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Review

# Emerging Approaches to Mitigate Neural Cell Degeneration with Nanoparticles-Enhanced Polyelectrolyte Systems

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## Abstract

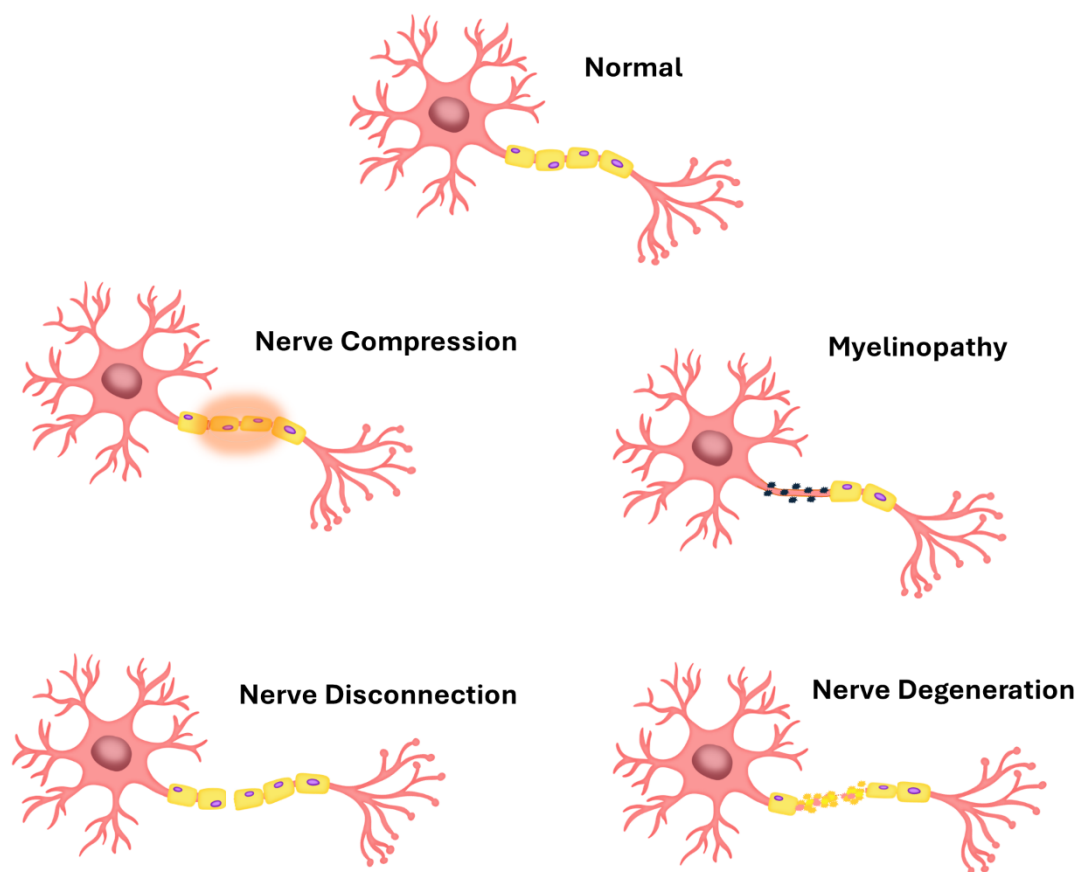
Counteracting neurodegenerative diseases (NDs) presents a multifaceted challenge in the aging societies of Western countries. Each year, millions of people worldwide are affected by such ailments as Parkinson's disease, PS; Alzheimer's disease, AD; Huntington's disease, HD; multiple sclerosis, MS; spinal cord injury; ischemic stroke; motor neuron disease; spinal muscular atrophy; spinocerebellar ataxia; and amyotrophic lateral sclerosis, ALS. Advancements in modern biomaterial technologies present substantial opportunities for the field of regenerative medicine. Nevertheless, limitations arise from the requirement that biomaterial design be tailored to the specific biological parameters of the target cell types with which they are intended to interact. Such an opportunity creates nanomaterials involving nanoparticles. The surface chemistry of nanoparticles, especially when functionalized with bioactive agents, enhances biocompatibility and facilitates interactions with nervous cells. Herein, we review contemporary strategies in the application of biomaterials for nerve regeneration, with particular emphasis on nanomaterials and biocompatible polyelectrolyte layers, which the authors identify as having the most significant potential to drive transformative advances in regenerative medicine shortly.

**Keywords:** polyelectrolyte membrane coating; neurodegenerative diseases; neural cells; nanoparticles

## 1. Introduction

Counteracting neurodegenerative diseases (NDs) presents a multifaceted challenge in the aging societies of Western countries. Each year, millions of people worldwide are affected by such ailments as Parkinson's disease, PS; Alzheimer's disease, AD; Huntington's disease, HD; multiple sclerosis, MS; spinal cord injury; ischemic stroke; motor neuron disease; spinal muscular atrophy; spinocerebellar ataxia; and amyotrophic lateral sclerosis, ALS [1–4]. A few years ago, it was estimated that approximately 15% of the Earth's population might suffer from neurodegenerative diseases [5,6]. In 2019 alone, NDs caused the death of 10 million individuals, whereas 349.2 million people were afflicted [7]. The latest reports are even more pessimistic; a study published in *The Lancet Neurology* shows that as many as 3.4 billion individuals could live with a neurological condition in 2021 [8]. Such a situation leaves a deep economic footprint as developed countries spend vast amounts of money on programs supporting the prevention and treatment of diseases of this type [5,9]. As a study from 2022 states, the total annual per capita cost of Alzheimer's disease alone ranged from US\$468.28 in mild cases to US\$171,283.80 in severe cases, with care-related expenses increasing nonlinearly with disease progression [10]. According to the report, including 93% of the world's population, Alzheimer's disease and other dementias are projected to impose an economic burden of \$14,513 billion in international dollars from 2020 to 2050, which corresponds to an average of 0.421% of the annual global gross domestic product (GDP) [11].

Neurodegenerative diseases are characterized by progressive damage to the nervous system's structures. These disorders are currently essentially incurable, as mature cells of the nervous system do not tend to regenerate. In Figure 1, the schematic representation of nervous cells' degeneration according to Waller is presented.



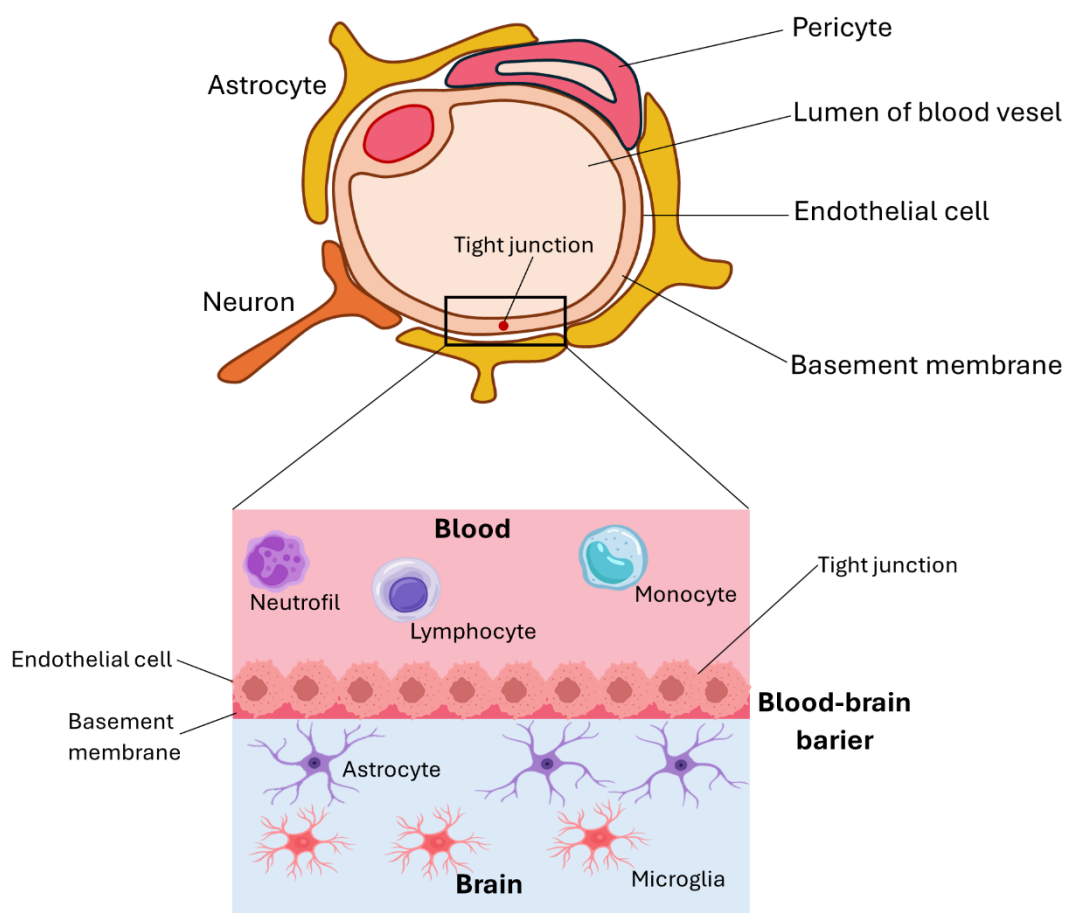
**Figure 1.** Nerve degeneration according to Waller.

Moreover, in addition to NDs, nerve damage can also occur as a result of traumatic brain injury, spinal cord injury, and peripheral nerve injury [12]. Nevertheless, regardless of the cause and type of damage, in each case, it is necessary to use regenerative medicine to restore the original, lost functions of nerve cells.

Neural tissue is the primary component of the nervous system and is found in the brain, spinal cord, and nerves. It is composed of two types of cells: neurons and neuroglial cells. There are three types of the latter: astrocytes, oligodendrocytes, and microglia, which are supposed to play a supporting role in neurons' activity. Moreover, they form myelin and provide neurons with nutrition. Neurons, the basic units of nervous tissue, generate and conduct impulses, which are responsible for cell-to-cell communication. The cells are composed of a soma, which includes the nucleus and other functional organelles, dendrites that receive signals from other neurons, and axons that receive signals from the cell body and transmit them to the axon terminals, where neurotransmitters are released to connect to muscles or glands. The dysfunction of neurons can be caused by neurodegeneration, resulting from the progressive loss of neurons and/or their functions, which is associated with dysfunction of the synapse and neural network [12,13].

