Simulating traumatic brain injury in vitro: developing high throughput models to test biomaterial-based therapies

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Abstract

Traumatic brain injuries are serious clinical incidents associated with some of the poorest outcomes in neurological practice. Coupled with the limited regenerative capacity of the brain, this has significant implications for patients, carers, and healthcare systems, and the requirement for life-long care in some cases. Clinical treatment currently focuses on limiting the initial neural damage with long-term care/support from multidisciplinary teams. Therapies targeting neuroprotection and neural regeneration are not currently available but are the focus of intensive research. Biomaterial-based interventions are gaining popularity for a range of applications including biomolecule and drug delivery, and to function as cellular scaffolds. Experimental investigations into the development of such novel therapeutics for traumatic brain injury will be critically underpinned by the availability of appropriate high throughput, facile, ethically viable, and pathomimetic biological model systems. This represents a significant challenge for researchers given the pathological complexity of traumatic brain injury. Specifically, there is a concerted post-injury response mounted by multiple neural cell types which includes microglial activation and astroglial scarring with the expression of a range of growth inhibitory molecules and cytokines in the lesion environment. Here, we review common models used for the study of traumatic brain injury (ranging from live animal models to in vitro systems), focusing on penetrating traumatic brain injury models. We discuss their relative advantages and drawbacks for the development and testing of biomaterial-based therapeutics.

Key Words: astroglial scar; biomaterial; cortical culture; in vitro model; microglial infiltration; multicellular model; penetrating injury; scaffold; traumatic brain injury

Introduction

The total global annual burden of traumatic brain injuries (TBIs) is an estimated US $400 billion (van Dijck et al., 2019). Such injuries can arise from blunt (closed) or penetrating trauma (open/TBIs). These are prevalent in civilian/military personnel in areas of a high incidence of terrorism/violence and are associated with the worst clinical outcome in head injury cases. An injury track created by a foreign body (e.g. fragments or gunshot rounds) causes cavitation, shearing, and compression of nerve fibers and blood vessels, with damage to neurons and glia including myelin damage (Oehmichen et al., 2001). There is focal and diffuse neuronal apoptosis/necrosis, during the primary and secondary injury phases, and cellular debris leads to a high concentration of damage-associated molecular patterns. Microglial infiltration of lesions and release of pro-inflammatory cytokines such as interferon-γ and tumor necrosis factor-α drive acute inflammation (Lively et al., 2018). Oligodendrocyte precursor cells and fibroblasts infiltrate the lesion and proliferate with early (within 24 hours) astrocyte activation. The latter extends into the surrounding brain tissue to lesion the lesion core which contains cellular debris and molecules inhibitory to neurite outgrowth (such as myelin-associated glycoprotein and oligodendrocyte myelin glycoprotein; Filbin et al., 2003). The subsequent failure of regeneration, chronic inflammation, and atrophy are suggested to underpin the poor clinical outcomes post-pTBI. Treatments have been refined over the years and include early debridement with or without craniotomy and supportive therapies such as anti-seizure medications, antibiotics, and intracranial pressure monitoring (Yakici et al., 2017). Such injuries have significant contamination (approximately 43% infection risk) so early broad-spectrum antibiotics use is key. Cerebrospinal fluid leaks are also encountered increasing contamination risk and requiring dural repair. Long-term management focuses on neurorehabilitation requiring multidisciplinary input including teams from neurosurgery, neurology, physiotherapy, speech, and language therapy, in addition to input from allied specialties depending on the systemic manifestation of clinical injury. Current clinical interventions are therefore supportive and truly regenerative/neuroprotective therapies post-injury do not exist, remaining a key goal for research in regenerative neurology.

In this context, the use of implantable biomaterials as therapeutic scaffolds to promote repair has been a major recent advance in regenerative medicine. Such matrices can be prepared from many different biomolecules, including numerous proteins/polysaccharides. These are highly versatile for neurological injury given their extracellular matrix-like structures, high porosity, and ease of fluid/nutrient movement, supporting the 3-D growth of cells including axon/blood vessel ingrowth from ‘host’ tissue into implants (Weightman et al., 2014). They offer benefits as drug/cell delivery devices enabling local, controlled release of a therapeutic and, significantly, the ability to modify the post-injury extracellular microenvironment. For example, there is evidence that biomaterials in situ reduce the expression of mRNA for inflammatory- and glial cell scarring-related genes in injury sites, limit immune cell infiltration, attenuate glial scarring, and reduce cystic cavitation post-injury (Krings et al., 2016; Basit et al., 2021). They have tissue-mimetic mechanical properties, relatively rapid biodegradability (within about 3 months allowing for gradual replacement with nascent tissue), and mouldability for surgical delivery (Chen et al., 2019). Using a pTBI model, Hou et al. (2005) found neurite outgrowth and angiogenesis into hyaluronic acid hydrogels modified with laminin, which had been implanted into the cortices of Sprague-Dawley rats, with decreased glial fibrillary acidic protein upregulation in areas of biomaterial contact. Similarly, Chen et al. (2019) demonstrated reduced infiltration of activated microglia in a collagen-glycosaminoglycan matrix hydrogel implanted into a surgical rat brain injury, with a modified inflammatory signaling environment (interleukin-6, tumor necrosis factor-α, and interleukin-10 expression).

