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# A Comprehensive Overview of Spinal Cord Injury (SCI) Experimental Models

Modinat Olushanu

## ABSTRACT

Spinal cord injury (SCI) is a multifaceted medical condition caused by trauma or disease, leading to sensory, motor, and autonomic dysfunction. The complex pathophysiology of SCI and the difficulties in developing effective treatments have driven the need for diverse experimental models that replicate various aspects of the injury observed in humans. These models are categorized into *in vivo* (animal), *ex vivo*, and *in vitro* (cellular) systems, each offering unique advantages tailored to specific research goals while also presenting inherent limitations. This review aims to provide a comprehensive overview of these experimental models, emphasizing their critical role in advancing the understanding of SCI mechanisms and facilitating the development of effective therapeutic strategies.

**Keywords:** Spinal cord injury, Experimental models, Secondary injury cascade, Contusion models, Regenerative therapies

## Introduction

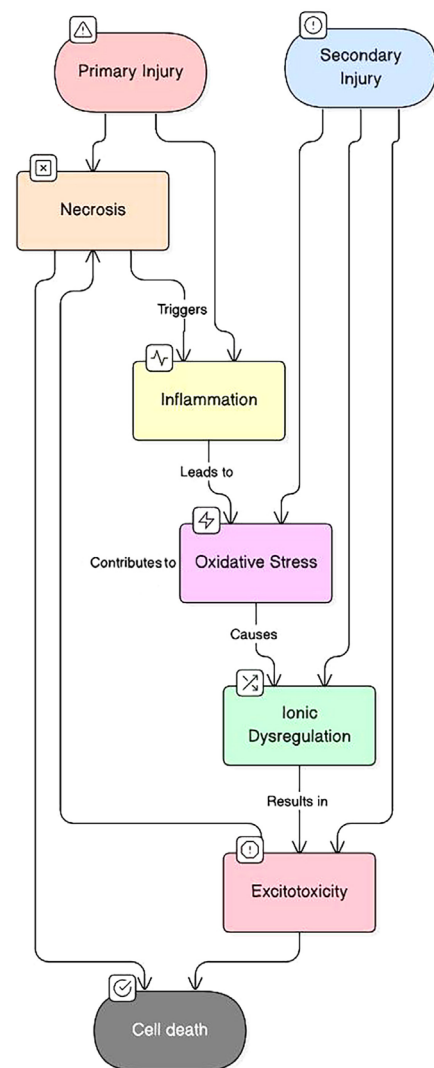
Spinal cord injury (SCI) is a complex condition that profoundly affects various physiological systems, leading to severe impairments in the sensory, motor, and autonomic functions.<sup>1-3</sup> SCI disrupts communication between the brain and body and affects the autonomic nervous system, leading to complications in cardiovascular, respiratory, urinary, gastrointestinal, and sexual functions.<sup>2,4</sup> Furthermore, SCI can lead to contradictory symptoms. For instance, although it generally causes motor paralysis and sensory loss, it can also result in spasticity, a condition characterized by increased muscle tone and exaggerated reflexes.<sup>5</sup>

The injury process begins with the primary mechanisms of trauma and involves the mechanical disruption of neural tissues, axons, and blood vessels.<sup>6</sup> This initial insult leads to immediate necrotic damage at the injury site, often accompanied by hemorrhage and edema. However, the extent of the damage is not solely defined by this primary trauma; it is significantly amplified by secondary mechanisms that unfold over the hours, days, and weeks following injury. This secondary injury cascade involves complex molecular and cellular events, including ischemia (restricted blood flow), inflammation, oxidative stress, ionic dysregulation, and excitotoxicity, caused by excessive glutamate release. Together, these processes contribute to widespread neural cell death, demyelination, and scarring, ultimately leading to functional deficits in patients with SCI (Figure 1).<sup>6-8</sup>

Secondary injury mechanisms are interconnected and self-perpetuating, resulting in progressive damage.<sup>9</sup> Inflammation exhibits a dual nature: while crucial for removing cellular debris, it releases

pro-inflammatory cytokines and reactive oxygen species, exacerbating tissue damage.<sup>7-11</sup> Oxidative stress, caused by an imbalance between free radicals and antioxidants, compromises cellular components including lipids, proteins, and DNA.<sup>12,13</sup> Mitochondrial dysfunction impairs energy production and initiates apoptotic pathways.<sup>14,15</sup> The accumulation of calcium ions within neurons disrupts cellular functions

## Spinal Cord Injury Mechanisms



**Fig 1 |** The mechanisms of SCI highlight the progression from primary and secondary injuries to cell death. The diagram illustrates how primary injury triggers necrosis and inflammation, which leads to oxidative stress and ionic dysregulation. These processes contribute to excitotoxicity and ultimately result in cell death. Secondary injury exacerbates these pathways, creating a feedback loop that amplifies damage

and contributes to neuronal death. Despite these deleterious mechanisms, certain factors may have regenerative potential. Specific inflammatory signals can stimulate the activation of endogenous neural stem and progenitor cells,<sup>16–19</sup> which migrate to the injury site and differentiate into neurons and glial cells. However, their regenerative potential remains insufficient without therapeutic intervention.<sup>7,16,20</sup> In SCI models, endogenous NSPCs are activated in the neural tissue adjacent to the injury and migrate to the epicenter.<sup>21</sup> Although NSPCs can differentiate into functional neurons *in vitro*, their *in vivo* neuronal differentiation is limited. Nakagomi et al. showed that in a stroke model, nestin-positive cells from the stroke-affected cortex migrated to the peri-infarct area and differentiated into glial cells; however, neuronal differentiation was not detected *in vivo*,<sup>22</sup> highlighting the influence of the microenvironment on NSPC fate.

