

Physicochemical Property Effects on Immune Modulating Polymeric Nanoparticles: Potential Applications in Spinal Cord Injury

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Abstract: Nanoparticles (NPs) offer promising potential as therapeutic agents for inflammation-related diseases, owing to their capabilities in drug delivery and immune modulation. In preclinical studies focusing on spinal cord injury (SCI), polymeric NPs have demonstrated the ability to reprogram innate immune cells. This reprogramming results in redirecting immune cells away from the injury site, downregulating pro-inflammatory signaling, and promoting a regenerative environment post-injury. However, to fully understand the mechanisms driving these effects and maximize therapeutic efficacy, it is crucial to assess NP interactions with innate immune cells. This review examines how the physicochemical properties of polymeric NPs influence their modulation of the immune system. To achieve this, the review delves into the roles played by innate immune cells in SCI and investigates how various NP properties influence cellular interactions and subsequent immune modulation. Key NP properties such as size, surface charge, molecular weight, shape/morphology, surface functionalization, and polymer composition are thoroughly examined. Furthermore, the review establishes connections between these properties and their effects on the immunomodulatory functions of NPs. Ultimately, this review suggests that leveraging NPs and their physicochemical properties could serve as a promising therapeutic strategy for treating SCI and potentially other inflammatory diseases.

Keywords: nanomaterials, biodegradable carriers, particle engineering, biomaterial-based therapies, neuroinflammation, nerve tissue repair

Introduction

Spinal cord injury (SCI) results in loss of sensation, movement, and autonomic functions of the body below the level of injury.^{1,2} The time course of SCI can be divided into two phases: the primary injury and prolonged secondary injury.^{3,4} The primary injury is defined as the initial mechanical trauma to the spinal cord initiating various secondary physiological processes.⁴⁻⁶ The secondary physiological disruptions following SCI include the disturbance of the blood-spinal cord barrier, inflammation, glial scarring, demyelination, compromised axonal regeneration, and neuronal death. These disruptions lead to various functional disorders such as locomotor and somatosensory dysfunction.⁷⁻⁹

Currently, treatment options for SCI remain limited with no FDA approved therapeutics that restore neurological function.¹⁰ Previously, glucocorticoids such as methylprednisolone were used to treat SCI due to their anti-inflammatory effects. However, they have since been linked to many severe side effects, leading to a decline in their use and creating a gap between patient needs and effective treatment options.⁴ Several treatments for SCI are currently in preclinical development. These include rehabilitation,¹¹ electrical stimulation of the motor cortex and spinal cord,¹² transplantation of stem cells,¹³ and the use of antibodies or pharmacological agents to target various axon growth inhibitory molecules in the central nervous system (CNS).¹⁴⁻¹⁸

Recently, polymeric nanoparticles (NPs) have emerged as a promising therapeutic treatment for SCI. NPs are broadly classified as particles with dimensions on the nanometer scale and can vary in multiple characteristics, such as base

material and physicochemical properties, which is a major focus of this review. Previously, NPs have been utilized for drug delivery owing to their ability to increase the safety and efficacy of various drugs.¹⁹ In addition to drug delivery, NPs have recently shown potential in modulating the immune response to SCI. Studies have demonstrated the ability of polymeric NPs to target the innate immune response after SCI, and a few preclinical studies have shown beneficial effects such as behavioral recovery and axonal regrowth in rodent models of SCI.^{20–22} The effects of NPs are influenced by their physicochemical properties, including size, surface charge, polymer molecular weight, surface functionalization, shape/morphology, and polymer composition (Table 1).^{23–25} These NP properties are key design features and have been shown to influence interactions with immune cells, resulting in varied therapeutic outcomes.^{23–25} In this review, we will investigate multiple physicochemical properties and their effects. Understanding the relationship between these properties and the ability of NPs to target specific outcomes in SCI enables the design of customized NP treatments. This tailored approach offers a promising therapeutic option for individuals with SCI.

Pathophysiology of SCI and Therapeutic Implications

The disruption of the blood-spinal cord barrier (BSCB) after initial traumatic SCI results in an inflammatory response, causing the invasion of circulating innate immune cells such as neutrophils, monocytes, and macrophages and the release of various inhibitory factors including pro-inflammatory factors at the injury site.⁶⁶

The inflammatory response after SCI consists of both adaptive and innate immune responses. Several cell types and inflammatory cytokines are involved in mediating the inflammatory response to SCI. Hellenbrand et al, has described the

Table 1 Properties of Nanoparticles and Their Major Functions

Nanoparticle Property	Function	Ref
Size	Cellular Internalization	[26,27]
	Immune Cell Activation	[19,28]
	Biodistribution	[29,30]
Surface Charge	Cytotoxicity	[26,31]
	Cellular Uptake	[32–34]
	Protein Attachment	[35,36]
Molecular Weight	Degradation	[37]
	Drug Release	[38]
	Cytokine Expression	[39,40]
	Trafficking	[22]
Surface Functionalization	Clearance	[41–44]
	Protein Binding	[41–44]
	Cellular Targeting	[45–52]
Shape and Morphology	Extent of Uptake	[53–59]
	Cellular Internalization	[53–59]
	Cellular Targeting	[60,61]
Polymer Composition	Degradation	[62,63]
	Hydration	[64]
	Drug Release	[65]

chemokine cascade and cell infiltration patterns after SCI.⁶⁶ During the early stages of injury, the resident microglia changes into pro-inflammatory cells that secrete cytokines and trigger peripheral immune cell infiltration.^{45,67} These changes contribute to myelin disruptions and fiber degeneration known as Wallerian degeneration (WD), leading to delayed sensorimotor dysfunction.³ Neutrophils are the first peripheral immune cell to arrive at the injury site within 4–6 hours and produce proteolytic and oxidative enzymes to prepare the area for repair.⁶⁸ During 6–12 hours post injury, polymorphonuclear leukocytes are seen at the injury site and release reactive oxygen and nitrogen species (ROS and NOS).⁶⁹ These ROS and NOS create widespread cell damage within the injury site, promoting an inflammatory and growth inhibiting environment, contributing to functional loss after SCI.⁷⁰ From 24 hours to 7 days, T cells, monocytes, and dendritic cells are recruited to the injury site. Beck et al, using flow cytometry in rats with SCI demonstrated that neutrophils peak after 1 day, macrophages and microglia peak after 7 days, and T cells peak after 9 days.⁷¹ Following infiltration, both B and T cells persist indefinitely within the injury site and continue to increase in number for at least 9 weeks.^{45,72–74} They continue to promote cytokine/chemokine imbalance within the injury site, promoting inflammation and immune cell activation.^{72,75} Additionally, they remain within the injury site following repair of the BSCB, suggesting that B and T cells are reactivated locally within the injury site, leading to long injury residence times.⁷³

