechanics of different injuries at the tissue and cellular levels to better understand trauma pathology and uncover new therapeutic targets. Due to the heterogeneous nature of clinical injuries, characterization of biophysical and molecular responses to distinct injury subtypes is important as a personalized therapeutic approach may become more relevant to treatment of TBI and SCI in lieu of a ‘one-fits-all’ standardized treatment.

### 4.1 Impact-based injury

Blunt injury to the brain or spinal cord is modeled using weight-drop-based impactors that use a controlled weight-drop mechanism to drive a stylus [7] or flat impactor head [8,9] directly onto *ex vivo* cultures or acute preparations of brain or spinal cord tissue (Fig. 2a). These models mimic focal contusion and compression injuries and are used to study severe TBI and SCI, typically using rodent tissue explants or slice preparations. Alternatively, engineered 3D neural tissue models can be used as surrogates for trauma studies [10,11]. 3D brain models, in which cell bodies and axonal tracts are compartmentalized, can provide crucial information about effects of injury on white matter processes. To investigate the effects of impact at the single cell level, a microfluidic device has been developed in which individual cells can be impacted and compressed with up to 90% strain by a nickel-iron armature and subsequently cultured or analyzed [12].

Recently, an innovative *in vitro* model was developed to mimic acceleration conditions during impact, without concomitant blunt force trauma-induced tissue damage or deformation. The ballistic pendulum injury system allows for study of neural networks cultured on microelectrode arrays (MEAs) after high accelerations (30 to 300 g) and

subsequent monitoring of cellular morphology and neural network electrophysiological function post-injury [13]. Investigation of alterations in network activity after rapid acceleration is of high significance for research and development of therapeutics for protection from concussion-induced deficits in neural network function.

### 4.2 Stretch-induced injury

Stretch-induced injury models aim to recreate traumatic brain and spinal cord deformation and are the most widely used *in vitro* TBI and SCI models. Typically, surrogate tissues or cultured cells are placed or grown on a flexible substrate, such as polydimethylsiloxane (PDMS), which is deformed by a hollow indenter [14–18] or pulses of compressed air [19] or nitrogen gas [20] (Fig.2b). Mechanical strain models have been extensively characterized and are commercially available as flexible silicone-based multi-well plates and accompanying validated injury controller systems [21–24]. Commercially available stretch culture systems have been developed over two decades and offer flexible membrane 6 and 24 multi-well plates, significantly increasing throughput capabilities of such systems. Specialized multi-well plates and apparatus modifications allow for induction of both equiaxial and uniaxial strain at a maximum of 20% strain. Moreover, a high-throughput 96 well stretch injury device driven by electromagnetic voice coils, which induces injury by stretching a silicone membrane with cultured cells onto hollow posts, was recently developed [25]. Applications of similar high-throughput systems will greatly improve future drug discovery and combination therapy screens. Importantly, as stretch models provide a high level of control and reproducibility, these models are becoming more widely used to study effects of repetitive TBI on brain function and recovery [16,17].

While the aforementioned models use stretch applied to whole organotypic slices or cultured cell monolayers, microfabricated microfluidic devices are used to compartmentalize and separate neuronal soma and axons for localized axonal stretch injury [26,27] (also reviewed in [28]). Diffuse axonal injury is a notable component of mild TBI neuropathology, and thus, these models induce stretch injury to axons alone to monitor post-injury axonal pathology at the subcellular level. Alternatively, a silicone barrier can be employed to compartmentalize neuronal soma and axons in commercially available elastic chambers [29]. After cell attachment, the barrier is removed, allowing axons to traverse through the previously blocked off area towards soma on the other side.

