

# Tissue Engineering: Current Research

## Chapter 2

# Tissue Engineering of Traumatic Brain Injury

*Xincheng Du<sup>1</sup>, Wen Zhao<sup>2</sup>, Jibo Wang<sup>2</sup>, Haowen Qiao<sup>2#</sup>, and Yu Zhou<sup>3#</sup>*

<sup>1</sup>*School of Sports and Health Engineering, Hebei University of Engineering, Handan 056038, P.R. China.*

<sup>2</sup>*Department of Biomedical Engineering, School of Basic Medical Sciences, Wuhan University, Wuhan 430071, P. R. China.*

<sup>3</sup>*Department of Neurosurgery, The Second Xiangya Hospital of Central South University, Changsha 410000, P. R. China.*

*Haowen Qiao<sup>#</sup> and Yu Zhou<sup>#</sup> contributed equally to this manuscript.*

**\*Correspondence to:**

**Haowen Qiao**, *Department of Biomedical Engineering, School of Basic Medical Sciences, Wuhan University, Wuhan 430071, P. R. China.*

*Email: 610188321@qq.com*

**Yu Zhou**, *Department of Neurosurgery, The Second Xiangya Hospital of Central South University, Changsha 410000, P. R. China.*

*Email: 507766@csu.edu.cn*

---

## Abstract

Traumatic brain injury (TBI) has considerable morbidity and mortality, which contributes to the loss of cognitive and motor function in patients. This is an urgent need for the generation of in vitro model to understand the pathophysiological mechanism of TBI and explore the potential therapeutic options. Tissue engineering of TBI in vitro has been placed on the role of attention due to its advantages in Bionic architecture and function. The in vitro TBI engineering models according to the different ways of injury are classified into 4 categories, including the impact injury models, stretch injury models, negative pressure drainage injury models, and blast injury models. In this chapter, we will present a unique perspective to introduce and compare these four models from their research hotspots.

**Citation:** Yu Zhou, (2021) *Tissue Engineering: Current Research, Vol. 1, Chapter 2, pp. 25-32.*

## 1. Introduction

Traumatic brain injury (TBI) is defined as an acute brain injury caused by mechanical energy to the head from external physical forces (mainly from external blows, impacts, or even explosions) [1-3]. TBI could affect the physical and mental health of the patient and lead the disability and death among young and middle-aged people [4,5]. It is reported that more than 50 million people worldwide suffer from TBI each year, and the cost of TBI is \$400 billion, which imposes a huge economic burden on families and society [6,7].

TBI has complex pathophysiological features, including primary neurological injuries (axonal damage, skeleton disruption, and enhanced cell membrane permeability of neurons) and secondary neurological injuries (excitotoxicity, oxidative stress, mitochondrial dysfunction, blood-brain barrier (BBB) disruption, and neuroinflammation caused by neurobiochemical cascade reactions [2]). Among them, 76% -83% of primary neurological injuries belong to mild TBI, however, it failed to attract enough attention for the timely and effective medical interventions in TBI patients [8]. What is more, the secondary neurological injury resulting from the primary neurological injuries can further aggravate brain lesions and lead to irreversible cognitive and motor dysfunction, with the increasing risk of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease [9-14]. Although many studies illustrated the changes of brain microenvironments during TBI, the molecular remains unknown [15-17]. Therefore, it is important to investigate the molecular mechanisms of TBI, develop relevant neuroprotective agents, and optimize therapeutic means.

Tissue engineering is an emerging subject based on biology and engineering for the generation of tissues or organs *in vitro* or *in vivo* [18]. This subject has broad application in TBI models. In this chapter, we describe recent applications of TBI models with a particular focus on impact injury models, stretch injury models, negative pressure drainage injury models, and blast injury models.