As mentioned above, nervous tissue is characterized by poor regenerative capacity; therefore, all approaches aimed at supporting its growth are under extensive evaluation. For example, neural stem cell-based therapies for central nervous system restoration have attracted growing interest recently due to their potential for reconstructing damaged tissue through exogenous neural cell

transplantation. The reason for this is that neural stem cells proliferate and are capable of long-term self-renewal, unlike mature neurons, which do not proliferate. Alternatively, some researchers place greater emphasis on advancements in materials engineering, proposing that the development of novel biomaterial surfaces capable of supporting neural regeneration may offer scalable and economically advantageous solutions for tissue repair. It may, in turn, enable the use of techniques from the field of nanotechnology and neurobiology, providing the opportunity to, among other things, thoroughly understand the structure and function of nervous tissue and influence the function of cells cultured on constructed substrates. Biomaterials engineering addresses nerve growth inhibition and loss of long-distance control problems, allowing for the expansion of therapy beyond cell-based approaches. Research has been conducted on materials that exhibit electrical and/or biomechanical properties, which trigger and enhance nerve regeneration. For that purpose, natural, synthetic, and conductive polymers are considered. Materials with incorporated nanoparticles are also employed; they are particularly valued for their ability to cross the blood-brain barrier (Figure 2 presents a diagram illustrating such a barrier). Moreover, biomaterial scaffolds in combination with growth factors, such as brain-derived neurotrophic factor (BDNF), basic fibroblast growth factor (bFGF), or vascular endothelial growth factor (VEGF), are reported to potentially stimulate neurogenesis directly at the injured site [14].



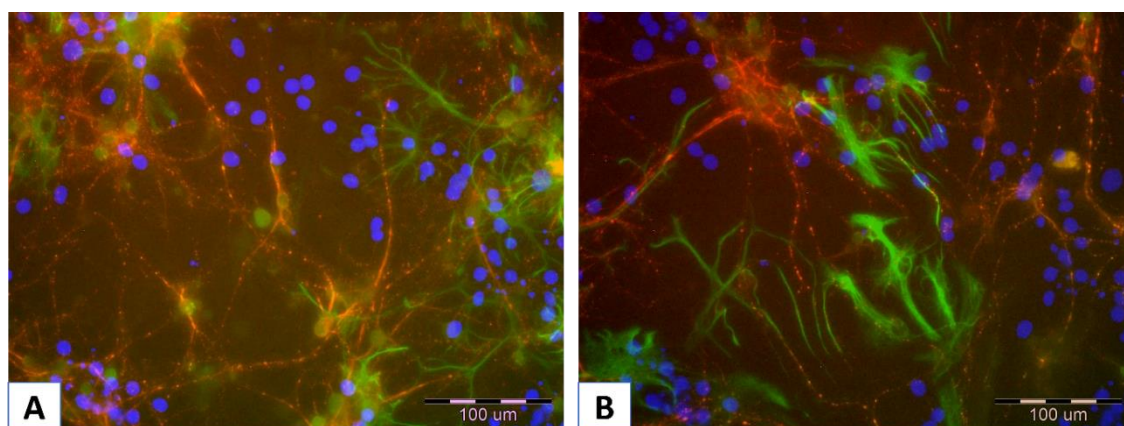
**Figure 2.** Blood-brain barrier.

Herein, we review contemporary strategies in the application of biomaterials for nerve regeneration, with particular emphasis on nanomaterials and biocompatible polyelectrolyte layers, which the authors identify as having the most significant potential to drive transformative advances in regenerative medicine shortly.



## 2. Trouble in Paradise – Short Overview of the Neuronal Cell Cultures

Regarding the aspect of cell culture, it is worth noting that neuronal cell culture provides a basis for studying the functioning of the nervous system. The fact that mature neurons do not undergo cell division encourages the use of immortalized secondary cell lines derived from neuronal tumors. Although an unlimited number of these cells can be obtained, they show physiological differences from the cell type from which they originate. These lines can be induced to exhibit a more neuronal phenotype by modifying the culture conditions, e.g., by adding specific growth factors. Among these lines, in which the expression of neuronal markers can be induced, we can mention the human neuroblastoma SH-SY5Y cell line, Ntera, a human neuronally committed teratocarcinoma cell line, and PC12, a pheochromocytoma of the adrenal medulla derived rat cell line. Also used as models for studying neuronal cells are mouse embryonic stem cells, as well as the F9 and P19 cell lines. Nevertheless, the chance to repeat the properties of neuronal cells *in vivo* is provided by the use of primary cell cultures, not derived from tumors. However, in this case, it is necessary to separate neuronal cells from other types of cells, such as astrocytes and oligodendrocytes [22]. In Figure 3, a and b, the exemplary pictures of murine neuronal cells isolated from the embryonic brain, immobilized on an alginate/chitosan polyelectrolyte bilayer, are presented.



**Figure 3.** Visualization of murine neuronal cells immobilized on alginate/chitosan polyelectrolyte bilayer after two weeks of culture. The red fluorescence shows neurons stained with anti-microtubule-associated protein 2 (MAP2), using a secondary antibody conjugated with the fluorochrome Alexa Fluor 555. The light green fluorescence indicates astrocytes stained with anti-glial fibrillary acidic protein (GFAP) and a secondary conjugated antibody labeled with Alexa Fluor 488 fluorochrome. Nuclei were dyed blue with Hoechst 33342 to enhance contrast under microscopy. Photos were taken using an Olympus IX71 fluorescence microscope.

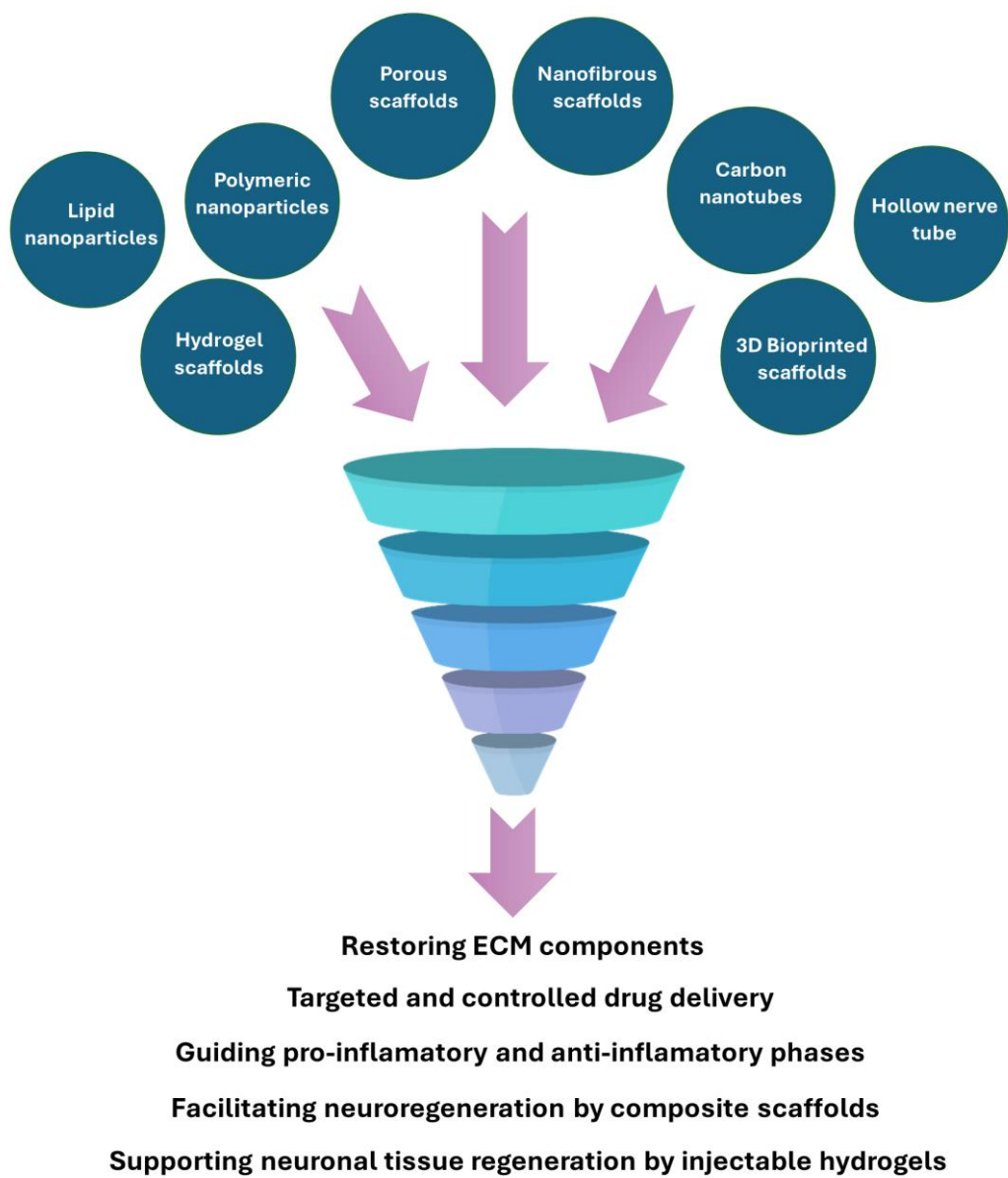
Neural cells (both isolated and cell lines) are characterized by poor adhesion to the surface of culture vessels. Their culture is commonly performed using substrate surfaces, such as those produced by poly-L-lysine (PLL) and poly-D-lysine (PDL) [16–18], which are applied as monolayers. Nevertheless, studies have been conducted to approach the polyelectrolytes and their combinations for interaction with neural cells.

## 3. Nanomaterials in Nerve Regeneration

It has long been recognized that nanoparticles representing nanomaterials, which have revolutionized medical science, offer new horizons in regenerative therapies, particularly at the cellular level [26,27]. The benefits of using nanoelements go far beyond structural reinforcement. Nanoparticles' unique size affects their diffusion properties, enabling them to penetrate tissues and consequently influence cellular uptake, which is vital for regeneration. On the other hand, the surface chemistry of nanoparticles, especially when functionalized with bioactive agents, enhances

biocompatibility and facilitates interactions with nervous cells. Surface charge also plays a crucial role in determining how cells interact with and internalize nanoparticles. Therefore, understanding how surface charge, chemistry, and size affect biological responses is essential to optimizing nanoparticle-based nerve regeneration therapies.

Additionally, the constantly increasing variety of available nanomaterials broadens the range of their possible usage in nerve regeneration therapies [28,29]. Figure 4 presents an overview of various examples of nanomaterials' share among biomaterials applied in nerve regeneration.