### 4.3 Transection and scratch injury

Transection and scratch injury models are commonly used to characterize trauma-induced axotomy and assess efficacy of therapeutics to promote axonal regeneration post-injury. While primary axotomy is relatively rare in TBI, damage to axonal tracts is more prevalent in SCI, especially in cases of complete SCI. It is important to develop models for axonal regeneration that accurately mimic the injury microenvironment and increase throughput relative to animal SCI models. Transection is typically performed by using a scalpel blade [30–32], laser [33], plastic stylet [34] or needle [35] to cut *ex vivo* tissue [30,31] or isolated axonal tracts [36] (Fig. 2c). A novel transection method was recently developed in which cultured neurons are transected with a custom-built tool while mounted on a light sheet microscope, allowing observation of post-injury actin reorganization, which is difficult to do

using conventional microscopy [35]. While induction of transection injury aims to mimic primary physical injury, it also leads to the activation of the secondary injury responses observed *in vivo*. The injury microenvironment is recapitulated through promotion of glutamate-induced excitotoxicity [36], release of proinflammatory cytokines [37], expression of growth factors and axonal growth inhibiting molecules, such as chondroitin sulfate proteoglycan [32,36,37], and alterations in cell metabolism and generation of reactive oxygen species [32].

Tissue or cultured cells can be transected in a dish or on a cell culture insert for studies of macroscopic injury in which damage is not localized or cell-specific. Microfabricated microfluidic devices can be utilized to compartmentalize or isolate neuronal dendrites and axons into separate chambers for localized transection. This type of microfluidics model was used to compartmentalize dissociated hippocampal neurons into somatodendritic and axonal compartments to investigate the effects of distal axotomy on synaptic remodeling [36]. Axons traverse from the somatodendritic compartment to the axonal compartment through 8μm wide microgrooves, which separate the two compartments [36]. To perform axotomy, fluid was aspirated from the axonal compartment, allowing for examination of the effects of axotomy on retrograde spine loss, synaptic vesicle release, and miniature excitatory postsynaptic currents of axotomized neurons [36].

An additional transection *in vitro* model is the scratch assay, in which primary neurons or astrocyte cultures or immortalized cell lines are scraped using a pipette tip [38] or plastic needle [39] to induce secondary damage and initiate secondary injury molecular cascades. This model is simple and accessible and is commonly used to induce astrocyte reactivity in studies of inflammation [37] and assess astrocytic response and wound closure following injury [38,40]. Moreover, because of its simplicity, the model is also used to induce generalized injury to neurons [34,39,41] or cell lines [42] as a means to investigate secondary injury.

## 5. Secondary injury models

In addition to modeling the primary impact that occurs as the first phase of CNS trauma, several groups have more directly assessed specific molecular pathologies associated with secondary injury. Many of the models used aim to characterize specific features of the injury microenvironment. Most commonly assessed secondary injury pathologies include perilesional tissue architecture, oxidative stress, and cell-cell interactions. In the case of tissue and lesion architecture models, 3D culturing methods have played an instrumental role in their development. Models of the glial scar, a hallmark of secondary SCI, are constructed by implanting mixed neural cell or astrocyte cultures in collagen or alginate gels [43–45] (Fig. 2d). These 3D models successfully recapitulate several important characteristics of glial scar pathophysiology and architecture, including hyperplasia [43,44], astrocyte scar clusters formation [44], changes in gene expression [45], and increased extracellular matrix production [45]. Thus, such models more accurately recapitulate the structure of injured tissue after CNS injury.

Structural alterations to the injury microenvironment are further augmented by biochemical changes. Oxidative stress, which occurs under ischemic conditions as a result of heightened ROS generation and release, is another molecular component of both secondary TBI and SCI. Ischemia is modeled through oxygen-glucose deprivation of dissociated neural cell [46,47] and organotypic slice cultures [48]. Subjecting microglial cultures to hypoxia/hypoglycemia (1% oxygen, 250μM glucose) followed by normoxia/normoglycemia (21% oxygen, 25mM glucose) was shown to induce targeted expression of interferon-stimulated genes [46], important signaling mediators of neuroinflammation and neuroimmune response.