Current research emphasizes targeting multiple pathways within the secondary injury cascade as single-mechanism approaches may not yield substantial clinical benefits.<sup>23–26</sup> Therapeutic interventions targeting inflammation, oxidative stress, and excitotoxicity show potential in preclinical studies,<sup>23,24</sup> including anti-inflammatory drugs, antioxidants, calcium channel blockers, and neuroprotective agents. Regenerative therapies, including stem cell transplantation, gene therapy, and bioengineered scaffolds, aim to enhance neural tissue repair.<sup>23,24,26,27</sup>

The complexity of SCI pathophysiology requires diverse laboratory models to investigate its mechanisms and evaluate interventions.<sup>28,29</sup> Animal models are categorized by injury conditions like contusion, compression, dislocation, transection, or chemical damage.<sup>28,30,31</sup> Rodents are widely used for accessibility, cost-effectiveness, and genetic manipulability,<sup>32</sup> while larger animals, including primates, porcine models, and canines, better approximate human SCI characteristics.<sup>29,33,34</sup> Each model provides insights into different aspects of SCI pathology.

Animal models have helped to identify therapeutic targets and evaluate experimental interventions, including pharmacological agents, stem cells, and neuroprosthetics. These preclinical studies have established the foundation for clinical trials, although translation to humans remains challenging owing to biological differences. Ongoing advances in understanding SCI pathophysiology and developing innovative therapies offer promise for improving outcomes in affected individuals.

### Methodology

A systematic literature review was conducted to construct a comprehensive overview of experimental SCI models. This review aimed to collect, synthesize, and critically evaluate the existing *in vivo*, *ex vivo*, and *in vitro* SCI models, emphasizing their translational relevance, experimental utility, and limitations.

### Search Strategy and Sources

The databases PubMed, Web of Science, Scopus, and Google Scholar were queried using combinations of

the following keywords: “*spinal cord injury*,” “*SCI models*,” “*contusion model*,” “*compression model*,” “*transection*,” “*organotypic culture*,” “*cell lines*,” “*3D co-culture*,” “*organ-on-chip*,” “*ex vivo*,” “*in vitro*,” “*animal model*,” “*rodent*,” “*primate*,” “*porcine*,” “*gene editing*,” and “*AI diagnostics*.”

### Inclusion Criteria:

- Peer-reviewed articles and systematic reviews
- Studies from 2000 to 2024, with emphasis on the last 10 years
- Literature focusing on the mechanistic modeling of SCI and/or therapeutic interventions
- Comparative studies evaluating different SCI models

### Exclusion Criteria:

- Non-English publications
- Abstracts, commentaries, and non-peer-reviewed gray literature
- Studies with insufficient methodological detail

### Selection and Analysis

In total, 1098 records were identified. After removing duplicates and screening the titles and abstracts, 234 articles were selected for full-text review. Of these, 119 were included based on relevance and methodological quality. The models were evaluated for replicability, cost, ethical feasibility, translational relevance, and capacity to mimic the key pathophysiological features of human SCI.

### In Vivo Models

*In vivo* models using live animals are essential to replicate SCI's mechanical and physiological aspects of SCI. Rodent models are invaluable for understanding mechanisms and evaluating therapeutic interventions.<sup>28</sup> Although rodents are most commonly used, larger animals, such as pigs and monkeys, are employed for translational research.<sup>33,35</sup>

The choice of species influences translational potential. Rodents are preferred for cost-effectiveness and genetic modifiability; however, their spinal cord size and immune responses differ from those of humans. Larger animals offer better anatomical similarities to humans despite ethical and cost challenges. Selecting appropriate species and models is crucial for research advancement. SCI models provide tools to explore injury and repair mechanisms through transection, hemisection, chemical lesions, and ischemic models. Although promising results exist, the complexity of SCI requires tailored approaches. Integrating insights from various models can help advance therapeutic interventions (Table 1).<sup>31,36–40</sup>