From 7 days to 2 weeks, there is proliferation of oligodendrocyte progenitor cells resulting in improper remyelination.⁷⁶ Infiltration of fibroblast-like cells and the formation of the fibrotic tissue matrix in mice occurs at 2 weeks.⁷⁷ After 2 weeks, astrocytes form the glial scar around the lesion expressing glial fibrillary acidic protein (GFAP).⁷⁸ Additionally, pericytes enter the lesion with blood vessel sprouts and form the stromal component of the scar tissue.⁷⁹ The scar itself inhibits multiple repair processes, including axonal regeneration, sprouting, conduction, and remyelination, preventing the reconnection of axons above and below the level of injury and contributing to paralysis.⁸⁰ The final glial scar has infiltrating immune cells in the center, microglia interacting with pericytes around the edges, and astrocytes surrounding the periphery (Figure 1). Collectively, both the innate and adaptive immune response along with changes to the intraspinal environment in response to injury lead to limited endogenous repair and ongoing secondary injury.

Given the complex pathophysiology of SCI and the limited availability of treatments, NP technology may offer minimally invasive and multimodal therapeutic tools to address both the neuroinflammatory responses and multifaceted immune dysfunction associated with SCI. Various NP types have been studied, each with unique mechanisms and benefits. Anti-inflammatory drug loaded NPs have shown potential in manipulating the immune response to SCI. One study using chitosan/poly-(γ -glutamic acid) based NPs loaded with the non-steroidal anti-inflammatory drug diclofenac demonstrated inhibition of prostaglandin E2 synthesis and IL-6 production in lipopolysaccharide (LPS)-treated human macrophages, leading to downregulation of inflammatory signaling.⁸¹ Despite their anti-inflammatory effects, these NPs failed to significantly impact pro-regenerative macrophage markers like IL-10, a key factor in promoting regeneration after SCI. In contrast, studies using antioxidant loaded NPs have been able to show both a reduction in inflammation and promotion of regeneration by minimizing oxidative stress. One study using curcumin-loaded poly(lactic-co-glycolic acid) (PLGA) NPs released from a gelatin/alginate scaffold in the spinal cord injury site of rat models found increased functional recovery and axonal regrowth following injury.⁸² In a separate study, curcumin-loaded lipid NPs were shown to regulate the TLR4/2-NF- κ B pathway in mice models for sepsis, which plays a major role in the immune response to SCI.⁸³ Additional antioxidants such as catechin from green tea have also demonstrated anti-inflammatory effects when delivered via NPs to mouse macrophages.⁸⁴

Despite the potential for anti-inflammatory drug and antioxidant-loaded NPs, other approaches have focused on using NPs to both directly and indirectly modulate the immune response to SCI without the delivery of additional compounds, providing a less invasive method and minimizing the potential for off-target effects. For example, we previously used intravenously injected PLGA NPs to reprogram the immune response after SCI in mice by interacting with circulating innate immune cells such as macrophages, monocytes, and neutrophils in the blood.²⁰ Systemically administered NPs can selectively target and reprogram immune cells, particularly cell populations with scavenger receptors such as Macrophage Receptor with Collagenous Structure (MARCO)-positive subsets.^{20,85} These NP-associated cells are sequestered in the spleen and undergo apoptotic change, thereby delaying the infiltration into the injury site. This limits intraspinal cell accumulation and decreases pathogenic inflammation.²⁰ Therefore, NPs indirectly attenuate the immune pathology in the inflamed area after injury.

