

Exploring the possibilities of using *in vitro* model for neuropathic pain studies

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Received: 16 December 2021; **Accepted:** 19 June 2022; **Published:** 27 June 2022

Edited by: Cheah Pike See (Universiti Putra Malaysia, Malaysia)

Reviewed by: Wan Mastura Shaik Mohamed Mossadeq (Universiti Putra Malaysia, Malaysia);

Paramita Basu (University of Pittsburgh School of Medicine, Pittsburgh, USA)

<https://doi.org/10.31117/neuroscirn.v5i3.144>

Abstract: Establishing experimental models to study neuropathic pain has been challenging due to the complex mechanism underlying the condition. Although *in vivo* models have been useful in the observation of behavioural pain responses, it should be acknowledged that species-to-species variance can lead to differences in terms of molecular mechanism and genetic expression. The study of molecular and signal transduction of neuropathic pain using *in vivo* models faces limitations due to ethical considerations involving pain induction in animals and the intricacy of molecular interactions in the pathophysiology of the condition. Hence, developing relevant *in vitro* models to study neuropathic pain is important, as it considers the physiological microenvironment and reduces the use of experimental animals. Several considerations should be taken into account in developing an *in vitro* model of neuropathic pain, including the use of either primary culture of cell lines with considerations to their origins; human or animal, the method of neuropathic pain-like induction and the relevant assays to assess pain. This review recapitulates previous research employing *in vitro* models in investigating the molecular mechanism of neuropathic pain, intending to provide an alternative to the growing concerns on *in vivo* neuropathic pain models.

Keywords: neuropathic pain; *in vitro*; receptors; inflammatory mediators;

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1.0 INTRODUCTION

Neuropathic pain is described as a painful sensation that is due to a lesion or disease of the somatosensory system ([“IASP Terminology - IASP,” 2017](#)). The prevalence of neuropathic pain within the global population was estimated at 7-10% ([van Hecke et al., 2014](#)). Current treatment of neuropathic pain involves tricyclic antidepressants such as amitriptyline, gabapentin and serotonin-noradrenaline reuptake inhibitors (SNRI) such as duloxetine, whereby these treatments manage to attenuate neuropathic pain symptoms but tend to cause adverse and side effects ([Nishikawa & Nomoto, 2017](#)). The management of neuropathic pain conditions is complex, and treatments are not a cure-all. The complex mechanism which underlies the pathophysiology of neuropathic pain involves various inflammatory mediators and changes in receptors and signalling molecules ([Finnerup et al., 2021](#)).

The study on neuropathic pain remains ambiguous relative to the pathophysiology and possible remedy in alleviating neuropathic pain. Neuropathic pain studies involving humans are ethically and practically challenging; thus, several *in vivo*, *in vitro* and *ex vivo* models are commonly used to mimic similar conditions or symptoms of neuropathic pain ([Hattangady & Rajadhyaksha, 2009](#); [Sousa et al., 2016](#); [Wang & Wang, 2003](#)). These neuropathic pain models demonstrated representative behaviour responses aligned with the pathophysiology of neuropathic pain ([Gregory et al., 2013](#)). Although limitations of these models have been addressed involving the observation of adverse and side effects due to species differences, the establishment of animal and cell culture models of neuropathic pain serves as a noteworthy tool in defining the pathophysiology ([Mogil, 2009](#)). In addition, the knowledge of establishing neuropathic pain models allows the development of clinically efficacious pain prophylactics.

2.0 THE VIRTUE OF EMPLOYING *IN VITRO* MODEL IN NEUROPATHIC PAIN STUDY

Among the experimental models employed in neuropathic pain studies, *in vivo* model of neuropathic pain is widely used to understand the pain behaviour of the condition. For instance, the commonly used animal models are the chronic constriction injury (CCI) induced neuropathic pain model, spinal nerve injury (SNI) model and spinal nerve ligation (SNL) model, as well as a streptozotocin-induced model as diabetic-induced neuropathic pain model ([Challa, 2015](#); [Kaliyaperumal et al., 2020](#); [Sambasevam, 2018](#)). These

animal models rely on the induction of central or peripheral nerve damage through drugs to mimic similar conditions observed in humans.