## 2. Injury Models

### 2.1 Impact Injury Models

Impact injury models have mainly been divided into weight-drop (WD) models and controlled cortical impact (CCI) models. WD models are caused by striking specific tissues with the weight falling freely. Usually, a catheter is used to guide the heavy object to fall freely. And the control of the degree of damage varies from the weight of the heavy object and the height of the fall. Chen et al [19]. utilized soft lithography to fabricate polydimethylsiloxane (PDMS) porous scaffold with a micro-pillar structure, and inoculated human pluripotent stem cells (hPSCs) on the surface of the scaffold. Subsequently, the falling body strike was used to construct the TBI model. Their results showed that the injury caused by the falling body blow promoted

the release of glutamate from glutamatergic neurons, which restored the pathophysiological characteristics of the human brain. This study further found that neuroprotective drugs could effectively inhibit the secretion of glutamate caused by injury and prevent secondary injury. Recently, Shi [20] generated the neurospheres derived from human induced pluripotent stem cells (hiPSCs) to construct a mild TBI model with drop-weight impact technology. In the mild TBI neurosphere models by the single weight impact, they showed minor pathological changes, such as a small amount of cell death; the mild TBIs by the multiple weight impacts, the neurospheres exhibited complex pathological phenotypes similar to the human brain, such as neuronal death, reactive astrocyte hyperplasia, and glial scar formation. To further investigate the effect of neuroinflammation on the mild TBI, Shi et al [20]. introduced microglia in this TBI neurosphere model. The results showed that the injury activated microglia and induced sustained release of inflammatory factors and chemokines. The model is simple in method and easy to control conditions and provides a good platform for high-throughput studies of molecular mechanisms, target identification, drug screening, and clinical translation of mild TBI. However, the accuracy of the strike force and the reproducibility need to be improved. The WD models with simple generation methods and easy control conditions provide a unique high-throughput platform for molecular mechanism, drug screening, and clinical translational research. However, the accuracy of the crackdown needs to be improved.

CCI models can adjust impact residence time, impact speed, and impact depth through an electronically controlled cortical impactor. Compared with the WD model, the strike force for CCI models is more precise. In the CCI model, the impact head could quickly retract after the impact, which avoids secondary uncontrollable damage caused by squeezing or rebounding from heavy objects. Liaudanskaya et al [13]. found that the neuronal degeneration in CCI models first appeared in the direct injury area and then spread to other areas over time. In addition, they also found that CCI induced the degradation of neural network structure and function, the release of glutamate, the high expression of pMLKL, and the inhibited expression of GSK3 $\beta$  protein in the AKT/mTOR signaling pathway. This study provided real-time monitoring CCI models for exploring the molecular mechanism of sports-induced cortical injury and secondary injury.

## 2.2. Stretch Injury Models

Stretch injury models can be divided into mild, moderate, and severe damage models according to the pressure applied. Salvador et al [21]. used compressed gas to induce different degrees of deformation of cultured neural, which eventually caused mild, moderate, and severe damage to the cells. However, at a higher rate of deformation of this model, the dishes or plates for culturing cells were prone to uneven deformation. With the development of microfluidic technology, stretch injury models based on a microfluidic chips have gradually been developed. Yap et al [22]. generated the stretch injury models based on microfluidic

chip with a pneumatic microvalve. The microchannel in the chip can direct the growth of axons, and pneumatic microvalves induce neuronal axon damage through the air pressure change. This study determined the correlation between the time of neuronal death and the degree of tensile injury by comparing the changes of cortical neuron axons in the microfluidic chip subjected to different degrees of tensile damage. In addition, it is reported that the degeneration of neurofilament in axons caused by injury is earlier than that of microtubules, and neurofilament changes may be the triggering factor of secondary injury [23]. The results of the study confirmed that more than 10% deformation could cause neuronal axon damage, thus contributing to the understanding of neuronal axon skeleton damage and degeneration in TBI research.

The incidence of recurrent mild TBI is highest in sports such as boxing, wrestling, and football. the symptoms of this disease are not obvious enough, it is not taken seriously by the public. However, it was reported that that the cumulative effects of recurrent injuries could increase the susceptibility to brain injury, leading to abnormal behavior and brain function defects in patients [24]. Yap et al [25]. investigated the effects of mild (5%), very mild (0.5%), and repetitive very mild ( $2 \times 0.5\%$ ) axonal stretch injury on primary cortical neurons and found that very mild and mild levels of stretch injury resulted in the formation of smaller growth cones at the tips of axons and a significantly higher number of collapsed structures compared to those present in uninjured cultures. This study helps to better understand the pathogenesis of mild and repetitive brain injury and provides potential support for testing the therapeutic effects. However, this model still has some limitations. For example, air bubbles or shear forces generated during the flow of the fluid may cause additional damage to the cells.