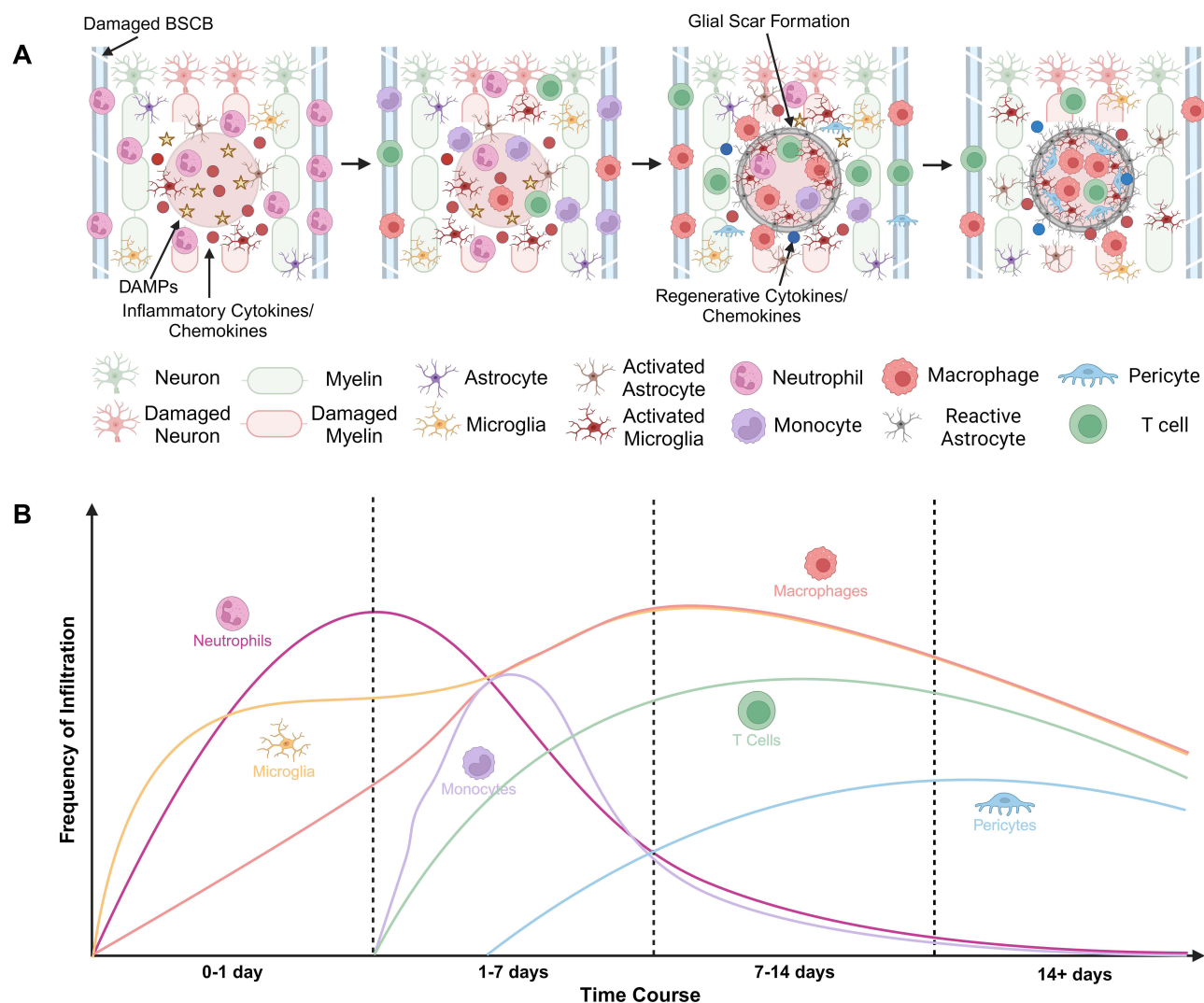


Figure 1 (A) Schematic depicting time course of SCI and infiltration of immune cells. (B) Graph illustrating the infiltration frequency of various immune cell populations over the time course of SCI. Created in BioRender. Kolpek, D (2024) <https://BioRender.com/w82h824>.

Generally, systemically administered NPs are taken up by immune cells in the vasculature and accumulate in the lysosome.⁸⁶ The PLGA-based NPs undergo lysosomal biodegradation, generating glycolic and lactic acids that are metabolized in the Krebs cycle and eliminated as carbon dioxide and water.⁸⁷ These byproducts effectively attenuate inflammatory responses by limiting NF- κ B nuclear localization and modifying effector proteins, resulting in reduced expression of multiple inflammatory factors after injury.⁸⁸ Published data demonstrate the accumulation of NP-positive cells at the injury site, which enables the upregulation of multiple pro-regenerative associated genes (RAGs) that create a more permissive environment after injury.^{20,21} Thus, this approach excludes invasive strategies such as surgical procedures and localized drug administration. In addition, the NP-mediated approach has more effective therapeutic strategies relative to antibody- and/or clodronate-encapsulated liposome-mediated approaches which can only employ a single factor or deplete entire systemic immune cell populations.^{89,90} Overall, NPs can serve as minimally invasive acute care of SCI, providing spinal tissue protection upon injury by reprogramming inflammatory immune cells, in turn reducing the development of an inhibitory environment and enhancing neurological outcomes after SCI.

As foreign substances, injected NPs rapidly engage the innate immune system, triggering tailored immune responses depending on their physicochemical properties. This underscores the potential of using engineered NP properties to modulate innate pro-inflammatory cell phenotypes, including inflammatory monocytes, neutrophils, and macrophages.²⁰

The physicochemical properties of NPs greatly influence the interactions between NPs and the innate immune system, particularly the downstream signaling for immunomodulatory events.^{25,26,91} Here we summarize the effects of physicochemical properties of NPs on immunomodulation and apply them to SCI.

Impact of Nanoparticle Size on Innate Immune Cells

The size of NP can significantly impact interactions with innate immune cells, influencing cellular internalization, immune cell activation, and biodistribution.^{27,92,93} Five distinct uptake pathways have been shown to play a role in the internalization of NPs. These include clathrin-mediated endocytosis (CME), fast endophilin-mediated endocytosis (FEME), clathrin-independent carrier (CLIC)/glycosylphosphatidylinositol-anchored protein enriched early endocytic compartment (GEEC) endocytosis, macropinocytosis, and phagocytosis. Furthermore, these pathways can all be sub-classified based on their clathrin and dynamin dependencies (Figure 2).⁸⁶ Previously, caveolar-dependent endocytosis was considered to be significant. However, it has been reported that while caveolae can be present on the plasma membrane, few nanocarriers rely on them for uptake. This discrepancy is due to the insufficient specificity of inhibitors targeting endocytic pathways.^{28,86}

The route of cellular uptake used among innate immune cells to internalize polymer-based NPs has been shown to vary widely with size. One study using 100 nm poly(methyl methacrylate) (PMMA) based NPs found LPS-activated microglia to selectively uptake NPs through CME.²⁹ A similar study using mouse macrophages showed that smaller 40 nm polystyrene (PS) NPs were taken up by a combination of CME and macropinocytosis. In contrast, only phagocytosis and macropinocytosis were used to take up larger 600 nm NPs.⁹⁴ The overall pattern indicates that immune cells employ a mix of CME, FEME, CLIC/GEEC, and macropinocytosis to internalize smaller NPs (<200 nm), whereas phagocytosis and macropinocytosis are utilized for larger NPs (>200 nm).⁸⁶

The processes of CME, FEME, and phagocytosis are specific pathways and are well controlled through receptors.⁸⁶ However, CLIC/GEEC and macropinocytosis are non-specific pathways that internalize both NPs and other fluids into the cell.^{30,86} Certain findings indicate that this variation in endocytic specificity may result in diverse immune responses. One study by Park et al found PLGA-based NPs with a diameter of 500 nm can effectively reprogram innate immune cells through association with scavenger receptors that rely on dynamin-dependent endocytosis and macropinocytosis.^{20,95} Furthermore, these NPs facilitated the differentiation of macrophages towards an anti-inflammatory phenotype following SCI in mice models.²⁰ In contrast, another study using smaller polypyrrole (PPy) based NPs ranging in size from 20 nm to 100 nm