Such research is encouraging and requires suitable preclinical experimental models to identify and test the most promising interventions for future medicine. Given the pathological complexity of pTBI, this does present a highly challenging goal. An appropriate model, in our view, should offer the following features: wide availability across experimental facilities; facile methods to facilitate researcher training, inexpensive, high throughput nature to test multiple experimental conditions simultaneously, patho-mimicry to accurately simulate in vivo pTBI; compatibility with a range of microscopic methods (including, light, fluorescence, time-lapse and electron microscopy);

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and applicability to a wide range of species including genetically modified animals. Importantly, any model should provide an ethically viable approach in line with the 3 Rs (Reduction, Replacement, and Refinement) of animal experimentation. Here, we review the literature on experimental models of pTBI and their utility for the study of biomaterial based therapeutics.

### Search Strategy and Selection Criteria

All years were chosen in the search. These searches were performed between June and December 2021 using the PubMed and Web of Science databases. Broad search terms such as traumatic brain injury, penetrating traumatic brain injury, in vitro model, neurological injury, biomaterial, organotypic, organotypic, and neuronal culture were used in various combinations.

### Models of Penetrating Traumatic Brain Injury Vary in Complexity

A variety of pTBI models have been deployed in experimental neurology. Large animal models typically involve researchers introducing penetrating brain lesions through gunshot/stab wounds in anesthetized sheep or monkeys (Finnie et al., 1993), with the evaluation of gross pTBI pathology. However, the ethical implications and rarity in procuring such animals within common research facilities have resulted in these models largely falling out of favor. Small animal rodent models deemed ‘less sentient’ have been developed (such as a penetratig ballistic-like brain injury–rifle pellet injury) (Passos et al., 2015). These are relatively inexpensive (versus live animal models), widely available in research facilities and offer ease of handling and standardized neurobehavioral tests to evaluate the regenerative benefits of any therapy. However, all live animal models of neurological injury result from ethically imposed limitations, given them. Hence, some of the most invasive models in experimental research. As such, there is a need for stringent regulation of such work, often requiring experimental licenses and specialist infrastructure and staff for the housing and care of animals. These models are inherently lower throughput, and technically challenging compared to in vitro alternatives. The significant ethical implications surrounding live animal use in medical research also continue to be a matter of extensive public and scientific debate.

In vitro organotypic (organ-like) brain slices provide a 3D alternative. These offer the controllable environment of in vitro preparations providing an ‘interface’ between high throughput screening and pre-clinical animal models. Neural cytoarchitecture, cell inter-relationships, a vascular network, and the extracellular matrix are maintained in such tissue, allowing for neural plasticity, cell migration, and axonal regeneration to be easily examined. Rodents, including transgenic models and higher species (rabbits, pigs, dogs, and humans) can be used as donor sources, and the application of advanced microscopy, electrophysiology, molecular and genomic methods to these models has greatly expanded their practical utility, including for the study of TBI. Our lab previously developed an in vitro spinal cord organotypic slice culture model with a penetrating (transecting) injury (Weightman et al., 2014). We showed that the model replicates stereotypical pathological responses seen after neurological injury in vivo, namely; (a) reactive gliosis with astrocytes forming a scar, a major barrier to nerve fiber regeneration; (b) gradual infiltration of lesions by microglia; and (c) decreasing nerve fiber outgrowth with increasing donor tissue age, in conjunction with glial scarring and reactive microgliosis in lesions. Whilst offering significant patho-mimicry, such models are moderate throughput at best, and can be technically challenging to establish and maintain.

The most basic in vitro models use immortalized cell lines, which are robust, inexpensive, and widely available but are often resistant to cell death and prone to cryptic contamination. These offer major advantages in terms of a more facile and controlled yet high throughput approach, for multiple experimental manipulations or measurements. Systems offering greater complexity include co-cultured or derived cell lines, which typically take the form of monolayer cultures. For example, an astrocyte scratch wound model (using primary cultures of astrocytes) has been used to simulate traumatic neural injury with glial fibrillary acidic protein up-regulation, hypertrophy of reactive astrocytes, and injury triggered calcium waves observed in the injury foci (Gao et al., 2013). In general, such models are high-throughput, facile, inexpensive, and widely available and contribute significantly to the reduction and refinement of animal experimentation, with ethical implications than in vivo systems. However, the flip side for reductionism has often meant these models are overly simplistic, not containing the major neural cell types (notably the immune cells) to simulate the complex pathological responses observed in neural pathology. Therefore, advanced in vitro systems are still required. Table 1 shows a comparison of in vitro neural cell models potentially adaptable to injury mechanisms.