### **2.3. Negative Pressure Drainage Injury Models**

The negative pressure drainage injury models based on microfluidic technology can mimic the damage of neuron axons by constructing negative pressure conditions. Jeon et al [26-28]. developed a microfluidic chip-based model that could accurately cause the damage of neuronal axons. This system contained two chambers connected by a microchannel. When the air bubbles generated by vacuum suction passed through the channel, the axons were broken to cause neural damage. The advantage of this model was that damage for the axon sites could be modeled specifically, thus it provided a novel platform for screening the potential drugs for axon regeneration [26,29]. At present, the negative pressure drainage injury models are also used to study the role of peroxisomal proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) in axon regeneration after neural injury [30]. PPAR $\gamma$  can participate in the regeneration of peripheral and central neurons caused by axon injury. The negative pressure drainage injury models based on microfluidic technology have high reproducibility and can be efficiently combined with techniques such as immunofluorescence staining, transcription analysis, and electrophysiology.

## 2.4. Blast-induced traumatic brain injury

Blast-induced traumatic brain injury (bTBI) refers to the craniocerebral injury caused by explosive shock waves and projectiles, which is the main type of injury in modern warfare. The advanced blast simulator (ABS) model is the most common type for bTBI. The model contains a cylindrical tube which is divided into two chambers (pressurized chamber and test chamber) by a special film. When the air pressure in the pressurized area rises to a certain extent, the shock wave generated by breaking the diaphragm will cause damage to the specific tissues placed in the test chamber. Campos-Pires et al [31] developed a novel blast traumatic brain injury model using C57BL/6N mouse organotypic hippocampal brain-slice cultures exposed to a single shockwave, with the resulting injury quantified using propidium iodide fluorescence. The results showed that the exposure to blast shockwave resulted in a significant injury that increased with peak-overpressure and impulse of the shockwave, and which exhibited a secondary injury development up to 72 h after trauma.

To accurately simulate the external impact on the human brain, Lai et al [32]. generated an acoustic radiation damage model in the brain organoids derived from hiPSCs. To accurately simulate the external impact on the brain and improve the bionic degree of the model, Lai et al [32]. constructed an acoustic radiation damage model. To accurately simulate the external impact on the brain as well as to improve the degree of model mimicry, Lai [32].

Constructed injury models by acoustic radiation force injury to brain-like organs of hiPSCs origin. Transcriptomic studies showed that injury upregulated glycolysis and protease inhibitors, recapitulating pathological features associated with neurodegenerative diseases, including elevated phosphorylated Tau protein and aggregation of cytoplasmic phosphorylated TDP-43. This model restores the hallmark processes of brain injury disease and the pathological features of neurodegenerative diseases caused by brain injury, laying the foundation for studying the molecular mechanisms and potential therapeutic options for acute and chronic brain injury. However, the maturity of brain-like organs is currently only equivalent to that of fetal life. However, with the development of brain-like organs, the construction of highly mature, fully functional, multi-brain region brain-like organs will provide a highly reliable protocol for studying adult TBI.

## 3. Challenges and prospects

The *in vitro* TBI model based on tissue engineering provides an effective means for further understanding the molecular mechanism of damage. It is worth noting that although the brain model based on tissue engineering has made effective progress, there are still significant differences in brain structure and function, including the lack of brain cells from the peripheral immune system, complex ECM, and functional BBB.

With the development of new related technologies, human pluripotent stem cells are used for the construction of the multi-cell lineage model [33]. In addition, combined with the tissue engineering and developmental biology, the construction of brain organoid-on-chips can precisely control the growth and development of the human brain in vitro, thus providing a new platform for TBI tissue engineering models [34-37]; the application of 3D printing and biological assembly technology in TBI tissue engineering models will help for the formation of blood [38,39]. In addition, combined with a micro electro mechanical system, it can help us to detect in situ the changes of relevant indicators and microenvironment parameters in the model in real time [40,41]. We envision that innovative biomaterials and engineering methodologies will continue to enhance tissue engineering of traumatic brain injury.