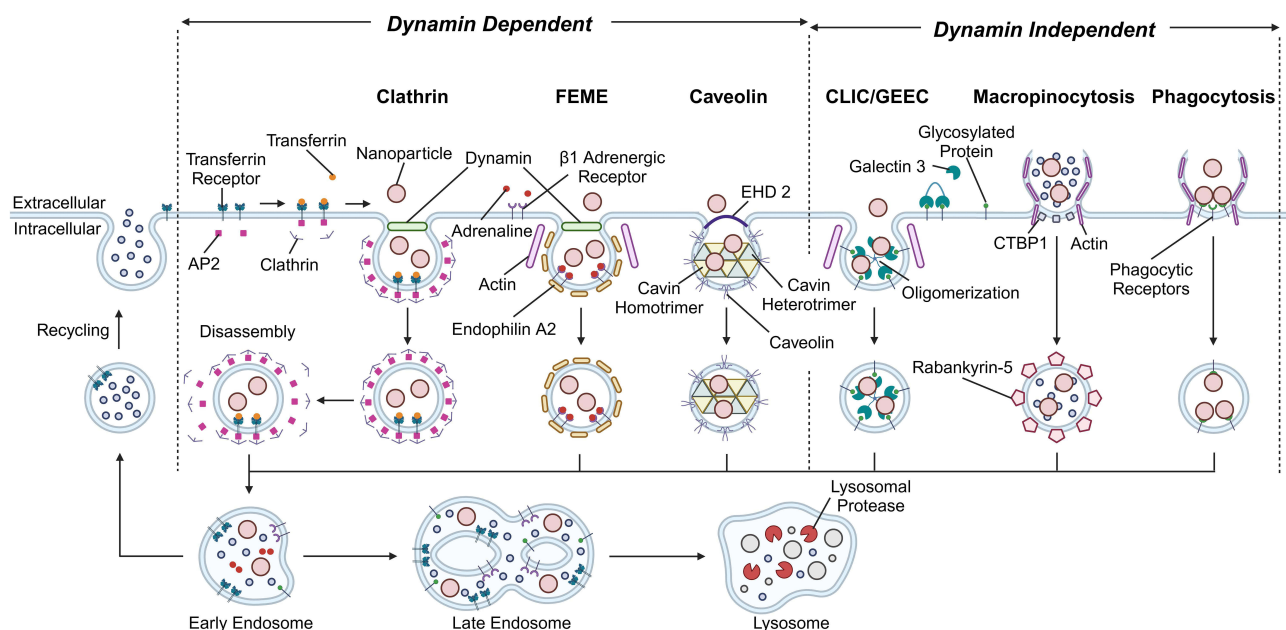


Figure 2 Primary uptake mechanisms used for NP internalization in cells. Created in BioRender. Kolpek, D (2024) <https://BioRender.com/o86q647>.

implicated the mid-sized 60 nm NPs to induce the highest production of ROS and costimulatory markers in macrophages.⁹⁶ While variations in specificity of the endocytic uptake route likely contribute to these results,⁸⁶ the exact mechanism remains unknown and requires further investigation before conclusions can be made.

In addition to cellular uptake route, particle size also acts as a major determinant for where NPs accumulate within the body and whether they reach the targeted cell population. One study comparing 50 nm and 500 nm PS NPs found higher agglomeration of larger (500 nm) NPs in the liver.⁹⁷ This result was linked with protein attachment to the NP surface, commonly referred to as the protein corona, and subsequent opsonization. The protein corona has been shown to influence multiple biodistribution factors including NP circulation time, clearance, and ability to cross biological barriers depending on the attached proteins.⁹⁸ These principles were demonstrated by a study using pegylated poly methoxypolyethyleneglycol cyanoacrylate-co-n-hexadecyl cyanoacrylate (PEG-PHDCA) NPs with varied size which found a strong correlation between NP size and protein absorption. Additionally, the results showed that smaller NPs with less protein absorption took twice as long to clear compared to larger NPs with greater protein absorption. Furthermore, the uptake percentage by murine macrophages was reported to decrease with increased NP size.³¹ Overall, larger NPs tend to have faster clearance due to hepatic filtration and opsonization following protein attachment, while smaller NPs remain in the blood longer and can extravasate into permeable tissues, increasing their uptake.⁹⁹

The Effects of Nanoparticle Surface Charge on Immune Interactions, Cytotoxicity, and Therapeutic Implications

Surface charge of polymeric NPs can vary widely, ranging from negative (below -10 mV), to neutral (-10 mV to $+10$ mV), to positive (above $+10$ mV).¹⁰⁰ Variations in charge are often accomplished through surface attachment of different surfactants during the fabrication process. Some commonly used surfactants include poly(ethylene-alt-maleic anhydride) (PEMA), poly(vinyl alcohol) (PVA), and Tween.^{20,67,101,102} Surface charge has been shown to affect immune cell interactions through alterations in cytotoxicity, cellular uptake, and protein attachment/opsonization. Each of these factors can greatly influence the overall immune response and resulting treatment efficacy, thus highlighting the need for a better understanding of the effects of NP surface charge on immune modulation.

NP cytotoxicity has been shown to differ significantly among treatments with varied surface charge. It is well established that positively charged NPs exhibit acute systemic toxicity and nonspecific stimulation of the immune system.⁹¹ One study using human neutrophils found that cationic NPs interact with the cell membrane to induce membrane disruption, calcium influx, oxidative stress, and cell death.³² The exact mechanism behind these effects remains unclear, but one potential cause is the activation of pattern-recognition receptors (PRRs) in immune cells via the cationic polymer base of the fabricated NPs.³³ PRRs are well known for their role in the inflammatory cascade following SCI. Specifically, the toll-like receptor (TLR) family of PRRs facilitate the immune response through signaling mechanisms in microglia, astrocytes, macrophages, neurons, and oligodendrocytes.³⁴ The activation of these cells via TLRs has been shown to inhibit neuronal regeneration, promote growth cone collapse, trigger neuronal cell death, and promote inflammatory signaling.³⁴ Despite their cytotoxicity, positively charged polymeric NPs have shown promise in pulmonary immunization and gene delivery.^{103,104} However, modifications are necessary to minimize cytotoxicity for these cationic particles to progress beyond in vitro studies. In contrast, both neutral and negatively charged polymeric NPs have shown minimal toxicity and promising immunomodulatory properties, such as downregulation of pro-inflammatory signaling and upregulation of pro-regenerative cytokines.^{20,101} These effects are attributed to the inhibition of the LPS-TLR4 cascade by negatively charged NPs, which leads to a decrease in the inflammatory response by macrophages following SCI.^{20,34} Overall, cationic polymeric NPs exhibit higher risk due to their greater toxicity through TLR signaling activation compared to neutral and negatively charged alternatives, which have shown minimal toxicity with promising immunomodulatory potential.