**Figure 4.** Different examples of biomaterial applications in nerve regeneration.

One way to use nanoparticles is as carriers of active substances (like growth factors [30], neurotrophic agents [31], or genes [32,33]) in targeted therapies or as support facilitating regenerated tissue growth. Liposomes [34–38] and dendrimers [33,39–42] are excellent examples of nanoparticles designed for targeted drug delivery. On the other hand, nanofibers [43–50], nanowires [51–54], and graphene derivatives [55–61] serve as structures simulating the extracellular matrix to support nerve cell growth. Furthermore, systems based on the antibacterial properties of nanoparticles are often developed to limit inflammatory reactions [62–64]. A different approach employs nanoparticles in theranostics; fluorescent quantum dots are commonly used to enable imaging and tracking [65–69]. However, scientists have become cautious about the involvement of nanoparticles in nerve

regeneration due to their potential toxicity [70–72], the risk of crossing the blood-brain barrier [73–75], and the accumulation of nanoparticles in organs during prolonged exposure [72,76–78].

### 3.1. *The Influence of Nanomaterial Structure on Its Role in Neuroregeneration*

The material's structure, characteristics, and toxicological profile determine the diverse functionalities of nanoparticles in neuroregeneration. For example, the specificity of metal nanoparticles (such as gold, silver, and iron) makes them suitable for nerve regeneration and stimulation, as they possess enhanced electrical conductivity that mimics natural conditions [79–81]. In contrast, polymeric nanoparticles, characterized by above-average biocompatibility, are excellent carriers for therapeutic agents as they allow precise and controlled drug release, supporting neuroprotection and modulation of neural growth simultaneously [82–86]. The separate class of materials is hybrid nanoparticles that combine metallic and polymeric elements to harness multiple functionalities. Such an amalgamate enables electrical conductivity and ensures direct delivery mechanisms, enhancing therapeutic efficacy in nerve renewal [87]. Combined metallic and polyelectrolyte elements, e.g., magnetic alginate microparticles obtained by water-in-oil emulsion crosslinking of sodium alginate and iron oxide nanoparticle mixture, can be applied as a sacrificial form of tubular structure to template construction composed of glycidyl methacrylate, hyaluronic acid, and collagen I to replicate the microarchitecture of the native nerve basal lamina [88].

Another critical aspect of nanoparticles' characteristics that should be considered before their application in nerve regeneration therapies is toxicity, as it influences the material's interaction with biological systems [89,90]. Material toxicity is a derivative of its composition, particle size, and surface properties [91]. Therefore, providing biocompatibility and minimizing potential adverse effects of applied nanoelements requires considering their physicochemical profile, which, in turn, is often determined by the chosen synthesis method. Two main strategies are applied to receive nanoparticles: bottom-up and top-down, each offering unique advantages for biomedical applications [92,93]. In the bottom-up approach, nanoparticles are constructed from the atomic or molecular level, allowing for precise control over their nature and simplified surface functionalization (functional groups, proteins, peptides, or antibodies can be attached to the surface to improve internalization, cellular uptake, and/or biocompatibility) [94]. Moreover, such modifications enable scientists to obtain stimuli-responsive materials, allowing for the precise release of drugs in response to environmental triggers, such as temperature or pH [95–97]. Among the bottom-up synthesis techniques used to obtain materials for biomedicine, chemical synthesis, emulsification, sol-gel methods, self-assembly layering, thin-film hydration, and chemical, physical, electrochemical, or atomic layer deposition can be listed [95,98,99]. For instance, gold and silver nanoparticles obtained by chemical synthesis have unique optical and electrical properties, which are beneficial for imaging and targeted delivery of therapeutic agents in nerve regeneration [100]. On the other hand, thin-film hydration of lipid-based nanoparticles provides carriers for bioactive molecules, facilitating their transport across the blood-brain barrier to support nerve reconstruction [99]. Finally, polymeric carriers of bioactive molecules, which enhance their transport across the blood-brain barrier while promoting cell growth, can be obtained by applying self-assembly or emulsion polymerization methods [101–104].

On the contrary, top-down synthesis techniques involve deconstructing bulk materials into nanoparticles by etching or managed fragmentation, offering scalability and precise size control. Examples include laser and electroablation, mechanical grinding (such as ball milling), sputtering, etching, and lithography, which are commonly used to fabricate nanoparticles with specific dimensions and shapes [105]. It is worth noting that top-down approaches are beneficial for creating nanoparticles with tailored physical properties, which are suitable for integration into scaffolds ideal for nerve reconstruction [106].

3.2. Multifaceted Approach to Nerve Regeneration Using Nanomaterials

Nanomaterials offer a multifaceted approach to nerve restoration by promoting axonal growth, supporting myelination, mitigating oxidative stress, modulating inflammation, and enhancing targeted drug delivery, which positions them as a key tool for advancing cell regeneration therapies. Selected examples of the usage of nanomaterials in different aspects serving nerve regeneration are collected in Table 1.

Table 1. Key Mechanisms of Neuroprotection/Regeneration.

Action	Description	Examples
Antioxidant and Anti-Inflammatory Actions		
Oxidative Stress Mitigation	NPs can scavenge reactive oxygen species (ROS), protecting neurons from oxidative damage.	<ul style="list-style-type: none"><li>• <b>Curcumin/poly (-)-epigallocatechin-3-gallate nanoparticles co-encapsulated within hyaluronic acid (HA-CurNPs)</b> eliminate reactive oxygen species (ROS) within the animal body and scavenge ROS both inside and outside cells; moreover, HA-CurNPs reduce cellular apoptosis, alleviate inflammation, and support motor function recovery, thereby enhancing neuronal repair in mice with spinal cord injury (SCI) [107].</li><li>• <b>Selenium nanoparticles (SeNPs)</b> derived from <i>Proteus mirabilis</i> YC801 alleviate oxidative stress and reduce cell apoptosis, thereby promoting axon regeneration after injury. Additionally, glutathione peroxidase (GSH-Px) from SeNPs functions as an antioxidant, protecting cells from oxidative stress [108].</li><li>• <b>Mannose-coated superparamagnetic iron oxide nanoparticles (mSPIONs)</b> have been shown to reduce ROS levels and alleviate neuropathic pain in sciatic nerve injuries in a rodent model [109].</li><li>• <b>Cerium oxide nanoparticles</b> loaded with therapeutic agents protect mitochondria and suppress neuronal apoptosis, allowing for suppression of neuroinflammatory processes in the middle cerebral artery embolization/recanalization mouse model [110].</li><li>• <b>Zein nanoparticles loaded with chloroquine (Zein-CQ NPs)</b> inhibit neuronal apoptosis, reduce neuroinflammation, and increase M2 phenotype microglia polarization, improving functional nerve recovery after SCI in rats [111].</li><li>• The <b>nanozyme hydrogel</b> delivery system loaded with drugs exhibited antioxidant and protective effects against oxidative stress-induced cell damage caused by ROS production, promoting nerve regeneration after SCI in mice [112].</li><li>• <b>Hollow Prussian blue nanozymes (HPBZs)</b> drive neuroprotection against ischemic stroke primarily by scavenging reactive oxygen and nitrogen species, suppressing apoptosis, and counteracting inflammation in a rat model of ischemic stroke [113].</li></ul>
Inflammation Modulation	NPs can decrease proinflammatory cytokines, creating a favorable environment for nerve regeneration.	
Enhancing neuronal viability	NPs can reduce cellular apoptosis.	
Promotion of Axonal Growth and Myelination		

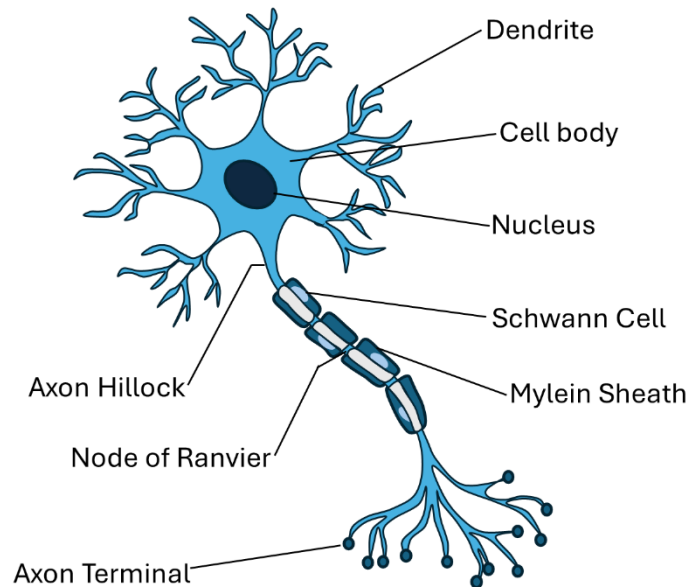


Action	Description	Examples
Axonal Regeneration	Surface-modified NPs can guide axonal growth by mimicking extracellular matrix structures.	<ul style="list-style-type: none"><li>Conductive topological scaffold – Morpho butterfly wing with reduced graphene oxide (rGO) nanosheets and methacrylate gelatin (GelMA) hydrogel with encapsulated brain-derived neurotrophic factor (BDNF) (<b>rGO/BDNF/GelMA-integrated Morpho butterfly wing</b>) enhances neurite elongation and functional recovery in a rat model [107].</li><li><b>Co-spin polycaprolactone (PCL) and graphene oxide-oriented hybrid nanofibrous scaffold (O-GO/PCL)</b> enhance axonal regeneration, increase remyelination, and elevate functional recovery in rats [108].</li><li>NPs delivering neurotrophic factors like BDNF or insulin-like growth factor 1 (IGF-1) have been shown to increase the density of myelinated axons and support muscle preservation post-injury [109,110].</li></ul>
Myelination Support	NPs can promote axon remyelination.	<ul style="list-style-type: none"><li><b>Iron oxide nanoparticles (IONPs)</b> combined with electromagnetic fields (MF) scavenge free radicals, lower demyelination, enhance the percentage of myelinated fibers, and induce axonal sprouting after SCI in rats [111].</li><li><b>Polyethylene Glycol-coated Gold Nanoparticles (PEG-Au NPs)</b> enhance the myelination of spared or regrown/sprouted axons after SCI in mice [112].</li><li><b>Poly(lactic-co-glycolic acid)-based nanoparticles</b> loaded with leukemia inhibitory factor (LIF) and functionalized with surface antibodies targeting NG2 chondroitin sulfate proteoglycan, expressed on oligodendrocyte precursor cells (OPCs), have been shown to promote myelin repair, increase the density of myelinated axons, and enhance myelin sheath thickness per axon [113].</li><li><b>CNM-Au8</b> (a clean-surfaced, faceted gold nanocrystal (AuNC)) promotes myelin generation in vivo animal models of multiple sclerosis [114].</li><li><b>Selenium-doped carbon quantum dots (Se-CQDs)</b> reduce nerve fibers' demyelination and neuronal cell apoptosis after traumatic spinal cord injury in rats [115].</li><li>Biomimetic <b>collagen gel with magnetic nanoparticles</b> (MNPs) coated with nerve growth factor (NGF) supports axon regeneration by promoting its growth [116].</li></ul>
Enhanced Drug Delivery		
Targeted Delivery	Functionalized NPs can cross biological barriers and deliver therapeutic agents directly to injury sites, minimizing off-target effects.	<ul style="list-style-type: none"><li><b>Thiolated trimethyl chitosan (TMCSH) nanoparticles grafted with the non-toxic fragment of tetanus neurotoxin (HC) – TMCSH-HC/BDNF</b> have been used for targeted delivery of plasmid DNA encoding brain-derived neurotrophic factor to peripheral neurons [110].</li><li><b>Chitosan-halloysite nanotube composite (NGCs)</b> featuring aligned microchannel pores have been designed to enable the sustained release of the neurogenic agent 4-aminopyridine (4-</li></ul>