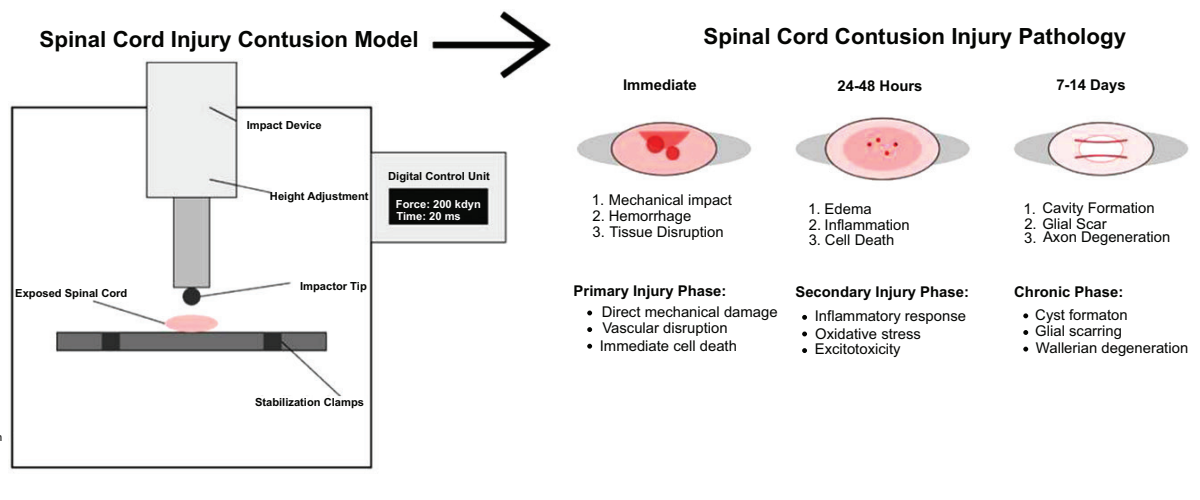
### Types of In Vivo Models

#### Contusion Models

Contusion SCI models provide a framework for studying the mechanisms and therapeutic interventions. The Allen weight-drop technique involves releasing a weight to induce injuries showing hemorrhagic

**Table 1 | Comparative analysis of animal models in SCI research**

Species	Anatomical/Physiological Features	Advantages	Limitations	Common Applications
Rodents (Mice/Rats)	<ul style="list-style-type: none"> <li>• Small spinal cord size</li> <li>• Basic anatomical similarities</li> <li>• Different immune response</li> </ul>	<ul style="list-style-type: none"> <li>• Cost-effective</li> <li>• Easy handling</li> <li>• Genetic modification possible</li> <li>• Large sample sizes feasible</li> <li>• Well-established protocols</li> </ul>	<ul style="list-style-type: none"> <li>• Small size limits surgical precision</li> <li>• Different immune response</li> <li>• Limited behavioral complexity</li> <li>• Anatomical differences from humans</li> </ul>	<ul style="list-style-type: none"> <li>• Initial screening studies</li> <li>• Genetic studies</li> <li>• Basic mechanism research</li> <li>• Drug testing</li> </ul>
Pigs	<ul style="list-style-type: none"> <li>• Similar cord size to humans</li> <li>• Comparable immune response</li> <li>• Similar anatomical features</li> </ul>	<ul style="list-style-type: none"> <li>• Size comparable to humans</li> <li>• Similar immune responses</li> <li>• Good anatomical correlation</li> <li>• Suitable for surgical technique development</li> </ul>	<ul style="list-style-type: none"> <li>• Higher costs</li> <li>• Complex handling</li> <li>• Limited genetic tools</li> <li>• Space requirements</li> </ul>	<ul style="list-style-type: none"> <li>• Surgical technique validation</li> <li>• Device testing</li> <li>• Translational studies</li> <li>• Safety assessments</li> </ul>
Non-human Primates	<ul style="list-style-type: none"> <li>• Most similar to humans</li> <li>• Complex neural systems</li> <li>• Similar immune response</li> </ul>	<ul style="list-style-type: none"> <li>• Closest to human anatomy</li> <li>• Similar immune system</li> <li>• Complex behavioral assessment possible</li> <li>• High translational value</li> </ul>	<ul style="list-style-type: none"> <li>• Highest costs</li> <li>• Ethical considerations</li> <li>• Limited availability</li> <li>• Complex regulatory requirements</li> </ul>	<ul style="list-style-type: none"> <li>• Final validation studies</li> <li>• Complex behavioral studies</li> <li>• Therapeutic translation</li> <li>• Clinical technique development</li> </ul>
Dogs	<ul style="list-style-type: none"> <li>• Intermediate cord size</li> <li>• Natural SCI occurrence</li> <li>• Similar pathophysiology</li> </ul>	<ul style="list-style-type: none"> <li>• Naturally occurring SCI models</li> <li>• Good size for surgical practice</li> <li>• Well-documented recovery patterns</li> </ul>	<ul style="list-style-type: none"> <li>• Ethical considerations</li> <li>• Genetic variability</li> <li>• Cost considerations</li> <li>• Limited control over injury type</li> </ul>	<ul style="list-style-type: none"> <li>• Natural injury studies</li> <li>• Long-term recovery studies</li> <li>• Rehabilitation research</li> </ul>
Cats	<ul style="list-style-type: none"> <li>• Well-studied nervous system</li> <li>• Good size for manipulation</li> <li>• Complex motor behavior</li> </ul>	<ul style="list-style-type: none"> <li>• Established neural circuitry knowledge</li> <li>• Good for motor studies- Moderate size</li> </ul>	<ul style="list-style-type: none"> <li>• Limited genetic tools</li> <li>• Ethical considerations- Less translational than larger models</li> </ul>	<ul style="list-style-type: none"> <li>• Motor control studies</li> <li>• Neural circuit research</li> <li>• Locomotion studies</li> </ul>