### Developing a Multicellular Model of Penetrating Traumatic Brain Injury In Vitro

We recently developed a reliable, technically simple, and high throughput pTBI model, using rodent cortices, to evaluate the effects of implantation of mixed glial scaffold (neural cells) (unpublished data). The model uses a variant of a widely used mixed glial culture system and contains the two major immunocompetent neural cell types in the central nervous system–namely the astrocytes and microglia in addition to oligodendrocyte precursor cells (Basit et al., 2021). We found evidence of both microglial invasion of lesions and reactive astrogliosis in peri-lesional astrogliosis (hypertrophic palisading astrocytes at the lesion edge) with glial scar formation and glial fibrillary acidic protein up-regulation at the lesion edge. Implantation of a surgical grade matrix Duragen Plus™ into the lesion resulted in microglial and astrocytic infiltration into the biomaterial. Therefore, the model broadly replicates in vivo features of TBI pathology. It also highlights the need for experimental models that can predict the responses of major neuroglial phenotypes to introduced biomaterials. This is of high importance given the critical role these cells play in determining the intra-central nervous system fate of therapeutic scaffold materials. Astrocytes, for example, can dramatically remodel biomaterials, and fibrillar contraction in these cells alters their biomechanical properties. Microglia--the intrinsic immune cells of the central nervous system, have roles in biomaterial clearance and digestion which impacts their biodegradability and potential toxicity of the materials. This in turn exerts a critical impact on the pro-regenerative properties of the implant. Accordingly, we consider the model can be used to investigate materials varying in their chemistry, stiffness, and porosity to identify those with the most promise prior to preclinical testing. A major limitation of the model is the lack of a neuronal component, meaning axonal outgrowth—a key aspect of neuroregeneration, cannot be assessed. To address this, we have recently developed an advanced version of the multi-glial model using a simple chemical switch to maintain the neural cells in vitro (unpublished data; Figure 1). Briefly, cortices from neonatal rodents were extracted and enzymatically dissociated, using a specialized chemical medium found to support the growth of both neurons and all major glial subpopulations, including the microglia. In terms of the cellular constitution, the culture consists of approximately 50% neurons with glial populations making up the remaining cells. 70% of the neurons were found to be gamma-aminobutyric acid (GABA) positive, the remaining neurons were not found to be glutamatergic, in our hands and their identity, remains to be established (Figure 2).
Table 1 | Possible in vitro systems for modelling brain tissue, traumatic injury mechanisms and biomaterial interventions – advantages and disadvantages, arranged from highest complexity to least complexity

| In vitro models | Description | Advantages | Disadvantages | References |
|-----------------|-------------|------------|---------------|------------|
| 3D ‘organotypic’ slices | Ex vivo brain tissue slices | -Retain in vivo cytoarchitecture | -Higher complexity | Morrison et al., 2000; Di Pietro et al., 2012; Bar-Kochba et al., 2016; Kling et al., 2016; Campos-Pires et al., 2018; Ucar et al., 2021 |
| Brain-on-a-chip | Microfluidic culture systems of 3D iPSC derived cultures | -Tissue-like physiology | -Scalability limitations | Dolle et al., 2014; Bang et al., 2019 |
| 3D hydrogel constructs | Cells encapsulated within a 3D matrix | -3D architecture resembling tissue-like environment | -Lack immune and vascular components | Haycock, 2010; Antoni et al., 2015; Raimondi et al., 2020 |
| 2D primary multicellular models | Complex multicellular cultures of brain dissociates | -Can encompass major brain cell types (including microglia and neurons) | -Lack immune component | Kumaria, 2017; Goshi et al., 2020; Basil et al., 2021 |
| Primary neural stem cell cultures | Cultures of differentiated stem cells isolated from neurogenic regions e.g. subventricular zone (SVZ) | -High throughput | -Lack immune component | Goa et al., 2013; Barbora et al., 2020; Vagaska et al., 2020; Mogas et al., 2021 |
| Induced pluripotent stem cells (iPSCs) | Stem cells genetically reprogrammed from adult cells | -Indefinite propagation | -Moderate throughput (long differentiation protocols) | Ulrich et al., 2001; Kang et al., 2017; Postolato et al., 2017; Tukler et al., 2018 |
| 2D primary pure cell cultures | Primary cultures from brain dissociates; purified through sequential shaking or specific media components | -High throughput | -Overly simplistic model of the brain | Geddes et al., 2003; Chen et al., 2007; Vells and Cole, 2011 |
| Cell lines: Pure cells, NSCs/ESCs (neural/embryonic stem cells) | Immortalised cell lines | -Indefinite propagation | -Genetically and phenotypically different from endogenous counterparts | Gordon et al., 2013; Carter and Shee, 2015; Tapia and Scholar, 2016 |

*: Cells undergo artificial responses to adapt to the flat, stiff surface of 2D cultures systems. Mechanical injury includes stretch, weight drop and penetrating injuries.