Action	Description	Examples
Controlled Release	NPs can provide sustained release of therapeutics, reducing the need for frequent dosing and maintaining effective drug concentrations at the injury site.	<p>AP), thereby promoting peripheral nerve regeneration in a rat model [117].</p> <ul style="list-style-type: none"><li>• Bioactive 3D Scaffolds (<b>Polycaprolactone hollow tubes called nerve guide conduits</b>) supply a sustained release of heparin-bound nerve growth factor (NGF) or brain-derived neurotrophic factor (BDNF) electrostatically immobilized onto an amine-functionalized surface to encourage neurite outgrowth and Schwann cell migration [118].</li><li>• <b>Multifunctionalized silk nanofibers</b> supply nerve growth factor (NGF) and ciliary neurotrophic factor (CNTF) to promote peripheral nervous system nerve regeneration [119].</li><li>• <b>A nanofibrous scaffold fabricated via emulsion electrospinning of poly(L-lactic acid) (PLLA)</b> has been developed for the controlled delivery of recombinant human nerve growth factor (NGF) and recombinant human vascular endothelial growth factor (VEGF), aiming to facilitate peripheral nerve regeneration [120].</li><li>• Electrospinning multilayered scaffolds (<b>polycaprolactone nanofibers with magnetic nanoparticles – Fe<sub>3</sub>O<sub>4</sub></b>) for sustainable release of melatonin for peripheral nerve regeneration [121].</li></ul>

Nanoparticles' ability to scavenge reactive oxygen species (ROS) and simultaneously reduce inflammation is commonly employed in nerve repair after spinal cord injuries (SCI) [100,101,122,123]. In general, excessive ROS production stimulates macrophage activation, engendering inflammation and intensifying the development of SCI. It is worth noting that macrophages in SCI are categorized into two subpopulations: M1 (proinflammatory) and M2 (anti-inflammatory). A shift between M1 and M2 polarization alters the balance of inflammation, influencing the progression and recovery of the injury as different cytokine types are released. Nanoparticles are used to regulate the balance of macrophages [124]. For instance, it has been shown that selenium nanoparticles (SeNPs) derived from *Proteus mirabilis* YC801 reduce M1 macrophages by suppressing proinflammatory factors (TNF- $\alpha$ , IL-1 $\beta$ ) and increase M2 macrophages by elevating anti-inflammatory cytokines (IL-4, IL-10), thereby modulating microglial responses to promote nerve recovery in rats with spinal cord injury [101]. A further example is curcumin/poly(-)-epigallocatechin-3-gallate-encapsulated nanoparticles (HA-CurNPs), which promote anti-inflammatory M2 microglial polarization and suppress proinflammatory M1 polarization by inhibiting CD74 expression. Studies have shown that such modulation reduces inflammation, facilitates neuronal repair, and supports motor function recovery in mice with spinal cord injury (SCI) [100]. In turn, scientists have demonstrated that mannose-coated magnetic nanoparticles (mSPIONs) reduce proinflammatory cytokines (IL-6 and TNF- $\alpha$ ), thereby creating a conducive environment for nerve regeneration [102].

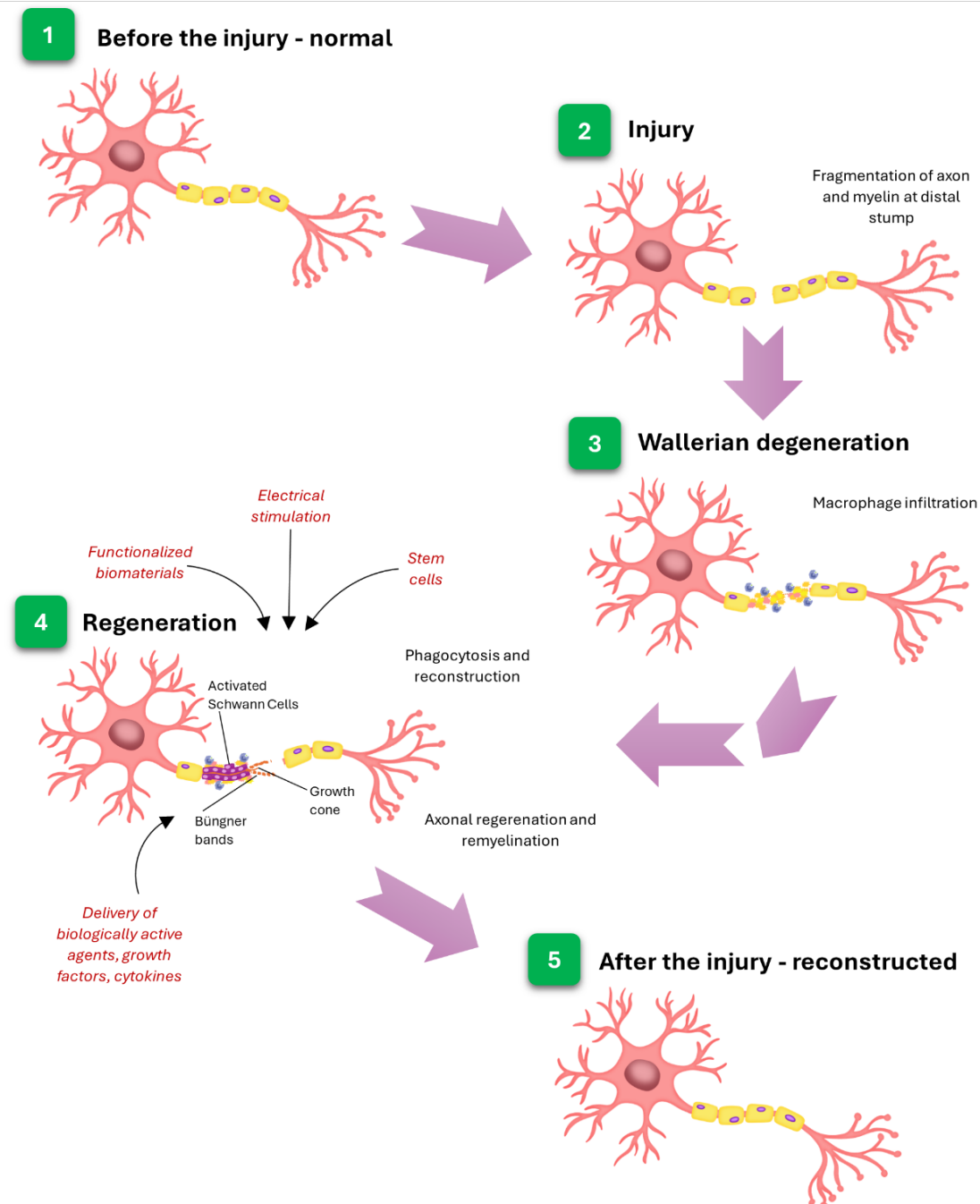
The promotion of remyelination and axonal growth after injury is an additional example of the beneficial effects of nanoparticles, which have applications in nerve regeneration [125]. Myelination is the biological process by which a protective myelin sheath is formed around axons, enhancing the speed and efficiency of electrical signal conduction along nerve fibers [126]. In Figure 3, the diagram shows the structure of a neuron with a myelin sheath and Schwann cells.



**Figure 5.** Neuron structure with myelin sheath and Schwann cells.

The injury can disrupt or inhibit regular mechanisms, resulting in incomplete myelination in damaged areas, which leads to conduction disturbances of nerve impulses. Therefore, it is not surprising that to re-establish cell function, it is necessary to restore myelination. Although research typically prioritizes axon regeneration over remyelination, in some instances, such as specific hereditary disorders or toxin-induced neuropathies, the myelin is primarily damaged, while the axons remain relatively intact, at least in the initial phases of the degeneration [125]. Thus, as the level of knowledge about the specificity of cellular impairment increases, the need to analyze remyelination dynamics becomes emphasized, as it is crucial for the development of effective therapy.

Interestingly, not all types of cells exhibit the same capacity for remyelination. For example, peripheral nerves, in contrast to central nervous system cells, are not only characterized by their excellent regeneration potential but also by the extraordinary ability to remyelinate. It is worth noting that myelination in the peripheral nervous system (PNS) is primarily carried out by Schwann cells, which originate from neural crest cells. Moreover, Schwann cells differentiate into either myelinating or non-myelinating types, with each myelinating Schwann cell enveloping a single axon segment. This one-to-one relationship is key for the proper functioning and maintenance of peripheral nerves [125]. Besides transdifferentiating into a repair phenotype promoting axonal regrowth [127], Schwann cells' role after nerve injury also includes clearing degenerated myelin and axonal debris (during such processes as Wallerian degeneration) and axon remyelination [128]. However, the regenerated myelin sheaths are typically shorter and thinner than the original [129]. In Figure 5, a schematic representation of nerve degeneration and regeneration after the injury is presented.



**Figure 6.** Nerve regeneration after the injury.

While Schwann cells are crucial in nerve regeneration, their proper functioning requires intercellular interactions with endothelial cells and fibroblasts, which support the guidance of axons and Schwann cells across damaged sites [130].