**Fig 2 | Experimental spinal cord contusion injury model and pathological progression.** The left panel illustrates the contusion model setup, highlighting key parameters such as the impact force (200–250 kdyn), duration (20 ms), height (12.5 mm), and tip diameter (2.5 mm). The right panel depicts the pathological timeline following SCI: immediate effects include mechanical impact, hemorrhage, and tissue disruption; within 24–48 h, secondary injury mechanisms such as edema, inflammation, and cell death occur; by 7–14 days, chronic phases manifest with cavity formation, glial scar development, and axon degeneration

necrosis, ischemia, inflammation, and central cavitation, which characterize human SCI pathology.<sup>31</sup> This method controls the compression and velocity variables.<sup>41</sup> Computer-controlled devices, such as ESCID, enabled precise displacement and impact modulation in murine models.<sup>42</sup> Pneumatic impactors independently regulate compression and contact velocity, generating distinct injury severities.<sup>43</sup> These refinements enhance our understanding of structural damage and functional impairment. Hemorrhagic necrosis correlates with contact velocity, whereas neuronal conduction loss is related to the degree of compression.<sup>43</sup> These insights demonstrate the complexity of SCI pathology and the importance of multiparameter models.<sup>28</sup>

Contusion models reveal cellular and molecular SCI responses, simulating spinal canal occlusion, and promoting specific cellular proliferation. McDonough et al. showed that contusion injuries affect the rostro-caudal extent of the spinal cord, causing lateral region proliferation with higher numbers of oligodendrocyte-fated cells compared to transection models.<sup>44</sup> Immunohistochemical analyses identified these changes and evaluated therapeutic strategies (Figure 2).<sup>45</sup>

Recent studies have also highlighted the evolution of contusion models. Smith et al. used the Infinite Horizon impactor to analyze microvascular changes post-injury.<sup>46</sup> A 2024 study using the NYU Impactor monitored motor potentials, demonstrating its utility for tracking recovery.<sup>47</sup> Studies have examined the influence of impact

variables on injury severity.<sup>48</sup> A 2019 review linked human SCI to animal model insights.<sup>49</sup>

Contusion models effectively replicate human SCI pathology, but cannot capture all injury types. Distraction and dislocation models produce unique axonal damage patterns that are absent in contusion injuries.<sup>30</sup>

Contusion SCI models are invaluable for replicating the clinical features of human SCI and testing therapies. However, integrating other models may be necessary for a comprehensive understanding of SCI pathology and treatment.

**Compression Models**

Compression models simulate spinal canal occlusion and secondary injury mechanisms, including ischemia, hemorrhagic necrosis, and inflammation.<sup>31,50</sup> Early decompression (within 6–12 h in rats, 8 h in humans) correlates with improved recovery outcomes,<sup>51–53</sup> emphasizing timely surgical intervention.

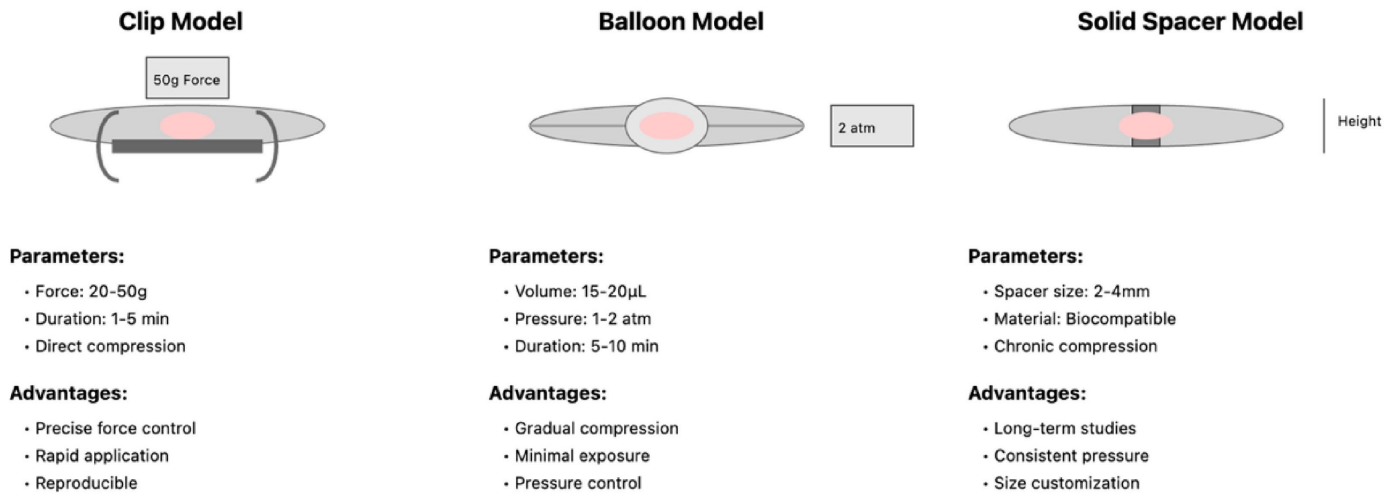
Clip compression models provide reproducible methods for studying secondary injury mechanisms,

showing dose-response relationships between clip forces and injury severity in rats.<sup>54</sup> Adapted for cervical SCI and mice, these models enable the study of cellular mechanisms and therapeutic interventions.<sup>55,56</sup>

Balloon compression models use catheter inflation in the epidural space to regulate compression duration and severity.<sup>57</sup> Using varying saline volumes, 15  $\mu$ L was found to induce recoverable paraplegia in rats. Finite element modeling showed the effects of posterior compression on spinal artery perfusion and ischemic risks,<sup>58</sup> helping to understand SCI pathophysiology (Figures 3 and 4).<sup>50,59</sup>

Solid spacer techniques simulate chronic spinal cord compression in animal models. Water-absorbable polyurethane polymer implantation in rats induces cervical compression and reduces neurological function,<sup>60,61</sup> whereas BMP implantation in rabbits shows no severe intramedullary pathologies.<sup>62</sup> These methods reveal the effects of compression on vascular and neurological outcomes.