The development and perpetuation of neuropathic pain are modulated by peripheral and central sensitisation. In general, neuronal sensitisation occurs when the neuronal sensitivity is altered due to various mechanisms. Peripheral sensitisation is distinguished by the abnormal sensitivity of afferent nociceptors to stimuli due to the effect of inflammatory mediators, ectopic discharges arising from dorsal root ganglion and ephaptic transmission from neighbouring uninjured nerve fibres ([Cohen & Mao, 2014](#); [Dureja et al., 2017](#)). At the cellular level, the transmission of pain signals is regulated by the ion channels, protein kinases, receptors and neurotransmitters. The action of inflammatory cytokines and chemokines will alter the expression of receptors and ion channels and the degeneration of nerve fibres, consequently bringing about neuronal hyperexcitability ([Meacham et al., 2017](#)).

Following the intense signals from the peripheral neurons, the central neurons within the brain and spinal cord will be sensitised to the signals—the central sensitisation results in the abnormal response to normal and low-threshold stimuli. Similar to the condition in peripheral sensitisation, alterations of the expression of ion channels and receptors occur, and changes in synapse and the release of neurotransmitter takes place coupled with the presence of pain-promoting mediators released by the microglial cells. This intensifies pain perception ([Gwak & Hulsebosch, 2011](#); [Meacham et al., 2017](#)).

The involvement of ion channels, receptors, neurotransmitters, cytokines and other signalling molecules in the mechanism of neuropathic pain has contributed to the challenge of alleviating the condition. Current therapeutic approaches are mostly limited to symptom management, with considerably fewer approaches targeting the mechanism.

2.1 Ethical considerations of pain induction in animals

Behavioural parameters, such as the presence of allodynia and hyperalgesia, were used as indicators of neuropathic pain in animal studies. However, the usage of animal models has ethical concerns due to the pain being imposed on the animals (**Table 1**). Furthermore, molecular and signal transduction study of neuropathic pain through *in vivo* models faces

limitations due to the intricate systems and interactions of intercellular signalling molecules ([Hattangady & Rajadhyaksha, 2009](#)). Hence, having *in vitro* models helps study the crosstalk of molecular signalling and reduces animal usage. Additionally, *in vitro* models can provide fundamental and preliminary data on the action of a particular compound or treatment target. The employment of *in vitro* models in this research area enables the diminution of inflated usage for *in vivo* models.

Table 1. *In vivo* models of neuropathic pain with the respective type of injury.

Model	Type of injury
Chronic constriction injury (CCI)-induced neuropathy	Nerve ligation
Spinal nerve injury (SNI)-induced neuropathy	• Nerve ligation • Mechanical compression of the nerves
Peripheral nerve injury-induced neuropathy	• Mechanical compression of the nerves • Nerve transection
Diabetes-induced neuropathy	• Streptozotocin induction • Genetic models
Drug-induced neuropathy	• Chemotherapeutic drugs (oxaliplatin, cisplatin) • Antiretroviral drugs (didanosine)
Sciatic inflammatory neuritis	Inflammation-induced pain (zymosan-induced inflammation on the sciatic nerve)
Human immunodeficiency virus (HIV)-induced neuropathy	• Genetic models • HIV-gp120-induced neuropathy
Alcoholic neuropathy	Chronic ethanol exposure through diets

3.0 PRIMARY CELL CULTURE AND TRANSFORMED CELL LINES

The use of *in vitro* models varies depending on the cell function to be studied and the cell culture type employed. There are two major cell culture types—primary and transformed cell lines. Primary cell culture involves acquiring the cells and culturing them under the optimum condition without modification to the cells ([Stacey, 2006](#)). On the other hand, the

development of transformed cell lines encompasses the alterations of genotype, enhancing the growth properties and immortalising the cells ([Dave et al., 2020](#); [Geraghty et al., 2014](#)). The use of primary cell culture and transformed cell lines will be further described in the context of neuropathic pain and its conditions.

3.1 Primary cell culture

Primary cell culture is commonly employed in research to retain the cell's original structure and physiology ([Gordon et al., 2013](#)). Primary cells are dorsal root ganglia (DRG) neurons, Schwann cells, neural crest cells, cortical neurons and human embryonic stem cells ([Berta et al., 2017](#); [Hattangady & Rajadhyaksha, 2009](#); [Jones et al., 2018](#)).

