A Systematic Review of the Effectiveness of Cell-Based Therapy in Repairing Peripheral Nerve Gap Defects

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Abstract: Nerve prostheses are widely utilized to reconstruct segmental (gap) defects in peripheral nerves as an alternative to nerve grafting. However, with increasing gap length, the effectiveness of a nerve prosthesis becomes sub-optimal, which subsequently has made repairing larger gaps in peripheral nerves a significant challenge in the field of regenerative medicine. Recently, the structure of nerve prostheses has been significantly revised, which interestingly, has provided a promising avenue for the housing and proliferation of supportive cells. In this systematic review, cell implantation in synthetic nerve prostheses to enhance the regenerative capability of an injured nerve with a focus on identifying the cell type and mode of cell delivery is discussed. Of interest are the studies employing supportive cells to bridge gaps greater than 10 mm without the aid of nerve growth factors. The results have shown that cell therapy in conjunction with nerve prostheses becomes inevitable and has dramatically boosted the ability of these prostheses to maintain sustainable nerve regeneration across larger gaps and helped to attain functional recovery, which is the ultimate goal. The statistical analysis supports the use of differentiated bone-marrow-derived mesenchymal stem cells suspended in oxygen-carrying hydrogels in chitosan prostheses for bridging gaps of up to 40 mm; however, based on the imperfect repair outcomes, nerve grafting should not yet be replaced altogether.

Keywords: nerve prosthesis; stem cells; axonal regeneration; nerve regeneration; nerve conduits

1. Introduction

The primary purpose of every nerve gap repair technique is to hold the transected nerve ends together to facilitate nerve continuity. Under the regime of surgical solutions for repairing completely transected peripheral nerves, microsurgical neurorrhaphy and nerve grafting are the most common techniques used in practice. However, the inherent shortcomings of these techniques which include nerve distortion, increased tension at the coaptation site, graft rejection, donor site morbidity, aberrant regeneration of axons, and unsatisfactory results has led to the development of neural prosthetic techniques [1]. The use of nerve prostheses to bridge irreducible large gaps in peripheral nerves is becoming popular nowadays due to their off-the-shelf availability in different shapes and sizes. Nerve prostheses, which are mainly synthesized from degradable biomaterials, are known to provide several benefits, such as enhanced nerve regeneration across smaller-to-medium-sized nerve defects (1 to 10 mm), mitigating scar tissue development, and preventing the leakage of intraneural fluid; however, the success rates become highly variable in gaps with greater lengths [2], where the proximal stump of
the injured nerve remains unable to find its distal counterpart and regenerating fibers become aberrant. This results in the formation of neuroma and scar tissues, which further impede the regeneration process, lengthens the recovery period and thereby jeopardizes the repair quality. Thus, this dilemma has stressed the need to find alternate ways to support nerve regeneration.

With the recent advances in tissue engineering, supportive cells are being considered as potential candidates for speeding up the quasi-static rate of peripheral nerve regeneration. Nerve prostheses seeded with a variety of stem cells, such as skin-derived stem cells (SdSCs), adipose-derived stem cells (ADSCs), and bone-marrow-derived stem cells (BMdSCs), are being tested regarding bridging nerve defects to observe their respective efficacy in promoting nerve regeneration over larger gaps; however, the majority of the studies have reported the use of cell transplantation techniques in bridging non-critical-sized gaps, i.e., 10 mm or less. Arguably, these smaller gaps can be repaired by traditional nerve repair techniques, and thus the therapeutic effect of the cell therapy cannot be fully realized. Therefore, to acknowledge the true potential of supportive cells used in gap repairs, a systematic literature search was performed according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, which resulted in the collection of 268 studies obtained from online research databases in the context of peripheral nerve gap management using neural prostheses (see Figure 1). A total of 21 studies were found eligible for inclusion in this systematic review (reported in Table 1), while the rest of the studies were excluded based on the following criteria:

(a) Studies that employed a nerve crush injury model, gapless repair model, or in vitro model.
(b) Studies that used non-synthetic or biological prostheses derived from nerves, muscles, or intestines.
(c) Studies where the segmental defect was 10 mm or less (the threshold for the length of a nerve gap was taken as 10 mm because most of the studies used a peripheral nerve injury model in rodents, and due to higher nerve regeneration capacity in smaller animals, the true efficacy of a nerve repair cannot be revealed over shorter nerve defects).
(d) Studies where nerve growth factors were applied either endo- or exogenously.