**Spinal Cord Compression Models Comparison**

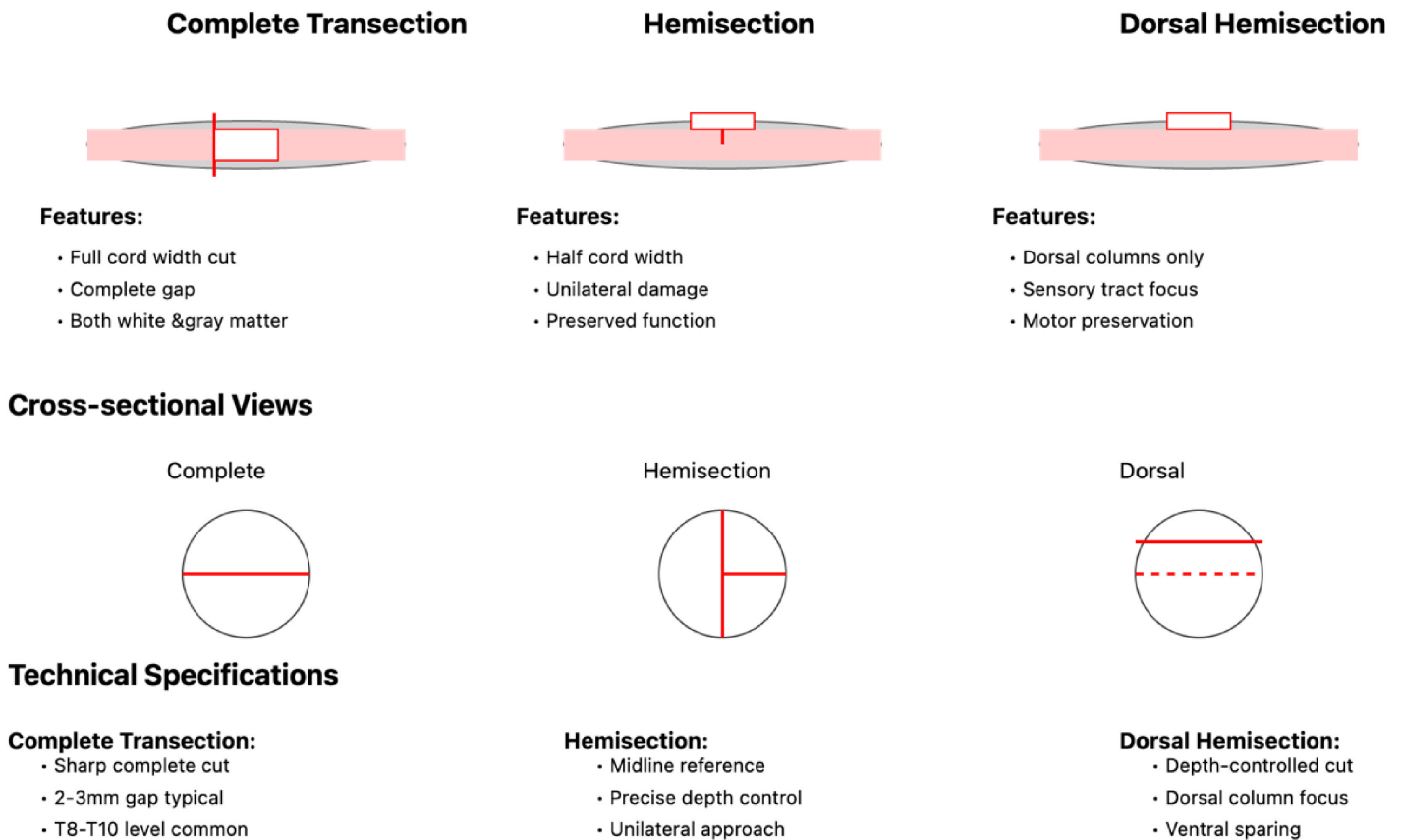


**Model Comparison**

Feature	Clip	Balloon	Solid Spacer
Application Time	Acute (minutes)	Sub-acute (minutes-hours)	Chronic (days-weeks)
Force Control	Direct measurement	Pressure controlled	Fixed displacement
Best Use	Acute injury studies	Progressive compression	Long-term studies
Technical Difficulty	Moderate	High	Low

Fig 3 | Comparison of spinal cord compression models: Clip, Balloon, and Solid Spacer. The top panel shows the parameters and advantages of each model. The Clip model delivers acute, direct compression with precise force control (20–50 g, 1–5 min), making it suitable for acute injury studies. The Balloon model provides gradual compression (15–20  $\mu$ L volume, 1–2 atm pressure, 5–10 min duration) and minimal tissue exposure, which is ideal for progressive compression research. The Solid Spacer model applies chronic compression (2–4 mm biocompatible spacer) with consistent pressure, making it suitable for long-term studies. The bottom panel presents a comparative table detailing each model’s application time, force control, best use, and technical difficulties