3.1.1 Dorsal root ganglia (DRG) neurons

Neuropathic pain pathophysiology involves both central and peripheral sensitisation. Dorsal root ganglia (DRG) play an important role in mediating peripheral sensitisation due to its neuronal formation. The DRG encompasses the sensory, motor, and autonomic nerves, surrounded by glial cells. Dorsal root ganglia were observed to serve as an excellent translational model for neuropathic pain because it contains large populations of sensory neurons ([Melli & Höke, 2009](#)). This is essential as most neuropathic pain patients exhibit sensory symptoms such as hyperalgesia and allodynia. These painful symptoms are also commonly reflected in neuropathic pain animal models ([Chia et al., 2020](#)). DRG neurons have diversified morphology and functions responsible for pain signal transduction and modulation ([Berta et al., 2017](#)). These neurons express various ion channels and receptors, which modulate pain signal transmission and modulation. The alterations were commonly observed in the transient receptor potential channels (TRP), voltage-gated ion channels, glutamate, and ATP-sensitive receptors ([Krames, 2014](#)). In addition to the morphological and protein expression analysis, DRG neurons were also employed in studies associated with the pathophysiology of ion channels ([Barkai et al., 2017](#); [Chung & Chung, 2002](#)).

Most experiments utilised DRG neurons from rodents since human DRG is unattainable ([Qi et al., 2011](#); [Vysokov et al., 2019](#)). To mimic the neuropathic pain-like condition, DRG neuronal cultures were subjected to axotomy, cultured in high glucose medium or induced with oxidative agents and virus ([Fernyhough et al., 2003](#); [Jones et al., 2018](#); [Vysokov et al., 2019](#)). In several neuropathic pain studies, morphological and

molecular assays were performed on DRG neuronal cultures to complement the findings of neuropathic pain tests on animals ([Chen et al., 2017](#); [Eldridge et al., 2019](#)). Furthermore, DRG neuronal culture is commonly co-cultured with glial cells, such as Schwann cells and satellite glial cells, which may explain the neuron-glia crosstalk underlying the pathophysiology of neuropathic pain ([Izzi et al., 2018](#)). Notwithstanding, the primary culture of DRG neurons has limitations as it is prone to damage due to the loss of adherent properties and low purity due to contamination by Schwann cells and fibroblasts. Besides, mature DRG neurons do not proliferate after extraction, contributing to the lower yield obtained ([Shen et al., 2019](#)).

3.1.2 Human embryonic stem cells (hESCs)

More recent studies have shown the potential of human embryonic stem cells (hESCs) as an *in vitro* model to study neuropathic pain ([Chen et al., 2019](#); [Hattangady & Rajadhyaksha, 2009](#); [Jones et al., 2018](#); [Srinivasan & Toh, 2019](#)). Human embryonic stem cells have been used in studies related to peripheral neuropathic pain due to their ability to be differentiated into physiologically functioning sensory neurons to study peripheral neuropathic pain ([Jones et al., 2018](#)). It has been observed in several studies that the induction of neuronal differentiation on hESCs produced sensory neuronal cells that express relevant ion channels, which are also expressed in DRG neuronal cells ([Jones et al., 2018](#); [Meyer & Kaspar, 2014](#)). Moreover, the electrophysiological analysis also shows that the differentiated neuronal cells exhibit functioning ion channels responsible for nociceptive signal modulation ([Lee et al., 2012](#); [Meyer & Kaspar, 2014](#)). The differentiated neuronal cells responded toward the induction of ATP and capsaicin, further supporting the development of sensory neuron characteristics ([Lampert et al., 2020](#)). Several neuronal differentiation methods were identified. For instance, hESCs were differentiated through a combination of dual-SMAD inhibition and early WNT activation coupled with small-molecule inhibition of Notch, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) signalling pathways ([Chambers et al., 2013](#); [Jones et al., 2018](#)).