The route of cellular internalization and extent of cellular uptake also heavily depends on the surface charge of the NP. Positively charged NPs exhibit increased interaction with the negatively charged cell membrane. These electrostatic interactions lead to increased uptake, which has been demonstrated in both macrophages and dendritic cells.^{105,106} Studies have also indicated a relationship between surface charge and cellular internalization pathways, with one finding positively charged particles to be taken up by clathrin-mediated endocytosis, while caveolin-mediated endocytosis was

the preferred endocytic route for neutral and negatively charged NPs.¹⁰⁷ How these differences influence the downstream effects of the NPs remains unknown and must be further studied for improved NP-based therapies.

Protein attachment to the surface of the NPs and the formation of the protein corona have also been shown to be impacted by surface charge.⁹⁸ Neutral NPs without a strong surface charge have been shown to absorb fewer proteins when compared to NPs with a strong charge.³⁵ Positively charged NPs tend to accumulate a thicker coating of plasma proteins compared to negatively charged NPs due to the abundance of negatively charged proteins in circulation.^{36,108} Beyond the extent of protein absorption, surface charge has been shown to influence the types of proteins absorbed, with basic proteins preferring negatively charged NPs and acidic proteins preferring positively charged NPs.¹⁰⁹ The increased protein attachment has also been shown to generate a greater rate of opsonization, leading to faster clearance for charged NPs compared to treatments involving more neutral NPs.³⁷ Recent research has achieved success by utilizing these effects to selectively enhance the attachment of specific proteins. One study using PS NPs with varied charge showed little variation in concentration of the most abundant corona proteins but found an increased presence of less abundant clusterin proteins in the negatively charged NP group.³⁸ Protein attachment due to surface charge has also been shown to influence the immune response to NPs. One study using silica NPs with varied surface chemistries in human whole blood found negatively charged NPs with hydrocarbon groups to bind albumin and increase pro-inflammatory signaling in macrophages.³⁹ Negative NPs functionalized with acid groups showed a similar inflammatory response due to complement and lower apoprotein binding. On the other hand, positively charged NPs functionalized with amine groups showed increased levels of anti-inflammatory marker expression.³⁹ These results highlight the value of manipulating the surface charge of NPs to create a more targeted treatment and underscore the need for additional research in the area.

The Role of Polymer Molecular Weight in the Immune Response

Many polymer bases used for NP fabrication come in a variety of molecular weights (MW). Some of the most prevalent examples include chitosan, PLGA, and polyethylene glycol (PEG).⁴⁰ MW has been shown to influence multiple factors, including degradation rate, hydration, and drug release rates.^{41,42,110} However, the role of polymer MW and its effects on immune modulation remains largely unexplored in the context of SCI.

Multiple studies using varied polymer MWs have demonstrated a clear relationship between MW and the resulting immune response. One study examining the effect of varied hyaluronic acid (HA) MW found high MW HA to decrease both nitric oxide (NO) production and gene expression of classical pro-inflammatory macrophage markers in LPS-activated RAW 264.7 macrophages, while medium MW HA increased the expression of these markers. Despite these contrasting pro-inflammatory responses, both high and medium MW increased anti-inflammatory gene expression.⁴³ Another study examining the immunomodulatory effects of polysaccharides isolated from *Chlorella ellipsoidea* found lower MW polysaccharides to reduce the production of NO in RAW 264.7 macrophages. Additionally, the lower MW polymers did not activate the pro-inflammatory NF- κ B, JNK, and p38 pathways.⁴⁴ These diverse results for different polymers highlight how the effects of MW can vary greatly between polymer bases.

The role of MW in polymer-based therapeutics has also been examined in the context of various inflammation-mediated disease states. A study by Casey et al examined how varied MW in PLGA-based NPs can affect TLR activation, which plays a major role in sepsis. Results showed that high MW NPs downregulated the expression of surface markers necessary for TLR activation in mouse bone marrow-derived macrophages, while low MW NPs had little to no effect. Additionally, high MW PLGA-based NPs showed greater suppression of pro-inflammatory cytokine production compared to their low MW counterparts.¹⁰¹ MW has also been studied in the context of experimental autoimmune encephalomyelitis (EAE) and cancer. Saito et al used low and high molecular weights of PLGA-based NPs to test their role in immune cell trafficking and therapeutic effect on EAE. Intravenous PLGA injections into EAE mice showed that high molecular weight PLGA had reduced clinical scores compared to controls and was associated with neutrophils accumulated in the spleen.²³ This research suggests that high molecular weight PLGA redirects trafficking of immune cells and reduces inflammation. It also highlights the ability of MW to influence the resulting immune modulation following NP treatments.

Overall, these studies clearly indicate that MW plays an important role in the immune modulation of polymer-based therapeutics and underscores the need for more research on the effects of varied polymeric NP MW on immune

modulation after SCI. However, as demonstrated by the varied results of these studies, each polymer must be examined individually to establish a relationship between MW and downstream immune response.

Surface Functionalization of Nanoparticles

NPs can modulate immune responses through interaction with immune cells. Surface functionalization of NPs by attachment of polymers or functional groups can enhance this interaction by controlling the surface properties.¹¹¹ PEGylation is a commonly used surface modification method to avoid clearance mechanisms and increase the circulation time of NPs. Conjugation of PEG to the NP surface hinders non-specific interactions with proteins through PEG's hydrophilic and steric repulsive effects. This reduces the opsonization of particles by the reticuloendothelial system.^{46–49} For example, PEGylated polycyanoacrylate NPs have been shown to increase uptake in neural tissues by improving blood circulation compared to non-PEGylated NPs.⁵⁰ In addition, conjugated PEG affects immune cell internalization. Microglia internalization was shown to vary depending on the amount of mPEG chemically bound to nanogel. This suggests that by tuning the nanogel coating, uptake from microglia can be changed.⁵¹