## Research Applications

- Complete: Regeneration studies, complete paralysis model
- Hemisection: Compensatory mechanisms, partial recovery
- Dorsal: Sensory pathway studies, neuropathic pain

**Fig 5 |** Spinal cord transection models include complete transection, hemisection, and dorsal hemisection models, each with distinct features, cross-sectional views, technical specifications, and research applications. Complete transection involves a full cord-width cut through the gray and white matter, typically creating a 2–3 mm gap at the T8–T10 level, and is commonly used for regeneration studies and complete paralysis models. Hemisection involves unilateral damage, affecting half of the cord while preserving function on the contralateral side. It is performed using a midline reference with precise depth control to study the compensatory mechanisms and partial recovery. The dorsal hemisection targets only the dorsal columns, sparing motor function, with depth-controlled cuts to focus on sensory tract studies and neuropathic pain research

appropriate models, as different injuries elicit distinct responses (Figure 6).

Ischemic models of SCI through spinal cord blood supply occlusion provide insight into vascular injury.<sup>38</sup> While these models study ischemia, compression and contusion models better replicate human SCI features, such as hemorrhagic necrosis and cavitation.<sup>31</sup>

Experimental SCI modeling is essential for understanding injury mechanisms and evaluating treatments. *In vivo* models, especially contusion and compression types, remain clinically relevant because they reproduce key human SCI features. Transection models study axonal regeneration, whereas chemical and ischemic models investigate specific pathologies.

However, *in vivo* models cannot fully replicate the heterogeneity of human SCI. *Ex vivo* systems maintain their native structure while allowing microenvironment manipulation, whereas *in vitro* models provide

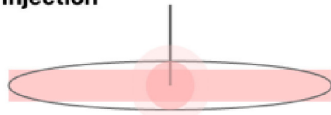
high-throughput capacity for mechanistic studies. Integrating these approaches enhances experimental rigor, and future advances depend on combining these models to understand the pathophysiology and therapeutic outcomes.

### Ex Vivo Models

*Ex vivo* SCI models provide platforms for investigating spinal cord dynamics in controlled environments by maintaining extracted tissue in culture systems. These models address the ethical challenges of *in vivo* studies using localized injury response analysis. Weightman et al. demonstrated organotypic slice arrays for replicating cellular responses to injury, including gliosis and nerve fiber outgrowth, thereby reducing invasive procedures.<sup>71</sup> Integrating aligned polylactic acid nanofiber meshes enabled the assessment of the regenerative potential of nanomaterials. Yan et al. developed a

### Chemical Lesion Models

#### Direct Injection



**Common Agents:**

- Kainate
- Glutamate
- Ethidium bromide
- Lysolecithin

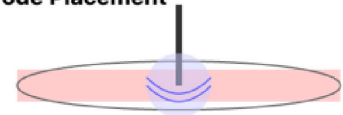
**Lesion Pattern:**

- Diffuse spread
- Gradient effect



### Electrolytic Lesion Models

#### Electrode Placement

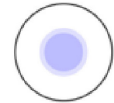


**Parameters:**

- Current: 1-2mA
- Duration: 10-20s
- Electrode size
- Depth control

**Lesion Pattern:**

- Precise boundary
- Controlled size



### Model Comparison

Feature	Chemical	Electrolytic
Precision	Moderate (diffusion-dependent)	High (current-controlled)
Size Control	Variable	Precise
Mechanism	Chemical toxicity	Current-induced damage
Time Course	Progressive	Immediate

**Key Considerations:**

- Chemical: Agent concentration, volume, and diffusion patterns critical
- Electrolytic: Current parameters and electrode positioning crucial

Fig 6 | Chemical and electrolytic spinal cord lesion models are illustrated along with their mechanisms, lesion patterns, and comparative analysis. Chemical lesion models involve the direct injection of agents such as kainate, glutamate, ethidium bromide, or lysolecithin, resulting in a diffuse spread and gradient effect due to chemical toxicity. Electrolytic lesion models utilize electrode placement with controlled parameters (current, 1–2 mA; duration, 10–20 s; depth control), producing precise and defined lesion boundaries through current-induced damage. The comparison table highlights key differences, with chemical models offering variable size control and progressive lesion development, whereas electrolytic models provide high precision and immediate effects. Key considerations emphasize the importance of the agent concentration and diffusion patterns for chemical models and electrode positioning for electrolytic models

micropatterned conductive nanofiber mesh with PCL and NGF, facilitating nerve stem cell differentiation while suppressing astrocyte formation.<sup>72</sup> The aligned nanofibers guided neurite outgrowth, mimicking *in vivo* patterns, and demonstrating translational relevance.

Explant models reveal neural regeneration processes including cell survival, differentiation, migration, and axonal regrowth. Dorsal root ganglia explants have shown that oxidized galectin-1 promotes axonal regeneration and Schwann cell migration.<sup>73</sup> A three-dimensional DRG model has demonstrated the role of the urokinase system in neural cell migration and axonal branching.<sup>74</sup> Studies on Schwann cell migration and axonal regrowth have shown conflicting

results regarding the process sequence after nerve transection.<sup>75</sup> Explant models help study neuronal-Schwann cell interactions and nerve regeneration factors.<sup>76,77</sup>

Human *ex vivo* spinal cord slice cultures provide a model to study neuronal populations and inflammatory responses. These cultures maintain neuronal subpopulations and preserve the tissue cytoarchitecture.<sup>78,79</sup> Ventral horn motoneurons decline after 3 days, while calbindin-positive neurons persist longer.<sup>80</sup> IL-1 $\beta$  can activate endogenous neural progenitors of oligodendrocyte lineage.<sup>81</sup> These models examine disease mechanisms such as neuromyelitis optica through immune cell-cytokine interactions.<sup>82</sup>

*Ex vivo* models, including organotypic slice cultures, offer key advantages. Controlling variables, such as

temperature and nutrients, enhances reproducibility and reduces confounding factors. These models are ideal for imaging and drug screening studies while reducing animal use.