Several approaches are being explored to augment the remyelination capacity of Schwann cells. Among these, gene- and cell-based therapies, pharmacological interventions (for instance, agents such as ascorbic acid have been demonstrated to facilitate myelination by supporting basal lamina formation [131]), and the application of biomaterial scaffolds can be listed. In the case of gene therapies, scientists target specific genetic mutations (e.g., the PMP22 gene, responsible for encoding peripheral myelin protein 22, which is critically implicated in the pathogenesis of Charcot-Marie-Tooth disease [132]) to correct aberrant protein expression. A different approach is to replace damaged Schwann cells *via* transplantation. Biomaterial scaffolds play a crucial role in the process, as they provide structural support for the transplanted cells, enhance their survival, and facilitate



integration [133–135]. Nanoparticles, engineered to mimic the natural environment of the extracellular matrix, can act as scaffolds and directional cues, creating conditions that facilitate Schwann cells' ability to rebuild the insulating myelin layers essential for nerve function [136]. They might also be used as agents providing cellular alignment in a 3D environment, such as superparamagnetic oleic acid-coated iron oxide nanoparticles (SPIONs) incorporated into poly-l-lactic acid electrospun fiber scaffolds, which were employed to receive oriented guidance for dorsal root ganglion (DRG) neurites [137]. However, the range of nanomaterials in nerve injury applications goes far beyond the 'typical support' role. Zong and coworkers presented an interesting strategy: they developed a biological conduit composed of neurotrophin-3-transfected Schwann cells combined with poly(lactic-co-glycolic acid) (PLGA) copolymer and transplanted it into rats. Nanoparticle liposomes were used as a neurotrophin-3 carrier in that work. The study results show that the designed system effectively enhances sciatic nerve regeneration and decreases motor neuron apoptosis [138].

Undoubtedly, nanoparticles are most notably recognized for their drug delivery capabilities, a property that is also extensively exploited in the treatment of neurodegenerative diseases [99,110,139–142]. For instance, NPs are highly effective carriers of neuroactive factors due to their ability to encapsulate these agents. Moreover, after functionalization, nanoparticles can cross biological barriers and deliver therapeutic agents precisely to injury sites, minimizing off-target effects [142–149]. In their extensive publication, Zha and coworkers compiled nanoparticle-based platforms for drug delivery that can cross the blood-brain barrier (BBB) through various transport mechanisms, including diffusion, carrier-, receptor-, or absorptive-mediated transcytosis, efflux pump, and osmosis, which are applied in the treatment of neurodegenerative diseases and cancer therapies. Authors devote considerable space for discussing functionalized polymeric, lipid, and metal nanomaterials that cross the BBB. Such materials are exceptionally suitable for the role thanks to their unique physicochemical characteristics and high biocompatibility [147]. As a result, they have attracted considerable attention within the scientific research community. An example is the assembled BBB-crossing lipid nanoparticles, which were applied to enable mRNA delivery to neurons and astrocytes in broad brain regions [150]. On the contrary, one non-standard approach to the subject is the engagement of microorganisms. Sun's team developed the so-called 'Trojan bacteria' – a drug delivery system based on bacteria (attenuated *Salmonella* strain VNP20009 and *Escherichia coli* 25922 were tested) loaded with glucose polymer (GC) and photosensitive indocyanine green (ICG)-silicon nanoparticles (GP-ICG-SiNPs) aimed at glioblastoma (GBM) photothermal immunotherapy. The studies on the orthotopic GBM mouse model have proven that the platform can bypass the BBB and penetrate cancer tissues, making them susceptible to treatment. Additionally, scientists observed that the designed system promotes innate immune responses due to the tumor-associated antigens (TAAs) released in response to applied irradiation [151].

On the other hand, nanoparticles provide sustained and controlled release of therapeutics, reducing the need for frequent dosing and maintaining effective drug concentrations at the injury site [152,153]. Lu and coworkers proposed a ligand-mediated drug/gene co-delivery platform for glioblastoma multiforme, which allows for controlled and sustained pH-triggered release of therapeutics. Co-encapsulating therapeutic agents developed the delivery system within a thermosensitive chitosan-g-poly(N-isopropylacrylamide) (CPN) hydrogel matrix. Specifically, stomatin-like protein 2 (SLP2) short hairpin RNA (shRNA) was combined with irinotecan (CPT-11) and loaded onto cetuximab (CET)-conjugated graphene oxide (GO-CET/CPT11), resulting in the formulation of a composite hydrogel designated as CPN@GO-CET/CPT11@shRNA. The effectiveness of the construct was proven both *in vitro* and *in vivo* (with a xenograft of U87 tumor-bearing nude mice). The results not only demonstrate the system's effectiveness in extended controlled drug release but also highlight its potential for broader applications [154].

Finally, due to the functionalization, nanoparticles demonstrate suitability for localized administration, offering improved selectivity while minimizing off-target effects [155–157]. Bai and coworkers presented phosphatidylserine (PS)- and transferrin (TF)-modified liposomes

encapsulating dexamethasone (DSS), designated as TF/PS/DSS-LPs, to enhance the treatment of ischemic stroke. Initially, TF molecules targeted the overexpressed transferrin receptor (TfR) in the blood-brain barrier. Upon entry into the brain, the PS modification enabled the liposomes to bind to phosphatidylserine-specific receptors (PSRs) on the surfaces of microglia and astrocytes. This modification also facilitated the uptake of TF/PS/DSS-LPs by these cells. Additionally, it promoted the polarization of astrocytes from the A1 to A2 phenotype and microglia from the M1 to M2 phenotype, thereby decreasing neuronal inflammation and boosting cerebral ischemic injury [158]. An illustrative example of a platform designed for targeted drug delivery to the central nervous system is the one developed by Vilella et al., comprising glycopeptide-functionalized poly(lactide-co-glycolide) nanoparticles (g7-NPs). This delivery system exhibited enhanced cellular uptake by both neurons and glial cells, accompanied by a region- and cell-type-specific distribution within the brain. Researchers also proved that the nanoparticles undergo clathrin-mediated endocytosis and localize within Rab5-positive early endosomes, with a direct correlation observed between Rab5 expression levels and nanoparticle accumulation [159].

Metal-based nanoparticles, particularly iron oxide and gold nanoparticles, represent a prominent class of nanomaterials that have been extensively explored for targeted drug delivery in the treatment of neurological disorders [160–163]. A recent study by Choi et al. introduces a transferrin-conjugated nanoparticle-based system designed to enhance delivery specificity to brain regions affected by amyloid aggregation in Alzheimer's disease. The platform, termed Tf-MeLioNs—comprising melittin and L-arginine-coated iron oxide nanoparticles—demonstrated reduced cytotoxicity and hemolytic activity *in vitro*. Moreover, *in vivo* experiments using 5XFAD transgenic mice revealed a significant reduction in amyloid plaque burden, particularly within the hippocampal region [164]. In a complementary approach, another research group synthesized gold nanoparticles (AuNPs) engineered for the selective targeting of tight junctions and showed that transcranial picosecond laser stimulation, following intravenous administration, significantly enhances blood-brain barrier permeability. Additionally, the applied modulation is dependent on laser intensity, reversible in nature, and promotes enhanced paracellular diffusion without impairing vasomotion or the structural integrity of the neurovascular unit. The method facilitates the trans-BBB delivery of viral vectors, immunoglobulins, and liposome-encapsulated therapeutics, presenting a promising nanotechnological strategy for drug delivery to light-accessible brain regions and expanding the scope for CNS drug screening and therapeutic applications [165].

Despite the encouraging outcomes associated with targeted therapies utilizing metallic nanoparticles, their clinical application remains constrained by concerns regarding potential toxicity. To address challenges related to bioavailability, a widely adopted strategy involves encapsulating these nanoparticles within biocompatible materials, such as polyelectrolytes. This approach not only improves biocompatibility and stability but also enables the functionalization of the nanoparticle surface with targeting ligands, thereby enhancing the precision of both imaging and therapeutic interventions.

#### 4. Polyelectrolytes Materials

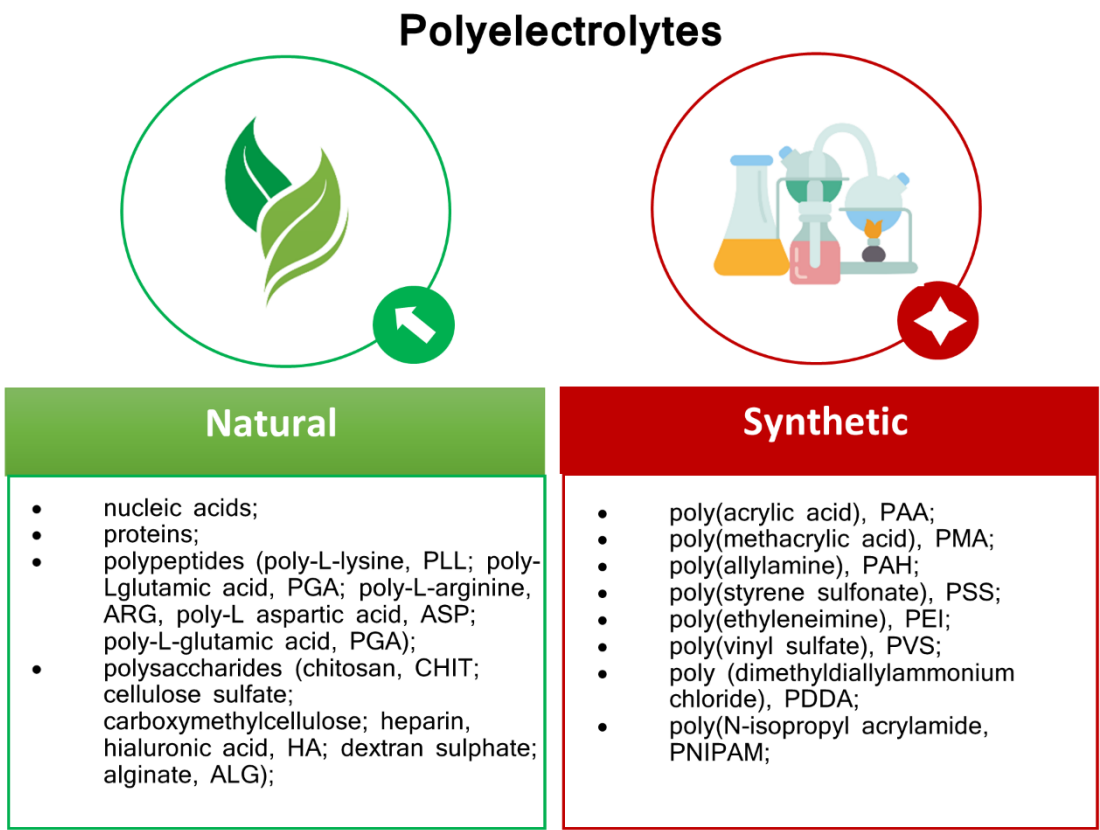
Among the diverse classes of biomaterials designed for cell interaction, polyelectrolytes (PEs) have emerged as up-and-coming candidates. Polyelectrolyte-based biomaterials have been employed for several decades across diverse industries, including pharmaceuticals, chemical processing, and food technology. Furthermore, they are employed in biomedicine for tissue engineering, bioimaging, biosensing, and drug delivery [166]. Polyelectrolytes participate in delivering regulatory factors, thereby facilitating cell functioning and tissue development, as well as stimulating processes such as differentiation, proliferation, and adhesion [167,168]. Another beneficial application is the use of polyelectrolytes as delivery systems for therapeutic agents (e.g., drugs and proteins) [169], as antibacterial factors [170], or in gene delivery systems [171].