Conjugating small molecules or peptides to the surface can target specific cells, including immune cells and neural tissues, and increase the therapeutic effects.^{52,112} Wu et al employed apamin, a honeybee-derived peptide known for its penetration and distribution to the CNS, and developed apamin-conjugated PEG-distearoylphosphatidyl-ethanolamine (PEG-DSPE) micelles. These apamin-conjugated micelles enhanced drug accumulation in the spinal cord, and showed high efficacy in tissue repair after SCI.¹¹³ Surnar et al engineered NPs by incorporating the triphenylphosphonium (TPP) cation that accumulates in the mitochondria of cells that are actively involved in respiration. NPs made with PLGA-b-PEG functionalized with TPP demonstrated the ability to accumulate in the brain and provide neuroprotection.¹¹⁴ Gao et al developed NPs with curcumin, which can reduce the infiltration of inflammatory macrophages and induce macrophage polarization. The NPs were fabricated by a co-loading approach combining retinoic acid and curcumin with bovine serum albumin. This NP was shown to polarize macrophages, inhibit the release of inflammatory mediators, promote functional neuron regeneration, and improve motor function.⁵³ Hua et al developed a nanocarrier as a combination treatment for SCI. This ROS-responsive nanocarrier mPEG-b-Lys-BECI-TCO can bind CD44 on the surface of human umbilical cord mesenchymal stem cells (Huc-MSCs) via Tz-A6 peptide. A combination of MSCs, NPs, and encapsulated Ferrostatin-1 promoted functional recovery in mice with SCI.⁵⁴ Wang et al used apocynin (APO), which has the ability to reduce secondary damage, and synthesized an amphipathic block copolymer (Allyloxypolyethyleneglycol-b-poly [2-(((4-acetyl-2-methoxyphenoxy)carbonyl)oxy)ethyl methacrylate], PAPO). They also introduced a scar tissue-homing peptide (cysteine-alanine-glutamine-lysine, CAQK) into PAPO. The NP fabricated using these polymers effectively alleviated secondary damage and led to sustained recovery of motor function after SCI through lesion tissue-specific delivery and sustained release of APO.⁵⁵ Kalashnikova et al conjugated miRNA to PLGA and formulated the NP with the excipient spermidine or polyethyleneimine. They used miRNA-129-5p, which has the ability to regulate microglia activation and checked the synergistic immunomodulatory effects between PLGA-based NPs and miRNA. These NPs exhibited sustained release of miRNAs, polarized activated microglia toward a more regenerative phenotype, and increased the production of pro-regenerative factors.⁶⁷ Moreover, conjugation of cell adhesion peptides can affect the function and regulation of immune cells,^{56,57} and conjugation of ligand that binds to Fas, a cell surface receptor that contributes to the regulation of T lymphocytes, can also control the immune response.⁵⁸

The Impact of Nanoparticle Geometry on Phagocytosis and Cellular Internalization in Innate Cells

Phagocytosis, a key component of innate immunity, involves antigen-presenting cells (APCs), such as macrophages internalizing antigen particles. The shape of NPs has a substantial impact on their cellular uptake. However, the role of NP geometry and its effects on immune modulation remains largely unexplored in the context of SCI.

In early investigations utilizing PS particles of varying sizes and shapes, NP shape was shown to exert a greater influence on absorption compared to its size. Specifically, the local shape of the particle at the point of initial contact with the macrophage was shown to play a crucial role in determining the initiation of phagocytosis. This is because the specific shapes of local particles have a direct impact on the actin structure, which is essential for the internalization process.⁵⁹

Particles with a high aspect ratio (AR), such as elongated particles, show reduced macrophage-induced phagocytosis compared to spherical particles. Thus, worm-like particles characterized by very high ARs (>20) demonstrate minimal phagocytosis compared to conventional spherical particles of the same volume.⁶⁰ Similarly, Geng et al found that longer worm-like micellar particles, known as filo micelles, exhibit reduced phagocytosis.⁶¹ Furthermore, other studies have indicated that high AR shapes contribute to increased NP residence time and decreased cellular internalization. The evaluation of human cell internalization of NPs with the same volume but different ARs showed that non-spherical particles had lower cellular absorption compared to spherical particles. This can be attributed to the average curvature radius of the particles adsorbed by the cell.⁶² In macrophages, the internalization of PS particles with three different shapes was studied utilizing spheres, oblate ellipsoids, and prolate ellipsoids. Spherical particles and oblate ellipsoids showed a greater degree of internalization than prolate ellipsoids. Phagocytosis is an actin-based process, so the extent of actin remodeling, an energy-intensive process, is related to internalization. Therefore, the shape that requires more actin remodeling induces slower internalization. The difference between oblate and prolate ellipsoids is likely due to differences in AR.⁶⁴ In a study comparing nanodisk and nanosphere using PS, nanodisk showed lower permeability in human cell membranes.⁶⁵

In contrast, some studies have shown different results. Using NPs larger than 100 nm, Gratton et al determined that rod-shaped particles showed the highest absorption, followed by spheres, cylinders, and cubes. Using particles made from cationic, cross-linked PEG hydrogel, rod-shaped high AR NPs (AR 3) were shown to internalize into human cells faster than symmetrical (AR 1) particles.⁶³ In a study by Kolhar et al using equal volumes of PS nanospheres and nanorods, nanorods with targeting peptides showed higher endothelial specificity and lower nonspecific accumulation compared to nanospheres.¹¹⁵ In addition, when investigating the impact of particle AR on phagocytosis by different human immune cells, it was observed that neutrophils, unlike macrophages, exhibited a preference for internalizing rod-shaped particles over spherical ones. These differences may be due to differences in phagocytic mechanisms, such as increased motility of neutrophils and the dispensation of phosphorylation during phagocytosis.¹¹⁶ The variation in internalization tendencies across different cells provides a valuable opportunity to customize the shape of NPs according to the target cell, offering a means for precise and effective design.

Polymeric Materials of Nanoparticles for Immunomodulation in Spinal Cord Injury

The composition of polymer base used in NPs has demonstrated its influence on various factors, including degradation rate, hydration, and drug release rates.^{117–119} In the context of SCI, various polymeric materials have been developed and employed.^{120,121} Among these, some polymeric materials have immunomodulatory effects, like the previously mentioned PLGA.