However, *ex vivo* models cannot replicate systemic interactions such as immune responses and hormonal signaling, limiting extrapolation to living organisms. However, the limited survival time of tissue cultures impedes long-term studies. These limitations necessitate combining *ex vivo* and *in vivo* approaches to understand the pathophysiology of SCI.

### ***In Vitro* Models**

*In vitro* SCI models use isolated cell cultures to investigate cellular and molecular mechanisms of injury and repair. These models provide controlled environments for understanding SCI processes and drug development. *In vitro* approaches include various systems with different applications and limitations.<sup>83-86</sup>

#### **Types of *In Vitro* Models**

##### **Primary Cell Cultures**

Primary cell cultures from spinal cord tissue are used to study cellular responses to SCI. Methods exist for isolating specific cell types: oligodendrocytes and astrocytes from embryonic rat spinal cords;<sup>87</sup> oligodendrocytes from adult human spinal cords;<sup>88</sup> and motor neurons, microglia, and astrocytes from mouse embryonic spinal cord.<sup>89</sup> Primary cultures replicate *in vivo* conditions better than immortalized cell lines,<sup>90,91</sup> with astrocyte cultures expressing higher levels of differentiated markers such as GFAP, S100B, AQP4, and ALDH1L1.<sup>90</sup>

##### **Immortalized Cell Lines**

Immortalized cell lines, such as PC12 and NSC-34, offer easy maintenance and reproducibility in SCI research. PC12 cells differentiate into neuron-like cells upon NGF

exposure,<sup>92</sup> while NSC-34 cells exhibit motor neuron characteristics (Anjum et al. 2024), which is useful for disease modeling.<sup>93</sup> These lines enable high-throughput screening and mechanistic studies.<sup>94,95</sup> However, their transformed nature may limit *in vivo* replication, which requires validation with primary cultures. Some cell lines can mimic primary cell responses, such as SV40-immortalized astrocytes showing ATP-induced calcium waves similar to primary astrocytes.<sup>96</sup> Although valuable for research, careful interpretation remains essential for clinical translation.

##### **Three-Dimensional Co-Culture Systems**

Three-dimensional (3D) co-culture systems replicate the native environment of the spinal cord better than two-dimensional (2D) cultures.<sup>97</sup> These systems integrate multiple cells within a 3D matrix to simulate cell-ECM interactions.<sup>98</sup> 3D spinal cord networks showed faster inhibitory synapse maturation and increased activity than 2D cultures. Creating ultrasoft 3D cultures mimicking spinal tissues requires solutions such as microfiber reinforcement.<sup>97</sup> These systems help to model glial scarring and axonal regeneration while evaluating drug efficacy.

##### **Organ-on-a-Chip Models**

Organ-on-a-chip technology has advanced SCI research by integrating spinal cells into microfluidic systems. These platforms simulate *in vivo* conditions, thereby enabling realistic SCI studies. Ao et al. developed a spinal organoid-on-a-chip for nociceptive drug screening,<sup>99</sup> whereas Su et al. created a microfluidic chip mimicking spinal tissues.<sup>100</sup> These systems bridge the gap between *in vitro* and *in vivo* models.

*In vitro* SCI models enable precise control of experimental variables and ensure reproducibility. They are cost-effective and are suitable for use in high-throughput studies. However, they lack vascular networks,

**Table 2 | Comparison of *in vivo*, *ex vivo*, and *in vitro* sci models**

Model Type	System	Key Features	Strengths	Limitations	Common Uses
<i>In Vivo</i>	Rodents (Mice, Rats)	Small size, genetically modifiable	Cost-effective, high-throughput, well-established	Limited anatomical/functional similarity to humans	Mechanistic studies, drug testing, regeneration
	Pigs	Anatomically similar to humans	Good surgical simulation, translational relevance	High cost, limited genetic tools	Surgical techniques, biomaterials, preclinical validation
	Non-human Primates	Closest CNS mimicry to humans	High behavioral and anatomical fidelity	Ethical constraints, regulatory hurdles	Complex interventions, behavior and recovery studies
	Dogs	Naturally occurring SCI	Good for chronic and recovery models	Ethical concerns, variability	Rehabilitation, veterinary SCI models
<i>Ex Vivo</i>	Organotypic Slice Culture	Maintains spinal cytoarchitecture	Reduces animal use, good for imaging	Short tissue viability, no systemic context	Injury modeling, neuroprotection, biomaterials testing
	Explant Models (e.g., DRG)	3D structure preserved	Insight into regeneration, axonal behavior	Short culture duration, low systemic mimicry	Schwann cell/axon interactions, migration studies
<i>In Vitro</i>	Primary Cultures	Derived directly from spinal tissue	High physiological relevance	Labor-intensive, limited scalability	Neuronal injury, astrocyte activation, screening
	Immortalized Cell Lines (e.g., PC12, NSC-34)	Long-lived, consistent behavior	High-throughput, easy maintenance	Lower physiological relevance	Mechanistic assays, neurotoxicity, neurogenesis
	3D Co-Culture	Matrix-based, multicellular	Mimics <i>in vivo</i> ECM structure, cell-cell signaling	Technically complex	Axonal growth, glial scarring, intercellular signaling
	Organ-on-a-Chip	Microfluidics + stem cells/organoids	Dynamic conditions, simulation of spinal biomechanics	High cost, still emerging tech	Personalized drug screening, <i>in vitro</i> clinical modeling