Additional studies on CNS injury implement co-culturing models to examine interactions between specific cell types within or proximal to the injured tissue. Macrophage-microglia studied with this type of model demonstrated that macrophages suppress pro-inflammatory and phagocytic functions of microglia [49]. Additionally, investigation of pericyte-oligodendrocyte dynamics demonstrated that pericytes promote the differentiation of oligodendrocyte precursor cells, which ultimately contribute to CNS remyelination [50]. Cumulatively, *in vitro* studies on secondary CNS injury have expanded our understanding of injury-associated signaling, gene and protein expression, and cell-type specific interactions. However, additional advancements in the field and the continued development of new systems are needed to provide more diverse and versatile models of secondary injury pathophysiology.

## 6. Limitations and future improvements

Although *in vitro* CNS trauma models are useful tools for investigation of injury neuropathology and discovery of new treatments, there are several limitations which hinder their utility. The most prominent is disparity between cultured primary cells and their counterparts *in vivo* due to differences in microenvironment. Preparation of *ex vivo* brain or spinal cord slices, especially using a vibratome, results in tissue and cell damage, which may affect cellular and molecular responses following experimental injury procedures and therapy treatments. Culture conditions, while mimicking the *in vivo* microenvironment, are still generally distinct from conditions *in vivo*, making it difficult to predict how patient cells will behave during the process of clinical translation. Isolation of primary cells and tissue may affect gene regulation, and thus, affect downstream gene expression during experimental procedures. Furthermore, it is difficult to deduce drug dosages for *in vivo* applications from drug concentrations applied *in vitro,* further complicating studies of toxicity and mechanism of action.

In addition to general constraints of *in vitro* studies, there are several drawbacks to *in vitro* trauma models, limiting their potential. While animal trauma models will remain necessary during pre-clinical drug development, *in vitro* models could be significantly improved to bolster existing strengths and further utility for different drug discovery applications. Few models, beside the commercially sold stretch microwell plates, offer medium to high throughput assessment capabilities. As *in vitro* models can serve as important tools in discovery of new drug and therapy candidates and in studies of drug toxicity, the ability to test a multitude of compounds and concentrations in one experiment becomes of significant importance. Medium- to high-throughput *in vitro* injury models should also be highly

controlled and reproducible, to ensure a similar level of injury across conditions and to decrease technical and biological variability. In addition, there is also a need for additional *in vitro* models that utilize co-culture of different cell types whereby groups of cells and their interactions can be controlled to further dissect the roles that different glial cell types play during the manifestation of post-injury sequalae. Future developments of *in vitro* CNS injury models should aim to improve cell culture conditions to better mimic the *in vivo* microenvironment, allowing for greater dynamic control over cellular signaling, behavior, and response, and the composition of and alterations in the extracellular environment in response to injury. Improvements to current *ex vivo* culture systems include incorporating technologies used for 3D culture to improve the congruence between current *in vitro* models and corresponding *in vivo* phenomena, promoting retention of tissue or explant 3D structure and cellular composition.