#### 4. Reference

1. Shultz, S. R. et al. Repeated Mild Lateral Fluid Percussion Brain Injury in the Rat Causes Cumulative Long-Term Behavioral Impairments, Neuroinflammation, and Cortical Loss in an Animal Model of Repeated Concussion. *J. Neurotrauma* 29, 281-294 (2012).
2. Lozano, D. et al. Neuroinflammatory responses to traumatic brain injury: etiology, clinical consequences, and therapeutic opportunities. *Neuropsychiatric disease and treatment* 11, 97 (2015).
3. Peterson, A. B. & Kegler, S. R. Deaths from fall-related traumatic brain injury—United States, 2008–2017. *Morbidity and Mortality Weekly Report* 69, 225 (2020).
4. Maas, A. I. et al. Traumatic brain injury: integrated approaches to improve prevention, clinical care, and research. *The Lancet Neurology* 16, 987-1048 (2017).
5. Khellaf, A., Khan, D. Z. & Helmy, A. Recent advances in traumatic brain injury. *Journal of neurology* 266, 2878-2889 (2019).
6. Jiang, J.-Y. et al. Traumatic brain injury in China. *The Lancet Neurology* 18, 286-295 (2019).
7. Faul, M., Wald, M. M., Xu, L. & Coronado, V. G. Traumatic brain injury in the United States; emergency department visits, hospitalizations, and deaths, 2002-2006. (2010).
8. Liu, Y. Research Progress of Mild Traumatic Brain Injury. *Chinese Journal of Clinical Neurosciences* 27, 182-185+215 (2019).
9. Li, Y. et al. Head injury as a risk factor for dementia and Alzheimer's disease: a systematic review and meta-analysis of 32 observational studies. *PloS one* 12, e0169650 (2017).
10. Blennow, K. et al. Traumatic brain injuries. *Nature reviews Disease primers* 2, 1-19 (2016).
11. Gardner, R. C. et al. Mild TBI and risk of Parkinson disease: a chronic effects of neurotrauma consortium study. *Neurology* 90, e1771-e1779 (2018).
12. Sagarkar, S. et al. Repeated mild traumatic brain injury causes persistent changes in histone deacetylase function in hippocampus: Implications in learning and memory deficits in rats. *Brain research* 1711, 183-192 (2019).
13. Liaudanskaya, V. et al. Modeling Controlled Cortical Impact Injury in 3D Brain-Like Tissue Cultures. *Advanced healthcare materials* 9, 2000122 (2020).
14. VanItallie, T. B. Traumatic brain injury (TBI) in collision sports: Possible mechanisms of transformation into chronic traumatic encephalopathy (CTE). *Metabolism* 100, 153943 (2019).