immune responses, and the structural complexity of *in vivo* systems, which require complementary studies for clinical applications (Table 2).

### Emerging Innovations in SCI Treatment

Emerging innovations in *in vitro* SCI research have overcome the limitations of traditional models by increasing their relevance and potential for clinical translation. These advancements provide enhanced platforms for understanding the mechanisms of injury and for developing therapies. Key approaches include the use of multipotent mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSCs), bioengineered models, and *in silico* simulations. MSCs are valuable in SCI research because of their regenerative properties, including neuroprotection, neuronal regeneration, angiogenesis, immunomodulation, and reduction of glial scarring, making them crucial for studying repair processes and testing therapies.<sup>101</sup>

iPSCs derived from patient-specific cells can differentiate into spinal cord cell types, including neurons, astrocytes, and oligodendrocytes.<sup>102,103</sup> They enable the study of personalized injury responses and genetic factors in SCI progression, helping to develop tailored therapies and testing interventions before clinical application.<sup>104,105</sup>

Bioengineered models using bioprinted scaffolds and hydrogels replicate the 3D architecture of the spinal cord, allowing the observation of cellular behaviors within a relevant microenvironment.<sup>106–108</sup> By mimicking spinal cord properties, these models provide platforms for studying cell-matrix interactions and drug delivery methods.<sup>106,109–111</sup>

*In silico* models use computational simulations to predict SCI progression and treatment outcomes. These models integrate experimental data into streamlined research designs and evaluate therapeutics.<sup>112,113</sup> They efficiently studied processes such as axonal degeneration, and assessed the impact of multiple variables on injury progression.<sup>113,114</sup>

Gene-editing technologies, especially CRISPR-Cas9, further amplify this progress by targeting genes involved in axonal regeneration, inflammation, and cell death, thus promoting neuroprotection and functional recovery.<sup>115,116</sup> Engineered stem cells modified through gene editing can enhance remyelination and secrete neurotrophic factors, whereas immune modulation via the genetic suppression of pro-inflammatory pathways reduces secondary damage.<sup>117</sup> Combined with AI, these strategies have become even more potent: AI can identify novel therapeutic gene targets through large-scale data analysis, optimize guide RNA design for improved precision, and enable real-time monitoring of therapeutic effects using biosensors.<sup>118,119</sup> Together, these innovations pave the way for highly personalized and adaptive SCI therapies.

### Conclusion

The study of SCI requires diverse models to address its complex pathophysiology and to support the development of effective therapeutic strategies. *In vivo*, *ex vivo*,

and *in vitro* models each provide unique advantages for exploring various aspects of SCI, including mechanisms of injury, repair processes, and therapeutic efficacy. *In vivo* models, particularly in rodents and larger animals, remain critical for replicating the physiological and mechanical characteristics of human SCI and understanding systemic factors such as inflammation, vascular responses, and functional recovery. *Ex vivo* systems, such as organotypic slice cultures and explant models, offer valuable tools for investigating localized cellular responses and regenerative processes within a controlled environment, while reducing reliance on live animals. *In vitro* models, including primary cell cultures, immortalized cell lines, and advanced systems such as organ-on-a-chip technologies, enable precise mechanistic studies and high-throughput screening for drug development.

Integrating emerging innovations such as iPSCs, bioengineered 3D cultures, and *in silico* simulations has transformed the landscape of SCI research. iPSC-derived platforms provide personalized approaches for disease modeling and therapeutic testing. In contrast, bioengineered systems replicate the structural and mechanical properties of the spinal cord and offer spatially relevant environments for regenerative studies. *In silico* models further complement experimental approaches by providing predictive insights that streamline experimental design and accelerate therapeutic development. These innovations address the limitations of traditional methods and improve the translational relevance of the preclinical findings.

However, each model has its inherent limitations. *In vivo* systems face inter-species differences and reproducibility challenges, whereas *in vitro* and *ex vivo* models lack systemic interactions, such as blood flow and immune responses, which are critical to SCI pathophysiology. A multifaceted approach that integrates complementary models is essential for overcoming these challenges. Combining the strengths of diverse methodologies will enable a comprehensive understanding of SCI mechanisms, facilitate the identification of therapeutic targets, and enhance the translation of promising interventions into the clinical setting.

In conclusion, continued advancement and refinement of SCI models and innovative technologies, such as bioengineered platforms, iPSCs, and computational simulations, are promising for improving the accuracy, relevance, and clinical applicability of preclinical research. By leveraging these tools, researchers can bridge the gap between experimental findings and clinical outcomes, ultimately paving the way for novel and effective therapeutic interventions to address the profound impact of SCIs.

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