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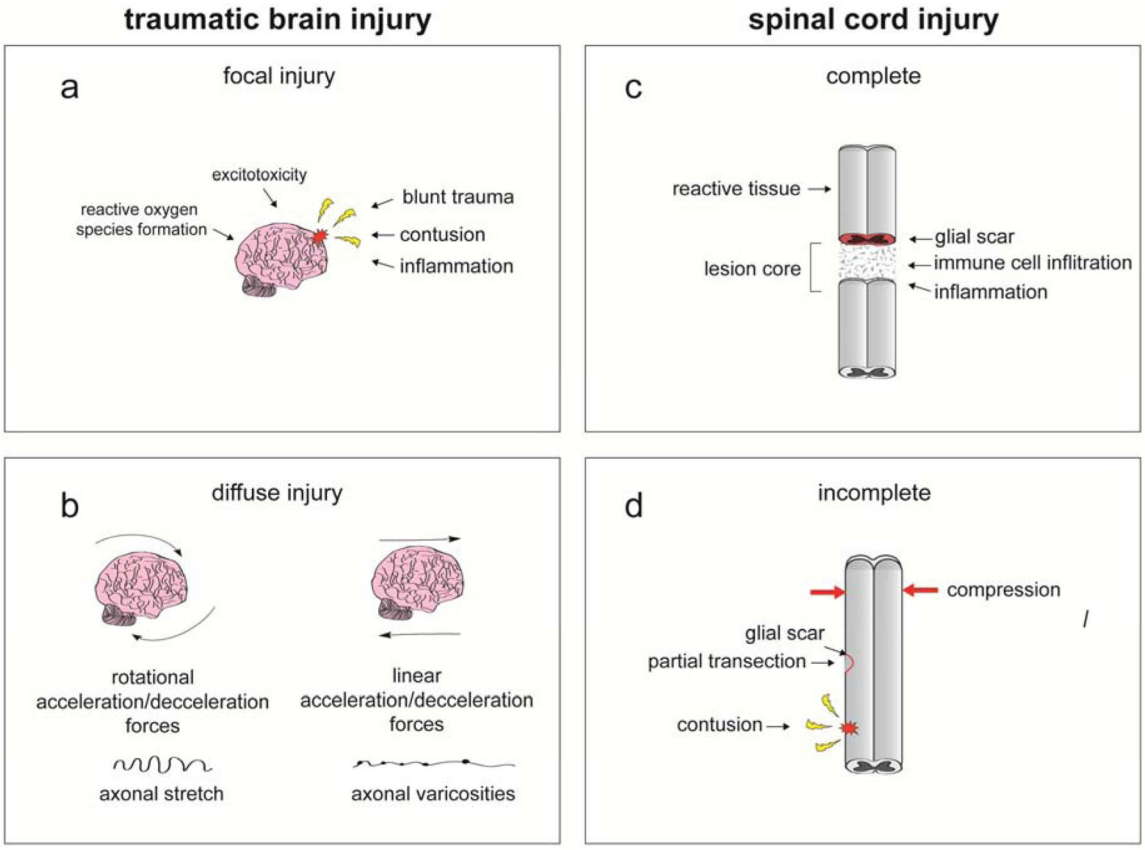


**Fig. 1. Aspects of TBI and SCI pathogenesis modeled *in vitro*.**

CNS injury is highly heterogenous and is categorized the biomechanics of the forces acting on the brain or spinal cord during trauma, injury severity, and other clinical complications. **a.** Focal injury results from direct impact to tissue and can lead to formation of a lesion. **b.** Diffuse injury results from rapid rotational and/or linear acceleration of brain or spinal cord that propagates shear, compression, and tension stress in multiple regions of the respective tissue. **c-d** Spinal cord injury can be further classified based on whether the spinal cord is completely severed (**c**) or is partially damaged (**d**). Investigators should utilize appropriate *in vitro* models of CNS trauma based on the severity and biomechanics of injury that is the focus of study.