Poly-Beta-Hydroxybutyrate (PHB)

PHB is a high molecular weight biopolyester that exists in the cytoplasm of many bacteria. Due to its biodegradability and non-toxicity, it has been widely used as a biomaterial for many years and has also been approved by the Food and Drug Administration (FDA).¹²² One study using a PHB scaffold showed the promotion of growth and differentiation of neural stem cells and supported marked axonal regeneration within the graft following SCI.^{123,124} In addition, PHB has shown immunomodulatory effects. For example, a study using a Poly(hydroxybutyrate-co-hydroxyvalerate)/polylactic acid/collagen (PHBV/PLA/Col) membrane reduced the infiltration of CD86+ macrophages to the lesion site.¹²⁵ Moreover, PHB is slowly decomposed in vivo to produce β -hydroxybutyric acid (BHB), which was shown to attenuate microglial and glial activation in SCI mice while suppressing SCI-induced NLRP3 inflammasome.¹²⁶ In another study, BHB attenuated neuroinflammation after SCI by downregulating the NLRP3 inflammasome and shifting the activation state of macrophages/microglia from an anti-regenerative to pro-regenerative phenotype.¹²⁷ Although research on PHB-based nanoparticles for SCI is limited, their demonstrated immunomodulatory effect suggests that PHB holds potential as a material for NP-based treatment of SCI.

Polysialic Acid (PSA)

PSA is an endogenous carbohydrate polymer composed of α -2,8-linked N-acetyl neuraminic acid units that frequently attach to neural cell adhesion molecules (NCAM).¹²⁸ PSA acts on cell-cell and cell-extracellular matrix interactions and

plays an important role in axon regeneration, nervous system development, and plasticity.^{129–131} Because of these properties, PSA is being studied for SCI treatment and applied to nanotechnology. One study using PSA-based minocycline-loaded nanodrug delivery systems showed anti-inflammatory and neuroprotective activities both in vitro and in vivo. These NPs also protected the neurons and myelin sheaths from damage, and promoted neural regeneration, thereby improving the locomotor function of SCI rats.¹³² In another study, a polycaprolactone (PCL)/PSA hybrid nanofiber scaffold encapsulating methylprednisolone was implanted in the lesion area of rat SCI models. This reduced proinflammatory cytokine secretion by suppressing the activity of immune cells, promoted axonal growth, and improved functional recovery after SCI.¹³³ Additionally, evidence of the role of PSA in immunomodulation has increased in recent years. Studies have shown that PSA decreases the activation of microglia and macrophages and attenuates the neuroinflammatory response by interacting with sialic acid-binding immunoglobulin-like lectins (Siglec). These results demonstrate the therapeutic potential of PSA as an immune modulating NP material for treatment of SCI.¹³⁴

Chitosan

Chitosan is a linear polysaccharide, produced mainly from the exoskeleton of crustaceans. It has gained attention in biomedical applications due to its beneficial properties including biodegradability and low immunogenicity.¹³⁵ Chitosan can be a promising material in treatment and management of inflammatory diseases. This is due to its anti-inflammatory activity by inhibiting activation of NF- κ B and caspase-3 and lowering secretion of pro-inflammatory cytokines such as TNF- α and IL-6, and expression of iNOS.^{136,137} It also has proven effects on sealing nerve cell membranes and inhibiting neurotoxicity.^{138,139} Chitosan can be utilized as a material for NPs. One study used chitosan-based NPs to treat acrolein-mediated cell injury. The NPs effectively reduced damage to membrane integrity, secondary oxidative stress, and lipid peroxidation.¹⁴⁰ Furthermore, chitosan-based NPs have shown the ability to restore nerve impulse transmission following SCI in guinea pigs.¹⁴¹ Glycol chitosan (GC), a derivative of chitosan, is recognized as a natural neuroprotective compound,¹⁴² and was employed to overcome the issue of chitosan's poor solubility in neutral pH aqueous solutions.¹⁴³ Ferulic acid-modified glycol chitosan NPs showed prolonged circulation time and effectively delivered both chitosan and ferulic acid to the damaged area. It significantly preserved axons and myelin while reducing inflammatory response at the lesion site, leading to improved locomotor recovery.¹⁴⁴ Overall, chitosan shows great promise as a potential therapeutic tool for NP treatment of SCI.

Hyaluronic Acid (HA)

HA is a normal constituent of the extracellular matrix surrounding the basement membrane of multiple endogenous cell types and has many beneficial properties including non-toxicity, biodegradability, biocompatibility, and non-immunogenicity.¹⁴⁵ Due to these properties, HA has great potential for use in immune modulating NPs. The HA receptor, CD44, is expressed on the majority of immune cells, thereby increasing interactions between NPs and CD44, and augmenting NP absorption into cells.^{146,147} Furthermore, the high molecular weight of HA exerts an anti-inflammatory effect through its interaction with immune cells. It diminishes proinflammatory cytokines and enhances the expression of anti-inflammatory genes, such as Arg1, IL10, and MRC1.^{43,148,149} Additionally, it has the ability to modify astrocyte proliferation and migration.¹⁵⁰ Based on these characteristics, HA-based NPs present a promising therapeutic approach to regulate inflammatory responses following SCI.

Conclusion

In conclusion, NPs have demonstrated significant potential as therapeutic agents in SCI due to their immunomodulatory properties. In this review, we have detailed the pathophysiology of SCI, the role of inflammation, and the involvement of innate immune cells, while detailing how the physicochemical properties of polymer NPs influence their interactions with these cells. Key NP characteristics such as size, surface charge, molecular weight, shape, surface functionalization, and polymer composition all play crucial roles in modulating the immune response to SCI (Table 2).

NP size acts as a major determinant of the cellular uptake route. These uptake routes include CME, FEME, CLIC/GLEEC, macropinocytosis, and phagocytosis.⁸⁶ Each route varies in specificity, leading to variations in immune cell interactions and responses.^{20,86} Additionally, NP size greatly affects the formation of the protein corona. This can lead to

Table 2 Representative Papers Exhibiting Physicochemical Property Effects on Immune Modulation