Polyelectrolyte (PE) gels and complexes have gained considerable attention due to their potential to facilitate direct cell contact. Notably, the presence of ionic groups within PEs promotes the

formation of stable aqueous dispersions, wherein the ionized polymer chains function as surface-active centers.

The immobilization of cells within polyelectrolyte-based biomaterials has garnered substantial interest in the field of biomedical engineering, owing to its capacity to enhance the regeneration of damaged tissues through the localized production of biologically active molecules, including cytokines and growth factors, which are often deficient or absent due to pathological dysfunction.

Considering the origin, two classes of polyelectrolytes are distinguished – natural and synthetic ones (Figure 6). Natural polyelectrolytes include polysaccharides (e.g., sodium alginate (ALG), chitosan (CHIT), hyaluronic acid (HA), cellulose sulfate, heparin, dextran sulfate, carboxymethylcellulose, proteins, polypeptides (like poly-L-aspartic acid (ASP), poly-L-glutamic acid (PGA), poly-L-lysine (PLL), poly-L-arginine (ARG), and nucleic acids [172]. On the contrary, among the synthetic polyelectrolytes poly(styrene sulfonate) (PSS), poly(allylamine) (PAH), poly(acrylic acid) (PAA), poly(methacrylic acid) (PMA), poly(ethyleneimine) (PEI), poly(N-isopropyl acrylamide) (PNIPAM), poly(dimethyldiallylammonium chloride) (PDDA) and poly(vinyl sulfate) (PVS) can be enumerated [173,174].



**Figure 7.** Polyelectrolytes classification.

*4.1. Mechanism Of Self-Assembly*

Layer-by-layer (LbL) self-assembly can be governed by various intermolecular forces, including electrostatic interactions between oppositely charged species [175], van der Waals forces [176,177], hydrogen interactions [178–180], hydrophobic interactions [181–183], covalent linkages [181], or synergistic combinations thereof. Multilayer formation through combined mechanisms may be initiated via hydrophobic interactions and subsequently stabilized by hydrogen bonding, resulting in the formation of ultrathin membrane layers. Moreover, electrostatic and hydrophobic forces, as well as electrostatic-hydrogen bonding [184], are commonly associated with the self-assembly

process [185]. The morphology of individual monolayers is predominantly influenced by the competitive balance between hydrogen interactions and van der Waals forces, with the resulting two-dimensional nanostructure reflecting this equilibrium [186]. Among these, electrostatic interactions are most widely utilized, enabling the sequential adsorption of oppositely charged polyions onto charged substrates to fabricate polyelectrolyte (PE) multilayer films [175,187] with adjustable thickness and surface topography [188–190]. Covalent bonding is typically reserved for specialized applications, such as cell immobilization, where it facilitates the attachment of polymers to cell surfaces via chemical or enzymatic conjugation [191–193]. Additionally, metabolic introduction may be employed. Amphiphilic polymers, such as temperature-sensitive succinylated pullulan-g-oligo(L-lactide) [194], have been demonstrated to mediate hydrophobic conjugation. Experimental evidence suggests that the interaction between ionic and hydrophobic forces is a crucial factor in both guiding self-assembly and modulating the functional characteristics of the resulting bioactive interface [195].

#### 4.2. Polyelectrolytes for Neural Cell Regeneration in Terms of Mechanical Properties and Potential

The limitations of newly developed materials primarily arise from the requirement that biomaterial design be tailored to the specific biological parameters of the target cell types with which they are intended to interact. The example here might be the potential. The action potential of neurons during the depolarization and repolarization cycle (occurring on a time scale of approximately 2 ms) ranges from 30 mV at the transmembrane potential when the neuron is depolarized to a resting potential of  $-70$  mV when the neuron is at rest. In the resting state, the increased number of positively charged sodium ions outside the cell compared to inside creates a concentration gradient. Voltage-gated sodium channels open, leading to the passive transport of positively charged sodium ions from the outside of the cell to the inside, thereby shifting the transmembrane potential toward a positive value. After depolarization, voltage-gated sodium channels close and voltage-gated potassium channels open, causing potassium ions to move from the inside of the cell to the outside, after which the cell repolarizes to its resting potential [196]. Therefore, it can be assumed that the most beneficial would be to work with biomaterials that provide potential values within the range of neuronal function.

The use of polyelectrolytes with various potential values for interaction with neural cells has been reported. Lastly, capsule shells built of poly(allylamine hydrochloride) and poly(4-styrene sulfonate) sodium salt (PAH/PSS) or biodegradable poly-L-arginine and dextran sulphate sodium salt (PArg/DS) with zeta-potential of 3-bilayered samples meanly  $-18.2$  mV and  $-16.3$  mV respectively were applied for enhance electrostatic interactions with the plasma membrane, potentially facilitating cellular uptake by neuroblastoma N2A cells [197]. Moreover, polyethylenimine-poly-L-lysine PEI/PLL bilayers, which involve mainly hydrogen interactions between layers, with a positive potential of both layers, meanly  $+14.9$  mV, and  $+14.1$  mV respectively; furthermore, alginate-chitosan bilayers, with a potential meanly  $-37.60$  and  $+27.44$  [mV] respectively were assessed for cooperation with cells isolated from embryonic (E19) Wistar rat brains. Evaluation indicates the importance of the potential of the material-cell interface itself, and not the entire membrane system [198].

Conversely, the mechanical properties of biomaterial scaffolds can alter the function of neural cells. Evidence suggested that the soft materials ( $0.1$ – $1$  kPa) promote the differentiation of neurons, whereas the stiffer ones ( $7$ – $10$  kPa) promote the glial differentiation [199,200]. Hydrogels represent a widely utilized class of biomaterials in the treatment of neural dysfunction, mainly due to their controllable mechanical properties. These properties can be precisely modulated by varying the concentration of polymer precursors during hydrogel preparation, a crucial feature for potential clinical applications [201,202].

#### 4.3. Polyelectrolytes for Interface with Neural Cells

Several studies have been conducted to explore the usage of polyelectrolytes and their combinations in conjunction with neural cells. As mentioned above, for neuronal cell culture, the commonly applied supports are poly-L-lysine (PLL) and poly-D-lysine (PDL) [16–18], which are



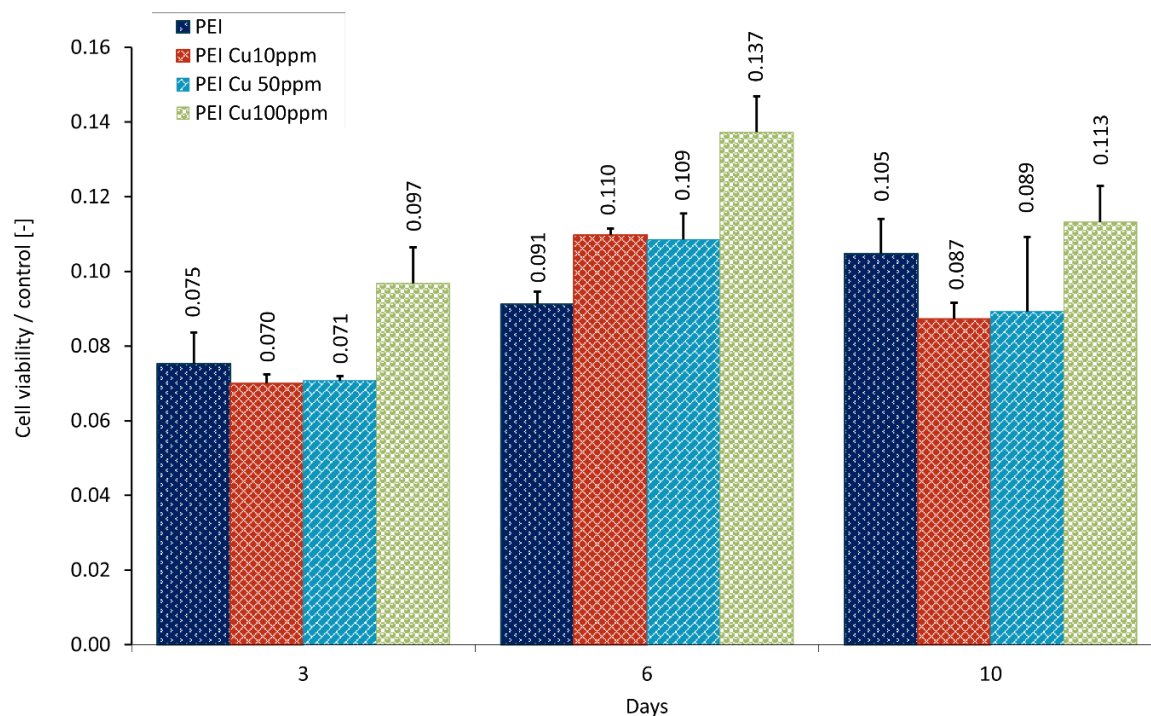
applied as monolayers. Poly-D-lysine and poly-L-lysine, enantiomeric forms of synthetic polylysine, are positively charged molecules known to enhance electrostatic interactions with negatively charged components of the cell membrane. Regarding the charge-related properties of these molecules, both are the same; however, there are reports that poly-L-lysine is subject to deterioration due to the action of proteases released by certain types of cells. Nevertheless, polymers such as HA [203], PAH, PAA, and PSS [204] have been identified as potential candidates for such applications. Dąbkowska and co-workers [205] utilized positively charged poly(diallyldimethylammonium chloride) (PDADMAC) and polyanion heparin sodium salt (HP) in their research to construct a polyelectrolyte membrane (PEM) scaffold for the adsorption of brain-derived neurotrophic factor (BDNF). The PEM scaffolds were fabricated using the layer-by-layer (LbL) technique, which relies on the successive deposition of polyelectrolytes with opposite charges. The impact of Brain-derived neurotrophic factor (BDNF) immobilized within poly(diallyldimethylammonium chloride)/ heparin PEMs was studied on the SH-SY5Y human neuroblastoma cell line. The SH-SY5Y cell line is heterogeneous, consisting of S-type (epithelial-like) and N-type (neuroblastic) undifferentiated cells. Studies have demonstrated that multilayers terminated with PDADMAC/HP may facilitate the formation of spheroid-like three-dimensional (3D) cell cultures, presenting a promising platform for future investigations as an *in vitro* model of neurodegenerative diseases. Moreover, polymers such as CHIT, ALG, PEI, and PLL were used to construct multilayer films to examine their interaction with rat neural cells [206]. PEMs multilayer scaffold constructed using the LbL technique, built of PLL as a polycation and poly-L-glutamine acid (PLGA) as a polyanion, was reported by Lee and Wu [16] to promote the differentiation of neural stem/progenitor cells (NSPCs) into neurons with synaptic functions. Those studies showed that NSPCs cultured within PEMs scaffolds without adding serum and growth factors could differentiate into functional neurons with significant neurite outgrowth. NSPCs are multipotent, self-renewing cells that can differentiate into the three principal neural lineages: astrocytes, oligodendrocytes, and neurons. Cerebral cortical NSPCs isolated from 14- to 15-day-old Wistar rat embryos were used to study the upregulation of the number of differentiated neurons and synaptic function with a PLL terminal layer. Some other studies reported the impact of PEMs scaffolds on neural stem/progenitor cells (NSPCs) derived from Wistar rat embryos on days 14-15 [207]. PEMs applied in the research were composed of cationic PAH and anionic PSS or PAA. It was observed that the PAH/PSS multilayer scaffolds stimulate extensive outgrowth and differentiation of NSPCs, primarily into neurons. The neurospheres were unable to attach and differentiate on the PAA-ended multilayers. That work indicates the NSPCs' dependence on the surface charge of PEM films.