Despite retaining human genetic features and exhibiting functioning sensory neurons, hESCs cultures require a long time to develop and differentiate before being subjected to downstream experiments ([Meyer & Kaspar, 2014](#); [Young et al., 2014](#)). Similar research has

also shown that the induction of sensory neurons from hESCs requires some growth factors, neurotrophic factors and signalling molecules. It is also important to note that the neuronal differentiation of hESCs entails characterisation for nociceptor or sensory phenotype as the differentiated cell culture may consist of cells that do not express neuronal morphology.

3.1.3 Schwann cells

Neuropathic pain development and maintenance do not only involve the alterations of the peripheral and central nerves. Within the peripheral nerve system, Schwann cells are one of the prominent glial cells which modulate neuropathic pain pathophysiology ([Wei et al., 2019](#)). Research has shown that in neuropathic pain conditions, Schwann cells displayed changes in morphology and activation of their cells following nerve injury and upregulation of several pro-inflammatory cytokines and chemokines ([Wei et al., 2019](#)). Moreover, Schwann cells were observed to express modulatory receptors, such as P2X4 receptors, a potential target for attenuation of pain signal transmission ([Su et al., 2019](#)).

Schwann cells used in neuropathic pain studies were often acquired from rodents or induced through differentiation from precursor cells, followed by the induction with oxidative agents, chemotherapy drugs, or cultured in a high-glucose medium to mimic neuropathic pain pathophysiology ([Imai et al., 2017](#); [Liu et al., 2016](#); [Su et al., 2019](#)). Common observation discerned in Schwann cell culture includes the viability of the cells, the expression of neuropathic-pain-related receptors, the production of inflammatory cytokines and chemokines, the morphology of the cells in regards to their polarity and the formation of the myelin sheath ([Imai et al., 2017](#); [Liu et al., 2016](#); [Logu et al., 2019](#)). Similarly, as it has been reported in sensory neuronal culture derived from hESCs, the differentiation of Schwann cells requires a number of growth and differentiating agents and a long period of cell differentiation. The differentiated precursor cells also require characterisation and culture purity validation following differentiation ([Kim et al., 2020](#)).

3.2 Transformed cell lines

Apart from primary cell culture, transformed cell lines are also used to establish *in vitro* models of neuropathic pain. Cell lines are preferred due to their high proliferative rate, allowing a continuous supply of cells and convenient culture, unlike primary cell culture, which usually requires the addition of several growth and neurotrophic factors in the culture media ([Stacey, 2006](#)). Commonly used cell lines are neuronal

cell lines, Schwann cell lines and microglial cells, which are sourced from humans or rodents.

3.2.1 SH-SY5Y cells

The commonly used neuronal cell line is the SH-SY5Y cells, a human neuroblastoma cell line (ATCC 2266TM). SH-SY5Y cells were first discovered in 1970, derived from a metastatic bone tumour biopsy, SK-N-SH cell line. SH-SY5Y cells are widely used as it exhibits noradrenergic, dopaminergic and cholinergic properties, and the cell line has been regarded as the gold standard for neuronal cell culture in neurobiology research ([Murillo et al., 2017](#)). Upon differentiation with brain-derived growth factors (BDNF) and/or retinoic acid, SH-SY5Y cells express functional receptors, ion channels and neurotransmitters involved in signal transmission ([Xicoy et al., 2017](#); [Yin et al., 2016](#)). The cells have been reported to express opioid receptors, transient receptor potential cation channel subfamily V member 1 (TRPV1), *N*-methyl-D-aspartate (NMDA) receptors, GABA receptors and alpha adrenergic receptors, by which these receptors play a significant role in the development and maintenance of neuropathic pain ([Chia et al., 2020](#); [Forrest et al., 2017](#); [Kovalevich & Langford, 2013](#); [Nopparat et al., 2017](#); [Rohm et al., 2018](#)). SH-SY5Y cells were also subjected to other differentiation methods and agents, such as serum deprivation, treatment of phorbol esters and dibutyryl cyclic AMP to induce neurite extension and branching and the expression of specific neuronal properties ([Kovalevich & Langford, 2013](#)).