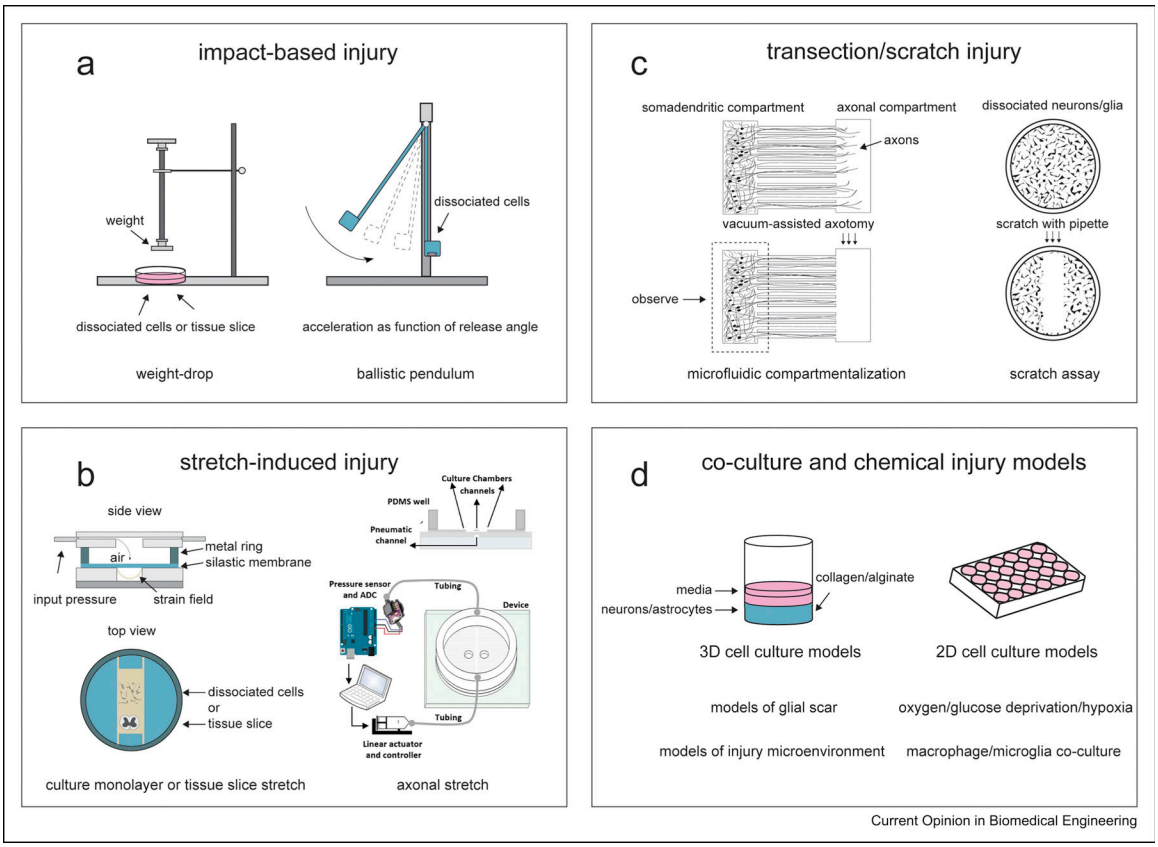


**Fig. 2. *In vitro* models of CNS trauma.**

Mechanical models of CNS injury aim to recreate the biomechanical parameters that underlie specific injury subtypes to recapitulate secondary injury mechanisms and the injury microenvironment. **a.** Impact-based models utilize a weight-drop or ballistic pendulum mechanism to induce blunt tissue damage or to recreate collisional impact, respectively. **b.** Stretch-induced injury models deform surrogate tissue or cells cultured on a flexible substrate, such as polydimethylsiloxane (PDMS), using compressed gas or by lowering the substrate onto an indenter platform. Biaxial and uniaxial strain can be applied based on the type of apparatus and setup employed. Microfabricated devices are used to compartmentalize and isolate axonal projections in microgrooves for investigation of axonal stretch injury. **c.** Transection models induce axotomy using laser or vacuum in investigations of axonal degeneration and regeneration. Microfabricated platforms with microgrooves are used in transection models to separate somatodendritic and axonal compartments to isolate axotomy and observe injury-induced alterations in synapse remodeling and synaptic function. **d.** Aspects of secondary injury pathogenesis are modeled in isolation to control for confounding pathology, especially in investigations of mechanism of action. 3D co-culture models have been developed using collagen or alginate to encapsulate primary neural cells in studies of perilesional tissue architecture alterations following injury. 2D monolayer cultures are used to investigate post-injury cell-cell interactions and injury-induced biochemical changes in neurons and glial cells. Modified culture conditions, such as oxygen/glucose deprivation, and treatments with chemicals are used to mimic the injury microenvironment and to control for confounding factors. Part of Figure 2b is adapted from