Figure 1. Literature search methodology according to PRISMA guidelines.
Table 1. Repairing critical-sized defects in peripheral nerves using a nerve prosthesis aided by cell-based therapy. PGA: Polyglycolic acid, PLA: Polylactic acid, PLGA: Poly(lactic-co-glycolic acid), PLLA: Poly(L-lactic acid), PLCL: Poly(L-lactide-ε-caprolactone), PTFE: Polytetrafluoroethylene, PFTBA: Perfluorotributylamine, TMC/CL: Trimethylenecarbonate-co-epsilon-caprolactone, d: differentiated, ud: undifferentiated, SCs: Schwann cells, MSCs: Mesenchymal stem cells, ADSCs: Adipose-derived stem cells, BMStCs: Bone marrow stromal cell, BMMnCs: Bone marrow mononuclear cells, GFP: Green fluorescent protein, SFI: Sciatic function index, NCV: Nerve conduction velocities, CMAP: Compound muscle action potential, CUiS: Contralateral uninjured side, CUiN: Contralateral uninjured nerve, FG: Fluorogold. Studies marked with an asterisk are of special interest (see Section 3 for further detail).

| Study No. | Cell Type | Mode of Cell Delivery | Gap (mm) | Animal Model | Assessment of Nerve Regeneration | Research Findings |
|-----------|-----------|-----------------------|----------|--------------|-------------------------------|-------------------|
|           |           |                       |          |              | Subject | Groups | Histological Assessments | Functional Assessments |                           |
|           |           |                       |          |              |                                   |                   |
| 1         | Human neural stem cells | Collagen prosthesis with an inner lining of engineered neural tissues (d-neural stem cells + collagen). | 12 | Rat sciatic nerve | G1: Autograft G2: Treatment G3: Prosthesis alone | Two months No. of Axons G1: 5000, G2: 4300, G3: 5200 Axonal diameter G1: 0.9, G2: 1.2, G3: 0 Myelin thickness G1: 0.65, G2: 0.57, G3: 0 No. of blood vessels G1: 18, G2: 23, G3: 0 Gastrocnemius weight (% of control) G1: 42, G2: 25, G3: 17 | Two months CMAP (gastrocnemius muscle) G1: 2.3, G2: 7.5, G3: 3.7 | Enhanced growth of neurites and vasculature along with reinnervation of the target muscle [3]. |
| 2         | MSCs      | Injection of d-MSCs into the lumen of the collagen prosthesis | 12 | Rat sciatic nerve | G1: Reverse autograft G2: Prosthesis with ud-MSCs G3: Prosthesis with d-MSCs G4: Prosthesis with SCs G5: Prosthesis alone | Three months No. of FG-labeled motoneurons G1: 1310, G2: 430, G3: 605, G4: 800, G5: 300 | Three months CMAP G1: 2.2, G2: 1.3, G3: 1.2, G4: 1.2, G5: 1.15, Control (healthy): 2.5 | Sufficiently supported limited axonal regeneration that was comparable to implant repair using SCs [4]. |
| 3         | SCs       | Injection of SCs in collagen gel into the lumen of the PLLA prosthesis | 12 | Rat sciatic nerve | G1: Reverse autograft G2: Prosthesis with 1 × 10^4 SC/mL G3: Prosthesis with 1 × 10^6 SC/mL G4: Prosthesis with gel only G5: Silicone prosthesis | Four months No. of Axons G1: 23,000, G2: 25,000, G3: 17,000, G4: 27,000, G5: 30,000 Gastrocnemius muscle weight G1: 1.5, G2: 1.25, G3: 1.1, G4: 1.2, G5: 1.1 | Four months Sciatic function index score G1: 86.80 G2: 84.74 G3: 89.92 G4: 83.74 G5: 74.43 | Regeneration in all groups with no significant difference between groups [5]. |
| 4         | d-ADSCs   | Sheet developed from ADSCs in collagen gel was placed inside Neura-Wrap™ | 15 | Rat sciatic nerve | G1: Autograft G2: Treatment G3: NeuraWrap alone | Two months Myelin thickness G1 and G3: Lower in G3 compared to the G1; G1 and G2: No difference | Not tested | Axonal regeneration in the distal stump was 3.5 times greater in G2 than G3 [6]. |
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|-----------|-----------|-----------------------|----------|--------------|----------------------------------|-------------------|
|           |           |                       |          | Subject      | Groups                           |                   |
|           |           |                       |          | No. of Axons | Histological Assessments         |                   |
|           |           |                       |          | No. of blood vessels | Functional Assessments |                   |
|           |           |                       |          | Functional Effect on the regeneration of nerve tissue in vivo [7]. |                   |                   |
|           |           |                       |          | Positive effect on the regeneration of nerve tissue in vivo [7]. |                   |                   |