Furthermore, the induction of neuropathic pain-like condition on SH-SY5Y cell culture has resulted in changes in the expression of inflammatory proteins and signalling molecules, namely TLR receptors, interleukins, NFκB and nitric oxide ([Amine et al., 2021](#); [Kaswan et al., 2020](#); [Lawrimore & Crews, 2017](#)). SH-SY5Y cells are also commonly co-cultured with microglial cell lines to study the neuron-glia interaction in chronic pain mechanisms ([Anand et al., 2015](#); [Pandur et al., 2018](#)). Although SH-SY5Y cells have been utilised as the gold standard model in neurobiology research, cell differentiation and appropriate markers are pertinent to ensure functional proteins and signalling molecules associated with neuropathic pain mechanism are expressed ([Encinas et al., 2000](#); [Teppola et al., 2016](#)).

3.2.2 Neuro-2a cells

Another example of tumour-derived cell lines is the Neuro-2a cells, a mouse neural-crest-derived cell line

(ATCC 131TM). Established in 1969, Neuro-2a cells have been used as an in vitro peripheral nerve model as it expresses dopaminergic and glutamatergic properties and potassium channels upon differentiation ([Elmann et al., 2017](#); [Pousinha et al., 2017](#); [Tremblay et al., 2010](#)). These characteristics are crucial for the study of molecular mechanisms of neuropathic pain. However, despite the convenience in cell culture and differentiation, it is important to acknowledge that Neuro-2a cells are of the neuroblastoma cell line, and the cells may possess species variance as they are derived from mice.

3.2.3 Schwann cell lines

Like the primary cultures, Schwann cell lines have been established for use in studies on neuropathic pain. Schwann cell lines are preferred to primary cell culture due to low yield upon isolation, caused by contamination of connective tissues and fibroblasts ([Sango et al., 2011](#)). Established Schwann cell lines are the S16 and IMS32, derived from the rat sciatic nerve and dorsal root ganglion of adult mice, respectively ([Sango et al., 2011](#); [Tsukahara & Ueda, 2016](#)). The cells express distinct mature Schwann cell morphology and secrete glial cell markers such as glial fibrillary acidic protein, neurotrophic factors and nerve growth factors, which closely resemble primary culture ([Hattangady & Rajadhyaksha, 2009](#)).

3.2.4 Considerations of employing transformed cell lines

Regardless of the tumour cell lines acquired from the human or murine origin, this type of cell line may not concur with normal primary cells. The tumour cell lines may possess altered genotypes, occasionally expressing unique genetic sequences, resulting in phenotypes that are inapposite to normal cell physiology ([Carter et al., 2015](#)). For instance, the differences could lie in the genetics or physiological function of the receptors ([Kaur & Dufour, 2012](#)). These characteristics may further be varied as the cells undergo sub-culturing or passaging due to the dynamic and evolvable properties of cancerous cells ([Greaves & Maley, 2012](#)). Moreover, it is important to note the results of the immortalisation method in several neuronal cell cultures had caused an increased in the differentiation capacity and the occurrence of spontaneous differentiation ([Magsood et al., 2013](#)). Thus, periodical validation is necessary in the employment of cells with increasing passage numbers.

4.0 INDUCTION OF NEUROPATHIC PAIN-LIKE CONDITION AND CORRESPONDING BIOLOGICAL ASSAYS

Neuropathic pain could result from viral infections like HIV and shingles, trauma, treatment of drugs and diseases like diabetes mellitus ([Alles & Smith, 2018](#); [Colloca et al., 2017](#)). As discussed previously, the pathophysiology of neuropathic pain is comprised of a number of mechanisms such as the build-up of oxidative stress, change in inflammatory mediator expression, alteration of receptors and ion channels' expression, as well as a change in neuron-non-neuronal cells interaction ([Alles & Smith, 2018](#); [Colloca et al., 2017](#); [Finnerup et al., 2021](#)). The mechanism underlying the induction of neuropathic pain-like symptoms differs among the *in vivo* models. The CCI animal model, for example, exemplifies peripheral neuropathic pain, while Allen's model mimics central neuropathic pain through spinal cord injury ([Jaggi et al., 2011](#)). Thus, the primary molecular mechanisms and induction factors are important in mimicking neuropathy *in vitro*.