Study/ Authors (Year)	NP Type	Physicochemical Property	Immune Target/Cells	Key Findings	Clinical Relevance	Relevant Figures	Ref #
Kim et al (2011)	PPy	Size	Macrophages	Mid-sized NPs (60 nm) induced the highest amount of ROS and costimulatory marker generation in NPs ranging from 20 nm to 100 nm	Demonstrates effect of NP size and cell internalization on immune responses	5–7	[96]
Casey et al (2019)	PLGA, PLA	Surface Charge, MW	Macrophages, Splenocytes	Negative NPs (~-45 mV) downregulated inflammatory signaling vs neutral NPs (~-25); High MW NPs had similar effect in macrophages but not splenocytes vs low MW NPs	Provides insights into surface charge and MW effects on inflammation	2,4	[101]
Saito et al. (2019)	PLGA	MW	Monocytes, Neutrophils	Low MW NPs showed greater association with circulating immune cells vs high MW NPs	Shows effect of MW on ability to interact with immune cells in circulation	2	[23]
Kalashnikova et al (2023)	PLGA	Surface Functionalization	Microglia	NPs conjugated with the immune-modulating mRNA demonstrated greater downregulation of pro-inflammatory signaling and upregulation of pro-regenerative signaling in microglia compared to mRNA alone	Illustrates ability of NPs to synergize with other treatments to provide greater therapeutic effect	7	[67]
Sharma et al (2010)	PS	Geometry	Macrophages	NPs exhibited variations in attachment, internalization, and phagocytosis depending on shape	Highlights the relevance of geometry as a determinant of cell interaction when designing NP treatments	4	[64]
Safari et al (2020)	PS	Size, Geometry	Monocytes, Neutrophils, Macrophages, DCs	Immune cells showed preferential uptake of NPs depending on geometry and size	Provides evidence for ability to target specific cell types by utilizing NP size and geometry	2,4,6	[116]
Park et al (2019)	PLGA	Polymer Composition	Macrophages	PLGA-based NPs demonstrated immunomodulation of spinal cord infiltrating macrophages following SCI	Demonstrates the ability of PLGA to modulate the immune response to SCI without additional therapeutics	2	[20]

variations in NP accumulation, uptake, and clearance, all of which are important factors for drug delivery.⁹⁸ Overall, NP size plays a critical role in determining uptake pathways, cellular interactions, and protein corona formation, highlighting its importance in the development of NP treatments for SCI and other therapeutic applications.

Similar to size, the surface charge of NPs can influence cellular uptake and protein corona formation. Variations in charge can lead to changes in interaction with the cell membrane, causing differences in extent of uptake and cellular uptake pathway.^{105–107} Also, certain types of proteins can be attracted to the surface of the NP depending on the surface charge.¹⁰⁹ This leads to changes in the protein corona composition and further downstream effects that impact the immune response to the NP.³⁹ In addition, the toxicity of NPs can be affected by their charge, particularly with positively charged NPs exhibiting increased cytotoxicity due to nonspecific immune system stimulation.⁹¹ These factors must be considered when designing a safe and efficacious NP treatment.

Polymer MW remains an understudied factor in NP treatment of SCI despite multiple studies demonstrating its relevance in other inflammatory diseases. In sepsis, NPs with varied MW showed differences in TLR activation.¹⁰¹ In EAE, MW influenced the trafficking of the innate immune cells and clinical scores of the experimental mice.²³ Given the similarities in inflammatory responses between SCI and these diseases, additional research on the effects of NP MW in the context of SCI is warranted.

Surface functionalization of NPs has shown strong potential for manipulating multiple factors including clearance, cellular interactions, and immune response. Surface conjugation with factors such as PEG can decrease nonspecific interactions with cells and opsonization, leading to greater circulation time.^{46–49} Additionally, multiple studies have demonstrated the ability of a surface conjugated peptide to traffic NPs to specific target sites such as the spinal cord and influence the downstream immune response following cell interaction.^{52,112} These studies highlight the value of surface functionalization and underscore the need for its utilization when developing effective NP treatments.

NP geometry is another critical factor in cellular uptake and immune modulation. Studies have identified the AR of a NP to be a major determinant of cellular uptake and absorption with certain immune cells preferring low AR or high AR NPs.^{60,63} This allows for the opportunity to engineer NPs targeted to specific immune cell populations. While the effects of NP geometry in SCI remain largely unexplored, investigating how different ARs influence spinal cord-infiltrating immune cells could lead to targeted treatments with improved therapeutic outcomes.

The polymer composition used when fabricating NPs considerably impacts the downstream effects of the NPs when considering cellular interactions in the context of SCI. The non-toxic and biodegradable PHB has been shown to modulate the immune response through changes in cellular trafficking and NLRP3 inflammasome activation.^{125,126} By utilizing its ability to interact with NCAM and influence cellular interactions, PSA has shown therapeutic potential as a drug delivery carrier and immunomodulator in SCI.^{128–133} Chitosan has the ability to inhibit multiple pro-inflammatory signaling pathways, and has been shown to minimize neurotoxicity, making it a promising NP material for SCI.^{136–139} Lastly, HA has the ability to modulate the immune response through CD44 interactions and has shown anti-inflammatory effects.^{146–149} By leveraging the characteristics of these various polymer bases, targeted NP treatments can be developed for both SCI and other inflammatory diseases.

Understanding these physicochemical properties and their specific interactions with immune cells is crucial for the engineering and design of targeted NPs that can be optimized for modulating the immune response to neurological diseases. However, despite a large base of knowledge connecting NP properties with immune modulation, studies applying this knowledge to SCI and related inflammatory diseases remain scarce.

Polymeric NPs offer significant clinical potential for immunomodulation in SCI. Using their physicochemical properties, NPs can be designed to target specific immune cell populations such as macrophages, microglia, and neutrophils.^{20,60,63,101} Through these interactions, the NPs can reduce the inflammatory response to SCI and polarize immune cells towards a more regenerative phenotype, allowing for greater recovery following SCI.^{20,101} Additionally, polymeric NPs have the capacity to act as drug carriers to the spinal cord injury site, where a controlled release of anti-inflammatory drugs, antioxidants, or genetic material can be facilitated.^{67,81,82} Through NP delivery, the degradation of these therapeutics can be minimized compared to more traditional pharmaceutical interventions.¹⁹ Another advantage polymeric NPs have is their minimal toxicity. Many polymeric NPs are composed of biodegradable components that are broken down to endogenous chemicals within the body, thus minimizing toxicity and off-target effects seen with other drugs.^{87,122,128,145} However, despite these benefits, polymeric NPs still face challenges such as potential long-term effects

and efficacy in human subjects.¹⁹ These shortcomings must be addressed before polymeric NPs can be applied in a clinical setting for treatment of SCI.

Future research should focus on applying these findings to the development and design of NPs specifically tailored for SCI, with the goal of targeting immune responses that maximize therapeutic outcomes. Further investigation is needed to explore how specific NP designs can target multiple facets of the immune response including different immune cell populations, distinct inflammatory pathways, and improved functional recovery in SCI patients. By advancing this research, there is an opportunity to refine current therapeutic strategies for SCI and extend the application of NPs to a broader range of inflammatory and neurodegenerative diseases.

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Disclosure

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