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|          |           |                       |          |              | Subject                          |                  |
|          |           |                       |          |              | Groups                           |                  |
|          |           |                       |          |              | Histological Assessments         |                  |
|          |           |                       |          |              | Functional Assessments           |                  |
|          |           |                       |          |              | Research Findings                |                  |
| 10       | SCs       | Injection of SCs into the lumen of a collage prosthesis | 18       | Rat sciatic nerve | G1: Prosthesis with >0.5 × 10⁶ SCs G2: Prosthesis <0.5 × 10⁶ SCs G3: Prosthesis with PBS | Six months No. of myelinated axons G1: 13,000, G2: 4800, G3: 0, Normal nerve: 6000 Twelve months SFI G1: ~70, G2: ~80, G3: 0 NCV operated side G1: 29, G2: 28, G3: 0 | Increasing the number of cells in the guides had a greater beneficial effect [12]. |
| 11       | SCs       | Injection of SCs in matrigel into the lumen of a TMC/CL prosthesis | 20       | Rat median nerve | G1: Reverse autograft G2: Treatment G3: Prosthesis alone | Nine months No. of Axons G1: 3453, G2: 4402, G3: 0, Normal: 2361 Myelin thickness G1: 1.086, G2: 0.954, G3: 0, Normal: 1.631 Nine months CMAP G1, G2: No difference G3: 0 | Functional recovery comparable to autograft repair [13]. |
| 12       | MSCs      | Induction of d-MSCs into PTFE prosthesis | 20       | Monkey median nerve | G1: Treatment G2: Prosthesis alone | Twelve months NF-positive area/total nerve area G1: S1.5, G2: 33.3 Twelve months CMAP G1: 7.5, G2: 2.1 NCV G1: 13.5, G2: 10.2, Control: 18 | Transplantation of stem cells was helpful for nerve regeneration [14]. |
| 13       | SCs       | Injection of SCs into the lumen of a polyglactin prosthesis | 20       | Rabbit sciatic nerve | G1: Treatment G2: Autograft | Two months No. of Axons G1: 900, G2: 1102 Not tested | Axonal regeneration was observed, even in the distal nerve stump [15]. |
| 14       | SCs       | SCs mixed in gelatin and pipetted into the lumen of the PGA prosthesis | 30       | Rabbit peroneal nerve | G1: Treatment G2: Prosthesis with gelatine only | Four months Scoring of myelinations (3—good, 2—fair, 1—poor, P—proximal, M—middle, D—distal) G1: 2.7 P, 1.5 M, 1.0 D G2: 3.0 P, 1.7 M, 1.4 D Four months NCV (% of the NCV of the CuIS) G1: 94.7, G2: 96.5 | Local initial effect on nerve regeneration; a gap of 30 mm was not enough to observe a significant difference [16]. |
| 15 *     | ud-BMSCs  | Injection of autologous ud-BMSCs into the lumen of a PLCL prosthesis | 30       | Dog ulnar nerve | G1: Reverse autograft G2: Prosthesis with ud-BMSCs | Six months No. of Axons G1: 7032, G2: 7165 Axonal diameter G1: 1.75, G2: 2.09 Six months CMAP G1: 25.3, G2: 10.9 NCV G1: 23.5, G2: 31.6 | A viable option for the treatment of peripheral nerve injuries [17]. |
| Study No. | Cell Type | Mode of Cell Delivery | Gap (mm) | Animal Model | Assessment of Nerve Regeneration | Research Findings |
|-----------|-----------|-----------------------|----------|--------------|----------------------------------|-------------------|
| 16 *      | BMMnCs    | Injection of autologous BMMnCs into the lumen of a chitosan prosthesis | 30       | Goat peroneal nerve | G1: Autograft G2: Prosthesis with BMMnCs G3: Prosthesis with Basal Medium Eagle | Twelve months Axonal diameter G1: 3.60, G2: 3.67, G3: 0, Normal: 6.12 Myelin thickness G1: 0.97, G2: 0.88, G3: 0, Normal: 1.32 Twelve months NCV G1: 51, G2: 37, G3: 0 | BMMnCs not only helped in bridging longer defects but also induced functional recovery [18]. |
| 17 *      | MSCs      | Injection of MSCs into the lumen of a collagen prosthesis | 35       | Dog sciatic nerve | G1: Autograft G2: Treatment G3: Prosthesis alone | Nine months Axonal diameter G1: 7.7, G2: 3.6, G3: 2.5 Thickness of neo-fibers G1: 0.75, G2: 0.33, G3: 0.08 Gastrocnemius weight (%) G1: 48, G2: 69, G3: 84, G4: 90 Nine months CMAP ratio G1: 77, G2: 68, G3: 46 | Axonal regeneration and improved functional recovery [19]. |
| 18 *      | MSCs      | Injection of an autologous bone marrow MSC suspension in a chitosan/PLGA prosthesis | 50       | Monkey median nerve | G1: Autograft G2: Treatment G3: Prosthesis alone | Twelve months Axons diameter G1: 5.7, G2: 4.7, G3: 4, Normal: 7.1 Myelin thickness G1: 1.1, G2: 0.8, G3: 0.6, Normal: 1.7 FG-labeled motoneurons G1: 15,800, G2: 15,700, G3: 15,050, Normal: 17,500 Twelve months CMAP G1: 6.8, G2: 3.9, G3: 3.8, Normal: 12.1 NCV G1: 30, G2: 19, G3: 13, Normal: 87 | Repair similar to autograft repair and better than the repair done using a prosthesis alone [20]. |
| 19 *      | MSCs      | Injection of an autologous bone marrow MSC suspension in a chitosan/PLGA prosthesis | 50       | Dog sciatic nerve | G1: Reverse autograft G2: Prosthesis alone G3: Treatment | Six months Myelin thickness G1: 1.3, G2: 1.1, G3: 1.4, Normal: 2.4 Density of myelinated fibers G1: 6, G2: 4.1, G3: 5.8, Normal: 11.2 Gastrocnemius muscle weight ratio G1: 0.92, G2: 0.79, G3: 0.86, G4: 0.6 Six months CMAP G1: 9, G2: 6, G3: 9.6, Normal: 15.8 NCV G1: 41, G2: 23, G3: 39, Normal: 101 | Nerve regeneration and functional recovery comparable to autograft repair [21]. |
Table 1. Cont.