4.1 Chemotherapy-Induced neuropathic pain condition

Patients undergoing chemotherapy reportedly experience neuropathic pain symptoms, although they have completed successful cancer treatments ([Eldridge et al., 2019](#)). Antineoplastic drugs have been observed to be effective in targeting cancerous cells, and these drugs have shown adverse effects on healthy normal cells, such as damaging nerve cells ([Zajackowska et al., 2019](#)). Several chemotherapy agents that cause chemotherapy-induced peripheral neuropathy are platinum-based drugs (oxaliplatin), vinca alkaloids (vincristine), immunomodulatory drugs (thalidomide) and epothilones (ixabepilone) ([Starobova & Vetter, 2017](#)). The neuropathy-causing mechanism of antineoplastic agents includes damage to sensory neurons, inhibition of DNA transcription, dysregulation of ion channels, disruption of mitochondria activities and inhibition of microtubule formation ([Starobova & Vetter, 2017](#); [Yamamoto & Egashira, 2021](#)). In addition, these chemotherapy agents affect neuronal morphology by reducing neurite outgrowth in response to axonal degeneration ([Eldridge et al., 2019](#); [Podratz et al., 2016](#)).

Several chemotherapy and other oxidative stress agents are employed *in vivo* and *in vitro* models of neuropathic pain. The parameters of chemotherapy-induced neuropathy research usually involve observation of neuronal and/or glial cell morphology,

such as neurite outgrowth, presence of neurofilament, cell viability and mitochondria activity via ATP production ([Lehmann et al., 2020](#)).

4.2 Diabetic-induced neuropathic pain condition

Another leading disease that leads to the development of neuropathic pain is diabetes mellitus. Diabetic patients experience hyperalgesia at moderate to severe intensity, whereby the symptoms are commonly felt in their legs ([Colloca et al., 2017](#); [Jay & Barkin, 2014](#)). The pathophysiology of diabetic neuropathy was proposed to encompass the alteration of blood vessels and blood supply to peripheral nerves, dysregulation of ion channels' expression, change in the descending inhibitory pain pathway and activation of glial cells associated with autoimmune disorders ([Schreiber et al., 2015](#)).

Diabetic neuropathy is mainly due to hyperglycaemia. Hence, high glucose treatment has been used to mimic neuropathic pain conditions *in vitro* models as it leads to several downstream mechanisms, such as glucose auto-oxidation. This leads to an increase in the production of reactive oxygen species, which results in neural dysfunction and neuronal apoptosis ([Afrazi et al., 2014](#); [Hattangady & Rajadhyaksha, 2009](#); [Kaeidi et al., 2011](#)). Aside from hyperglycaemia, streptozotocin (STZ) is also used to induce diabetic neuropathy. STZ is an antimicrobial and chemotherapy agent which brings about the necrosis of pancreatic β -cells, causing hyperinsulinemia and hyperglycaemia ([Damasceno et al., 2014](#)). The development of *in vitro* model of STZ-induced neuropathy is done by injection of STZ into animals prior to neuronal cell culture. Another approach to employing STZ to mimic diabetic neuropathy is directly treating neuronal cell culture with STZ ([Hattangady & Rajadhyaksha, 2009](#); [Sun et al., 2018](#)).

The parameter observed in the *in vitro* model of diabetic neuropathy include the oxidative stress on neuronal and glial cells. This includes the analysis on the expression of ion channels and reactive oxidative species, as well as proteins and signalling molecules within the oxidation pathway ([Gardiner & Freeman, 2016](#)). Electrophysiology of neuronal culture as hyperglycaemia may alter the expression of ion channels on neuronal cells. Another hallmark of diabetic neuropathy is the retraction of neurite extension, hence this is an important output measure for the *in vitro* model ([Lu et al., 2019](#)).