However, the above-mentioned materials are not among the FDA- and/or CE-approved natural and synthetic materials used in clinical trials as components of DDS systems or as catheters for the delivery of cells intended to produce CNTF. To date, nerve conduits, wraps, and cuffs that have received FDA or CE approval are collagen or polyester – based, like RevolNerv® (Orthomed S.A.S., Lyon, France); NeuraGen® and NeuraWrap™ (Integra Life Sciences Corp., Plainsboro, NJ, USA); NeuroMatrix™, Neuroflex™ and NeuroMend™ (Collagen Matrix, Inc., Franklin Lakes, NJ, USA) or Neurolac® nerve guidance (Polyganics B.V., Groningen, The Netherlands) and Neurotube® nerve guidance (Synovis Micro Companies Alliance, Birmingham, AL, USA) respectively [208,209].

## 5. PE and PE-Based Nanocomposites Application for Neuronal Cell Immobilization

It is noteworthy that, among various scaffolding materials, polymeric hydrogel scaffolds are employed owing to their biocompatibility and structural resemblance to tissue [210]; however, they are distinguished by low mechanical strength and limited biological activity. Thus, investigations have been conducted on materials with enhanced physicochemical properties and/or bioactive features. In scaffolds made of polyelectrolyte membranes, various substances can be incorporated, including anticancer factors [211], DNA [212], graphene [213,214], inorganic materials [215], drugs [190,216], and proteins [217], as well as different types of nanomaterials.

There has been a marked increase in research activity concerning the incorporation of metallic nanoparticles into biomaterial systems. Metal-based NPs employed as additives allow nanocomposites to produce improved mechanical strength, involving additional biological features like antibacterial and antiviral activity [218,219]. Bacteriostatic materials for use in conjunction with neuronal cells have been continually developed. For example, incorporating polyethylenimine CuNPs has been verified to cooperate with the mice's neural stem cell line NE-4C. The effect of different concentrations of CuNPs incorporated within the membrane shells was verified. The results of the mitochondrial activity evaluation of NE-4C cells indicated that the involvement of CuNPs up to 100 ppm did not decline mitochondrial activity by more than 13% compared with the control (cells cultured without membrane addition) (Figure 8).



**Figure 8.** The mitochondrial activity of NE-4C cells immobilized on the coating layer assessed over a 10-day culture period, with results expressed as the ratio relative to the control based on formazan production, as measured by absorbance. Key to the symbols: PEI – polyethylenimine, PEI Cu10ppm – polyethylenimine 10ppm CuNPs incorporating, PEI Cu50ppm – polyethylenimine 50ppm CuNPs incorporating, PEI Cu 100ppm – polyethylenimine 100ppm CuNPs incorporating. The values are presented as mean  $\pm$  SD.

It is worth noting that some metal-based NPs may be considered as electrostimulating factors for theranostic applications. For example, copper nanoparticles (CuNP), which show high conductivity (Cu,  $\sigma = 5.96 \times 10^7 \text{ Sm}^{-1}$ ) [219].

Electrical stimulation (ES) is a widely discussed strategy for treating the nervous tissue. The principle of ES involves the application of electrical impulses to activate neural cells, which are inherently responsive to electrical signals. The demonstrated efficacy of electrical stimulation in modulating neural cell activity and enhancing tissue regeneration presents promising implications for clinical applications. Thus, conductive materials like graphene, carbon nanotubes, silk fibroin-based biomaterials, and metallic nanoparticles can be considered. Besides metallic nanoparticles, various polymers are used as conductive scaffolds, including HA, ALG, poly(pyrrole) (Ppy), polyaniline (PANI), poly(3,4-ethylene dioxothiophene) (PEDOT), and PSS, which are also utilized as components of PEM construction [220–223].

It can be noted, that those conductive materials for nerves bridging in case of peripheral nerve injury with minor defects have the form of nerve guidance conduits (NGCs) that can mimic the conductivity of natural nerves or and can be applied with electrical stimulation E.g. the conductive NGCs in form of fiber formed by coprocessing the conductive polymers PEDOT/ PSS and CHIT via electrospinning technology [224].

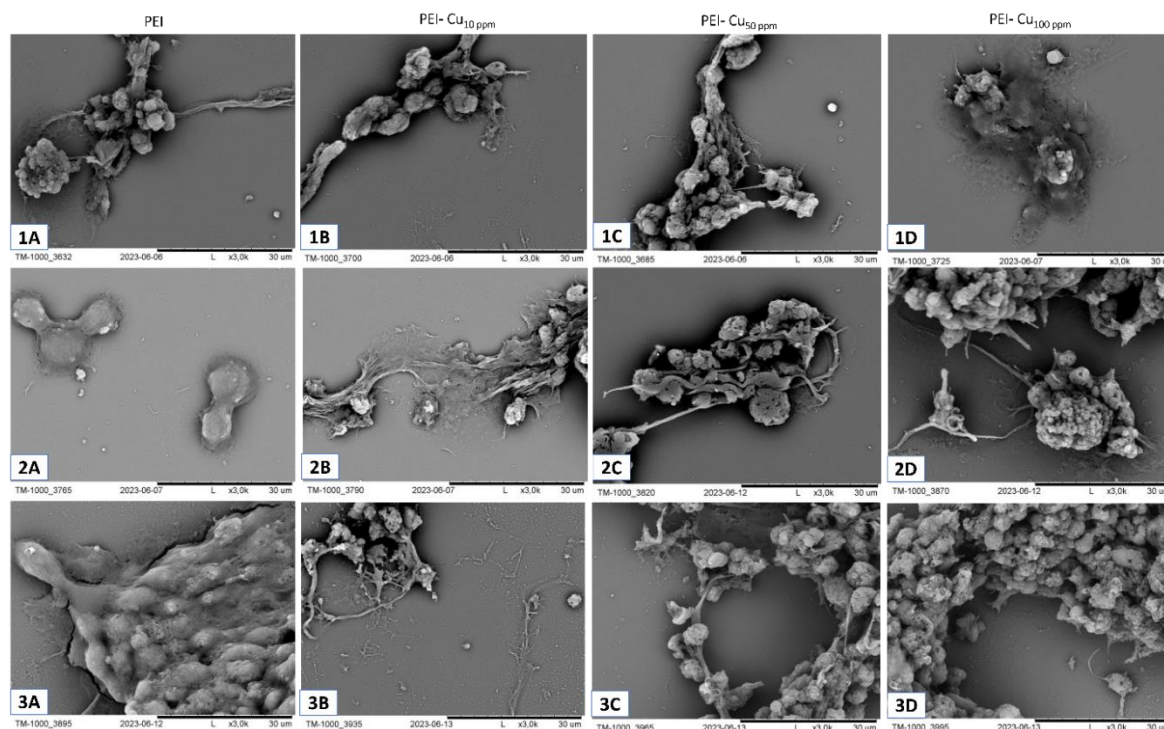
The integration of these two approaches—facilitating the directed differentiation of nerve cells on polyelectrolyte substrates and applying electrostimulation—could efficiently promote nerve tissue reconstruction and advance the elucidation of nerve cell regeneration mechanisms. Furthermore, metallic nanoparticles not only support electrostimulation but also serve as effective antimicrobial agents or drug delivery factors.

Microscopic observation of the morphology of neural cells in a system with immobilizing material can be a simple way to provide insight into their interaction with the immobilizing substrate used.

Below, we present an exemplary microscopic analysis of the function of neural cells immobilized in membranes composed of selected polyelectrolytes with incorporated metallic nanoparticles.

### 5.1. Visualization of Layer Coating Scaffold-Neuronal Cells Systems

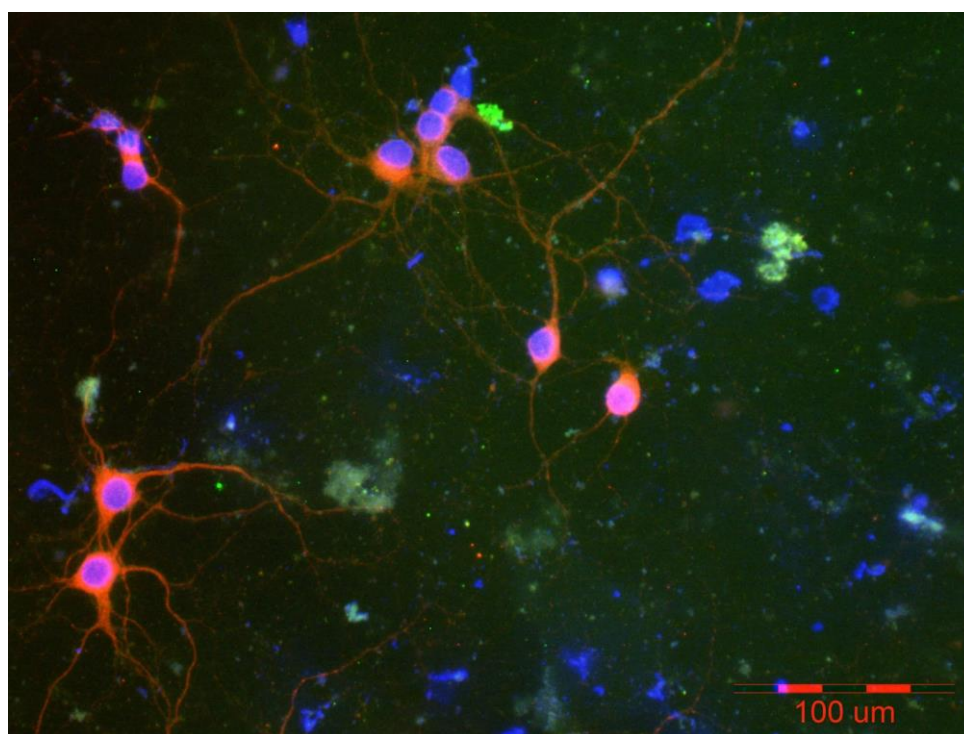
Scanning electron microscopy (SEM) is a valuable technique for examining the morphological features of layer membrane coating scaffold-cells systems, particularly polyelectrolyte (PE) membrane-immobilized cells. Figure 9 presents the example morphology of PE membranes, particularly a PEI-based coating layer incorporating copper nanoparticles (CuNPs) at different concentrations (10, 50, and 100 ppm), after 3-, 6-, and 10-day culture periods. No noticeable alterations in cell morphology may be observed during a 10-day culture period in the presence of nanocomposites containing varying CuNPs content. SEM analysis of cellular morphology corroborated the findings of the MTT assay (Figure 8), indicating no significant differences in cellular function in the presence of composites with differing CuNP share.