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|-----------|-----------|-----------------------|----------|--------------|----------------------------------|-------------------|
|           |           |                       |          |              | Subject | Groups | Histological Assessments | Functional Assessments |                      |
| 20 *      | MSCs      | Injection of an autologous MSC suspension in a chitosan/PLGA prosthesis | 60       | Dog sciatic nerve | G1: Autograft G2: Prosthesis with MSCs G3: Prosthesis alone | Twelve months Axonal diameter G1: 5.2, G2: 4.1, G3: 4.0, CUiS: 8.1 Myelin thickness G1: 1.3, G2: 1.1, G3: 0.8, CUiS: 1.7 Muscle weight ratio Gastronomes G1: 0.82, G2: 0.78, G3: 0.76, G4: 0.25 FG-labeled motoneurons G1: 18,500, G2: 17,500, G3: 15,000, CUiS: 21,000 |         |
| 21 *      | MSCs      | Injection of bone-marrow-derived mesenchymal stem cells into the lumen of a chitosan/silk prosthesis | 60       | Dog sciatic nerve | G1: Reverse autograft G2: Prosthesis with BMSCs | Twelve months Axonal diameter G1: 6.2, G2: 6.1, CUiS: 10.1 Myelin thickness G1: 1.3, G2: 1.2, CUiS: 2.2 FG-labeled motoneurons G1: 14,000, G2: 13,000, CUiS: 21,000 Muscle weight ratio (%) Gastronomes G1: 67, G2: 43, G3: 25 |         |

Note: All results are reported relative to the findings in proximal stumps. Axonal diameter, axonal growth (neurite length), and myelin thickness are reported in micrometers (µm); NCV is reported in meters per second (m/s); and CMAP is reported in millivolts (mV).
2. Scope of the Manuscript

The primary objective of this review was to appraise the efficacy of cell implantation in nerve prostheses to achieve successful nerve regeneration along with functional recovery across critical-sized defects (>10 mm) in peripheral nerves. The secondary objective was to make recommendations about prosthetic materials and the source of stem cells for developing an optimal and reliable therapeutic modality.