15. Baeten, K. M. & Akassoglou, K. Extracellular matrix and matrix receptors in blood–brain barrier formation and stroke. *Developmental neurobiology* 71, 1018-1039 (2011).
16. Hemphill, M. A., Dauth, S., Yu, C. J., Dabiri, B. E. & Parker, K. K. Traumatic brain injury and the neuronal microenvironment: a potential role for neuropathological mechanotransduction. *Neuron* 85, 1177-1192 (2015).
17. Kan, E. M., Ling, E.-A. & Lu, J. Microenvironment changes in mild traumatic brain injury. *Brain research bulletin* 87, 359-372 (2012).
18. Lanza, R., Langer, R., Vacanti, J. P. & Atala, A. *Principles of tissue engineering*. (Academic press, 2020).
19. Chen, C. et al. Develop a 3D neurological disease model of human cortical glutamatergic neurons using micropillar-based scaffolds. *Acta Pharmaceutica Sinica B* 9, 557-564 (2019).
20. Shi, W. et al. Design and evaluation of an in vitro mild traumatic brain injury modeling system using 3D printed mini impact device on the 3D cultured human iPSC derived neural progenitor cells. *Advanced Healthcare Materials*, 2100180 (2021).
21. Salvador, E., Burek, M. & Förster, C. Y. Stretch and/or oxygen glucose deprivation (OGD) in an in vitro traumatic brain injury (TBI) model induces calcium alteration and inflammatory cascade. *Frontiers in cellular neuroscience* 9, 323 (2015).
22. Yap, Y. C., Dickson, T. C., King, A. E., Breadmore, M. C. & Guijt, R. M. Microfluidic culture platform for studying neuronal response to mild to very mild axonal stretch injury. *Biomicrofluidics* 8, 044110 (2014).
23. Fournier, A. J., Rajbhandari, L., Shrestha, S., Venkatesan, A. & Ramesh, K. In vitro and in situ visualization of cytoskeletal deformation under load: traumatic axonal injury. *The FASEB Journal* 28, 5277-5287 (2014).
24. Fehily, B. & Fitzgerald, M. Repeated mild traumatic brain injury: potential mechanisms of damage. *Cell transplantation* 26, 1131-1155 (2017).
25. Yap, Y. C. et al. Mild and repetitive very mild axonal stretch injury triggers cytoskeletal mislocalization and growth cone collapse. *PloS one* 12, e0176997 (2017).
26. Taylor, A. M. et al. A microfluidic culture platform for CNS axonal injury, regeneration and transport. *Nat Methods* 2, 599-605, doi:10.1038/nmeth777 (2005).
27. Park, J. W., Vahidi, B., Taylor, A. M., Rhee, S. W. & Jeon, N. L. Microfluidic culture platform for neuroscience research. *Nat Protoc* 1, 2128-2136, doi:10.1038/nprot.2006.316 (2006).
28. Taylor, A. M. et al. Microfluidic Multicompartment Device for Neuroscience Research. *Langmuir* 19, 1551-1556, doi:10.1021/la026417v (2003).
29. Kim, H. J. et al. Integrated microfluidics platforms for investigating injury and regeneration of CNS axons. *Ann Biomed Eng* 40, 1268-1276, doi:10.1007/s10439-012-0515-6 (2012).
30. Lezana, J. P. et al. Axonal PPARgamma promotes neuronal regeneration after injury. *Dev Neurobiol* 76, 688-701, doi:10.1002/dneu.22353 (2016).
31. Campos-Pires, R. et al. Xenon protects against blast-induced traumatic brain injury in an in vitro model. *Journal of neurotrauma* 35, 1037-1044 (2018).
32. Lai, J. D. et al. A model of traumatic brain injury using human iPSC-derived cortical brain organoids. *BioRxiv* (2020).
33. Lacalle-Auriolles, M., de Camps, C. C., Zorca, C. E., Beitel, L. K. & Durcan, T. M. Applying hiPSCs and biomaterials towards an understanding and treatment of traumatic brain injury. *Frontiers in Cellular Neuroscience* 14 (2020).

34. Qiao, H., Zhang, Y. S. & Chen, P. Commentary: Human brain organoid-on-a-chip to model prenatal nicotine exposure. *Frontiers in bioengineering and biotechnology* 6, 138 (2018).
35. Wang, Y., Wang, L., Zhu, Y. & Qin, J. Human brain organoid-on-a-chip to model prenatal nicotine exposure. *Lab on a Chip* 18, 851-860 (2018).
36. Wang, Z., He, X., Qiao, H. & Chen, P. Global trends of organoid and organ-on-a-chip in the past decade: a bibliometric and comparative study. *Tissue Engineering Part A* 26, 656-671 (2020).
37. Ren, T., Chen, P., Gu, L., Ogut, M. G. & Demirci, U. Soft Ring-Shaped Cellu-Robots with Simultaneous Locomotion in Batches. *Advanced Materials* 32, 1905713 (2020).
38. Xie, R., Zheng, W., Guan, L., Ai, Y. & Liang, Q. Engineering of hydrogel materials with perfusable microchannels for building vascularized tissues. *Small* 16, 1902838 (2020).
39. Pham, M. T. et al. Generation of human vascularized brain organoids. *Neuroreport* 29, 588 (2018).
40. Santra, T. S. *Microfluidics and Bio-MEMS: Devices and Applications*. (CRC Press, 2020).
41. Kwon, Y. W., Jun, Y. S., Park, Y.-G., Jang, J. & Park, J.-U. Recent advances in electronic devices for monitoring and modulation of brain. *Nano Research*, 1-26 (2021).