**Figure 9.** SEM images of NE-4C cells adsorbed within the designed membranes after a third day of culture. Key to the symbols: PEI – polyethyleneimine, PEI-Cu<sub>10ppm</sub> – polyethyleneimine 10ppm CuNPs incorporating, PEI-Cu<sub>50ppm</sub> – polyethyleneimine 50ppm CuNPs incorporating, PEI-Cu<sub>100ppm</sub> – polyethyleneimine 100ppm CuNPs incorporating.



The application of selective dyes that distinguish between neurons and astrocytes enables visualization of the functional architecture of neural cells within the scaffolds. For example, cells can be simultaneously labeled with anti-microtubule-associated protein 2 (MAP2), a marker for the neural skeleton, and anti-glial fibrillary acidic protein (GFAP), an indicator of astrocytes. Furthermore, fluorescent labeling can be accomplished by conjugating fluorochromes such as Alexa Fluor 488 and Alexa Fluor 555, facilitating the identification and delineation of specific cellular components under fluorescence microscopy. Figure 5 illustrates the neural cells encapsulated within a nanocomposite scaffolding matrix following one week of culture (5% CO<sub>2</sub>, 37°C). An examination of the system using immunostaining demonstrated the presence of neural cell populations. The system layer coating scaffold, designed explicitly for NE-4C cells, supports the growth of neurons within the neuronal cell population. The light green fluorescence demonstrates the presence of cells containing glial fibrillary protein, and the red fluorescence reveals the presence of neurons. The obtained results indicate that the demonstrated scaffolds enable the survival of neurons during culture, which can be considered for regenerating neural tissue.

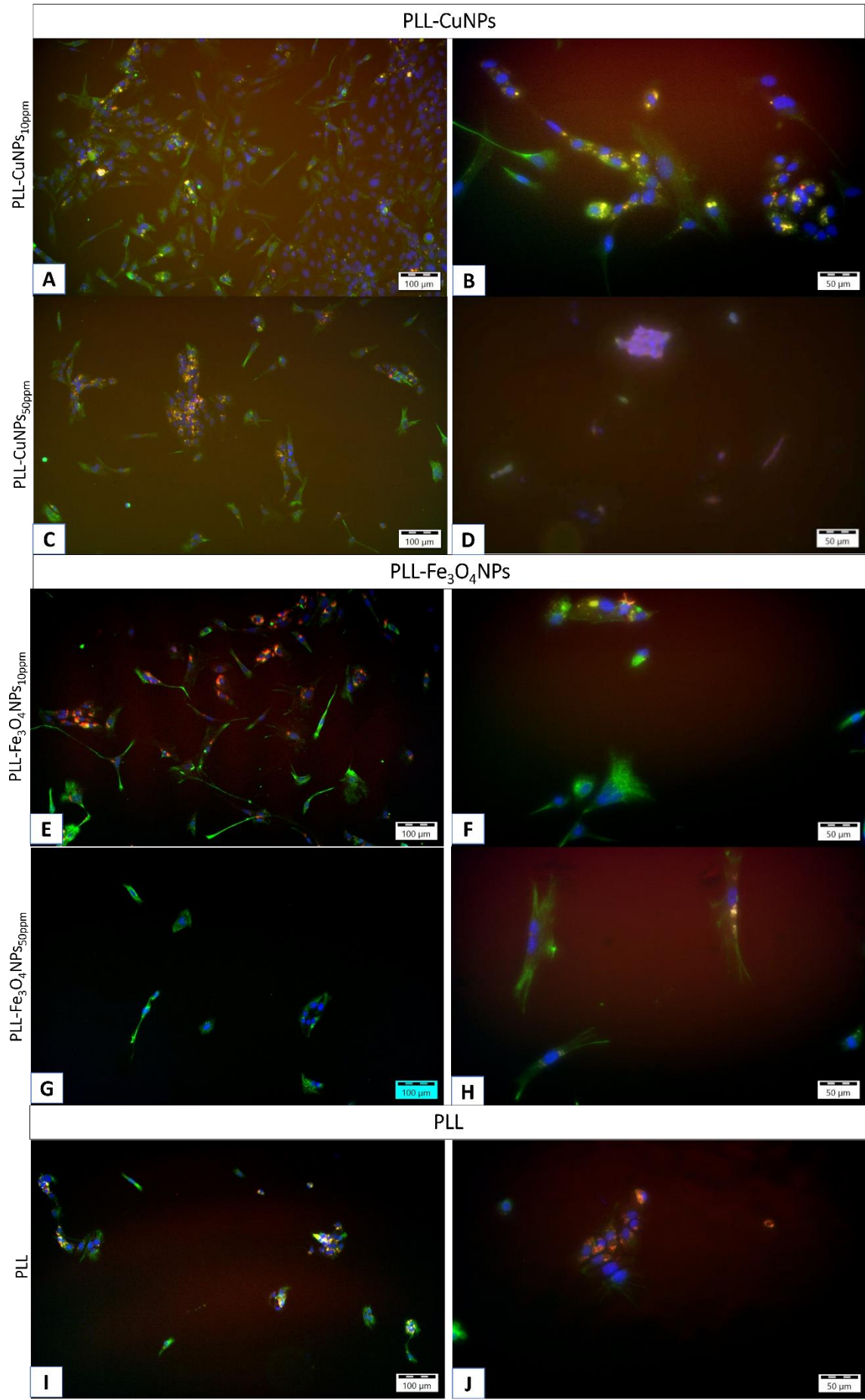


**Figure 10.** Visualization of cells immobilized within PEM membrane scaffolds after a week of culture. The red fluorescence indicates neurons stained with anti-microtubule-associated protein 2 (MAP2), using a secondary antibody conjugated with the fluorochrome Alexa Fluor 555. The light green fluorescence shows astrocytes stained with anti-glial fibrillary acidic protein (GFAP) and a secondary antibody conjugated with Alexa Fluor 488 fluorochrome. Cell nuclei were visualized using Hoechst 33342, a fluorescent dye that emits blue fluorescence upon binding to DNA. Photos were taken using an Olympus IX71 fluorescence microscope.

Poly-L-lysine may be another example of a polyelectrolyte, evaluated for its potential use in conjunction with neural cells.

The efficacy assessment of scaffolds composed of poly-L-lysine incorporating copper or iron oxide nanoparticles is presented below. Figure 11 shows the neural cells immobilized within the nanocomposite scaffolding shell after two weeks of culture (5% CO<sub>2</sub>, 37°C). An examination of the system using immunostaining demonstrated the presence of neural cell populations. The light green fluorescence indicates the presence of cells containing glial fibrillary protein, while the red fluorescence reveals the presence of neurons.





**Figure 11.** Visualization after two-week culture of cells immobilized within layer membrane scaffold built of poly-L-lysine with incorporated: CuNPs in share of 10ppm (a, b) and 50ppm (c, d); and with incorporated Fe<sub>3</sub>O<sub>4</sub>NPs in share of 10ppm share (e, f), and 50ppm (g, h), and poly-L-lysine alone (i, j). The red fluorescence indicates neurons stained with anti-microtubule-associated protein 2 (MAP2), using a secondary antibody conjugated with the fluorochrome Alexa Fluor 555. The light green fluorescence shows astrocytes stained with anti-glial fibrillary acidic protein (GFAP) and a secondary antibody conjugated with Alexa Fluor 488 fluorochrome. The nuclei were tagged with Hoechst 33342 for imaging. Photos were taken using an Olympus IX71 fluorescence microscope.

However, microscopic observations confirmed the presence of both astrocytes and neurons on all selected scaffolds; the morphology of the neurons differed from the typical characteristics of this cell type. Cells with a bundle shape predominate, with a few showing a tendency to form a spindle shape. The culture of the material containing Fe<sub>3</sub>O<sub>4</sub>NPs indicates a more significant share of neurons in the cell population than the material containing CuNPs.

It should be noted that the use of selected metallic nanoparticles affects both the morphology and function of neural cells, in addition to the anticipated antibacterial effects and the possibility of mediating electrical stimulation of neural cells.

## 6. Perspectives

Repairing the nervous system is a constant challenge for modern medicine. Research is focused on both cell therapies and biomaterials, as well as their associated technologies. The goal in the latter case is to obtain materials that influence the function, growth, and differentiation of neural cells. Polyelectrolyte materials offer some promise in this regard.

Polyelectrolyte layer membrane coatings offer a large number of possibilities for differing membrane properties, e.g., thickness, morphology, or wettability, allowing the control of cell culture outgrowth, differentiation, and development, which may serve as elements of the scaffolds for immobilization of cells, giving the ability to adjust cell interaction with the material at the material-host interface. Polyelectrolyte-based scaffolds incorporating bacteriostatic components can be considered an integral part of the system for amplifying nervous tissue and supporting the reconstruction of functions lost by neural tissue.

It is worth noting that layered coating presents some challenges to material stability. The PE materials may undergo deterioration by endocytosis. Also, dissociation from the surrounding medium or body fluids may occur. Thus, only the temporary usability of the systems may be considered. The spatial complexity of neural cells and the optimization of operating time are also factors that limit the usefulness of materials. However, the use of nanotechnology in the construction of nanocomposite materials based on polyelectrolytes allows for the modification and strengthening of interactions at the material-cell interface.

At the same time, continuous assessment of toxicity or unintended immune reactions is necessary to protect against adverse effects.

It can be assumed that future work on systems of polyelectrolyte membrane nanocomposites with immobilized cells will focus on developing functional materials incorporating biological activity to enhance/modulate cell function, as well as novel cell types.

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