4.3 Lipopolysaccharides-Induced neuropathic pain condition

Lipopolysaccharides (LPS), found on the outer membrane of Gram-negative bacteria, is also used to induce neuropathic pain condition *in vitro* models. LPS is not a common trigger of neuropathic pain in clinical settings. However, induction of LPS triggers various mechanisms that mimic neuropathic pain pathophysiology. Induction of LPS results in an increase of pro-inflammatory mediators and reactive oxidative species, which alters the expression of proteins and signalling molecules underlying the transmission of pain signals ([Pandur et al., 2018](#)). It has been observed that the treatment of LPS on neuronal and glial cells upregulates the expression of interleukin 6 (IL-6), tumour necrosis factor α (TNF- α), vanilloid receptor 1 (TRPV1) and *N*-methyl-*D*-aspartate (NMDA) subunit NR2B ([Chia et al., 2020](#)). TRPV1 and NMDA NR2B receptors are the leading player underlying hyperalgesia and allodynia in neuropathic pain conditions ([Carrasco et al., 2018](#); [Chia et al., 2020](#)). In addition, LPS could also induce changes in reactive oxidative stress (ROS), a similar condition observed in chemotherapy-induced and diabetic-induced neuropathic pain, as previously discussed ([Chanchal et al., 2016](#); [Kaswan et al., 2020](#)). The utilisation of LPS in inducing neuropathic pain pathophysiology *in vitro* model has been considered comprehensive as it can induce an inflammatory response and alters the leading players of nociceptive pathways such as the ion channels and receptors.

4.4 Human Immunodeficiency Virus (HIV)-induced neuropathic pain condition

Another common aetiology of neuropathic pain is a viral infection, such as the human immunodeficiency virus (HIV). HIV has been regarded as a chronic condition with a significant neurological complication: distal symmetric polyneuropathy ([Schütz & Robinson-Papp, 2013](#)). Patients with HIV reported symptoms such as hyperalgesia and allodynia, whereby the symptoms were reported to be within the neck, joints and leg area ([Addis et al., 2020](#)). The main structure causing neuropathy was the glycoprotein wrapping the virus, gp120. Treatment of gp120 in DRG neuronal culture resulted in cell lysis ([Datta et al., 20019](#)). The induction of gp120 also activates glial cells, such as Schwann cells, which further cause an increase in the expression of pro-inflammatory mediators from both neuronal and glial cells in a co-culture setting. The upregulated expression of TNF- α triggers neuronal apoptosis due to the neurotoxicity effect ([Moss et al., 2015](#); [Zhao et al., 2017](#)).

Another output measure in analysing HIV-induced neuropathy in the *in vitro* model is observing neuronal cell morphology. Induction of gp120 causes axonal injury and cutaneous denervation of the neurons ([Yuan et al., 2014](#); [Zhao et al., 2017](#)). Additionally, evidence has shown mitochondrial damage in patients with HIV-induced neuropathic pain. Thus, mitochondrial dysfunction could be an important variable to be studied *in vitro* models of HIV-induced neuropathic pain ([Cotto et al., 2019](#)). A number of studies observed the alterations of neurite outgrowth and changes in the expression of P2X4 receptors and chemokines receptors, suggesting these as notable output measures for the *in vitro* model ([Datta et al., 2019](#); [Kamerman et al., 2012](#); [Yuan et al., 2014](#)).

5.0 FUTURE PROSPECTS OF *IN VITRO* MODEL OF NEUROPATHIC PAIN

Research on developing an *in vitro* model from human cortical neurons is currently in progress. Chen *et al.* (2019) designed a three-dimensional (3D) cell culture of human cortical neurons on a polydimethylsiloxane (PDMS) micropillar surface to mimic the 3D microenvironment of a brain. The cells were differentiated into mature neurons, which express glutamatergic properties. The 3D neuronal culture was also induced with traumatic brain injury (TBI) through the weight-dropping method, which increases injury-induced glutamine, concurring with *in vivo* chronic pain models ([Chen et al., 2019](#)). Another 3D model identified as having a good prospect is culturing neuronal and glial cells using capillary alginate gel. The culture method utilises capillary microarchitecture to create a 3D cellular culture, which has been proposed to translate better the nervous system environment ([Anderson et al., 2018](#)).

In vitro models of neuropathic pain could be an ideal tool to understand the pathophysiology in a controlled environment. Nevertheless, it is important for researchers to effectively design the cellular environment, which may require differentiation of cells and more comprehensive output measures to observe. Researchers need to note the limitations of *in vitro* models, where additional research from *in vivo* and clinical studies is required better to understand the pathophysiology and possible treatments of neuropathic pain.

Author Contributions: All authors contributed, have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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