The model is pathomimetic, inexpensive, high throughput and contains all of the central nervous system cell types. Penetrating lesions can easily be induced within the culture system, and potential therapeutic interventions such as biomaterial implantation or nanoparticle delivery assessed. There are very few reports that focus on the response of all major neural cell types simultaneously and thereby overlook the multi-dimensional cellular response, despite the known importance of all glial and neuronal cell functions in neuroinflammation, therapeutic assessment, and regeneration. Our model offers significant benefits in this regard. All the cell types were homogeneously distributed throughout the cultures allowing sufficient evaluation of the multiple cell responses to injury. The potential to study the therapeutic impact on all major neural cell types simultaneously has significant benefits when considering a high throughput, facile brain tissue model. For example, Goshi et al. (2020) reported that astrocytes and neurons respond differently to neuroinflammatory stimulators and neurotrauma in the presence of microglia. This reinforces the importance of the immune component in mixed neural cultures, and in turn the significance of the microdynamics between all neural cell types. Accordingly, we consider our new model offers a versatile adaptable platform for the study of a range of TBI mechanisms, therapies, and drug testing within the neuro-regenerative field, using a simple but scalable system. This robust brain tissue-modeling platform would be adaptable to multiple injury mechanisms including weight.
drop contusions, glutamate-induced excitotoxicity, lipo polysaccharide stimulated neuroinflammation and hypoxia, to understand concurrently the response of all brain cell types.

Interestingly, we have found that the model can be adapted into a 3D format using cell seeding into a hydrogel matrix, thereby expanding its utility to study more complex in vitro models, such as pellet simulating ballistic injuries or crush injuries through weight drop (unpublished data). Further refinements in the future could include the addition of endothelial cells to simulate the blood-brain barrier, and the inclusion of peripheral immune cells, to enhance the pathophysiometric potential of the approach. A more detailed assessment of the lesion pathology is also needed, for example, the use of proteomic methods to study the spatial and temporal molecular expression profiles in the injuries (for comparison to in vivo data), or an assessment of the extent of myelination/demyelination in the injuries, including use of high-resolution electron microscopy. Whilst such pTBI models can never outright replace preclinical testing, we believe they are of high value for the developmental testing and identification of promising biomaterial scaffold-therapeutic interventions. Their versatility also allows them to be deployed to test other promising neuro therapies such as novel nano-pharmaceuticals and glial scar attenuation/immunomodulatory treatments.

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Recent studies have also demonstrated the potential of utilizing in vitro models to study traumatic brain injury (TBI) resulting from missile or other mechanical injuries. These models can be used to investigate the pathophysiological mechanisms underlying the injury, including the roles of inflammatory cells, neurodegeneration, and neuroplasticity. For example, the use of organotypic hippocampal slices in an in vitro model of traumatic brain injury (TBI) has allowed researchers to study the immediate and delayed effects of TBI on neuronal function and morphology (1). Similarly, the use of microfluidic devices to simulate the blood-brain barrier has provided insights into the mechanisms of injury-induced neuroinflammation (2).

In addition, the use of iPSC-derived brain organoids has emerged as a promising tool for modeling TBI (3). These organoids are derived from human induced pluripotent stem cells (iPSCs) and can be differentiated into various brain cell types, including neurons, astrocytes, and microglia. They can be used to study the effects of TBI on these cell types and to identify potential therapeutic targets.

Future perspectives

The development of more advanced in vitro models for TBI is likely to continue, with a focus on improving the fidelity and predictive validity of these models. The use of additional cell types, such as peripheral immune cells and glial scar cells, may be incorporated to further enhance the complexity of these models. Additionally, the incorporation of additional biological matrices, such as collagen gels, may provide a more realistic simulation of the brain tissue environment.

In conclusion, the use of in vitro models to study traumatic brain injury has become an important tool in the field of neuroscience. These models have the potential to provide insights into the mechanisms of injury and to identify potential therapeutic targets. However, further refinement and validation of these models will be required to improve their predictive validity and to guide the development of novel therapies for TBI.

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Review

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