3. Effectiveness of Cell Therapy

A neural prosthetic technique is a process of encasing stumps of a transected nerve in a hollow conduit to create a favorable environment for regenerating axons. Fundamentally, a nerve prosthesis allows the proximal nerve stump to grow and orientate toward the distal nerve stump in a guided manner, and therefore, essentially provide a bridging scaffold for regenerating nerve fibers without necessitating graft material. Earlier, we highlighted the importance of developing biodegradable nerve prostheses from polyglycolic acid (PGA), chitosan, and collagen to support nerve regeneration across smaller-to-medium-sized nerve gap defects [24]; however, for larger gap defects, traditional prostheses consistently failed to maintain sustainable nerve regeneration and functional outcomes remained generally poor [25]. Although it is evident that nerve prostheses are being widely utilized to reconstruct nerve defects, no clinically available prostheses are known to promote nerve regeneration across larger gaps and guarantee full functional recovery; subsequently, this has made the reconstruction of larger nerve defects a significant clinical challenge.

Nerve regeneration has a multi-stage neural tissue repair process involving Wallerian degeneration, recruiting macrophages for sweeping up the axonal degradation products (myelin debris), the formation of Bünger bands by Schwann cells for axonal guiding, and finally, the regeneration of axons [26,27]. Schwann cells (SCs) are the key player in this repair process as they provide neurotrophic support to regenerating fibers by secreting several neurotrophic factors, such as brain- and glial-derived neurotrophic factor [28]. Schwann cells are also involved in the myelination of the regenerating axons [29], which facilitates their pathway toward the distal end [30], thereby improving nerve regeneration [31]; and hence their presence appears to be dispensable for sustainable nerve regeneration over longer segmental defects [32]. Therefore, at the injury site, Schwann cells aids the onset of healthy nerve regeneration, resulting in enhanced axonal regeneration [31], which expedites the repair process [33], and ultimately helps to attain functional recovery comparable to nerve grafting that is better than repair performed using prostheses alone [13,34].

Recently, nerve prostheses manufactured in various structural variations to mimic a nerve’s native intracellular matrix structure to provide a native nerve-like environment to regenerating axons are becoming popular. The structural variations mostly involve texturing the internal walls of the prostheses, controlling the membrane porosity and mechanical stiffness, and building guidance channels in the lumen of the prostheses [35]. Interestingly these structural enhancements support a promising avenue for the adhesion and proliferation of SCs, which could accelerate the nerve regeneration process. However, apart from prosthesis design, the method of harvesting and refining SCs is also of prime importance. A repair might yield non-significant results if cultured SCs remained functionally inactive in a nerve prosthesis [16], which could result in local hypoxia in the early stages of axonal regeneration. This problem can be prevented by infusing SCs with oxygen-carrying hydrogels, such as fibrin-based hydrogels, to increase the sustained oxygen delivery to SCs [9]; however, despite the popularity of SCs, their harvesting remains invasive and difficult [7]. Moreover, the use of SCs has several ethical and clinical limitations, including the reduced proliferative capacity of SCs with no readily accessible source.

Advancement in the tissue engineering field has enabled researchers to transform stem cells sourced from adipose tissues, skin tissues, bone marrow, umbilical cord, etc., into cells having an SC-like phenotype and properties using less invasive procedures [36]. In particular, stem cells derived from non-neural tissue, such as mesenchymal stem cells (MSCs), have attracted great interest because
of their remarkable differentiation potential [37,38]. Stem cells, for instance, skin-derived stem cells (SdSCs) in a collagen prosthesis, have shown the ability to improve peripheral nerve regeneration in terms of an increased number of myelinated axons [11]. Similarly, bone-marrow-derived stem cells (BMdSCs) are known to be helpful in successfully bridging nerve gaps by creating an adequate and favorable environment for the growth and myelination of regenerating axons, as well as the enhanced differentiation of BMdSCs into SCs [23]. In another study, the efficacy of the BMdSCs was assessed using a prosthesis filled with autologous BMdSCs, where axonal regeneration (myelinated axon number, the diameter of the regenerated fibers, and muscle weight), and functional recovery (amplitude and conduction velocity) were found to be comparable to autografting [17]. Similarly, bone marrow mononuclear cells (BMMnCs) seeded in a chitosan prosthesis, besides bridging the nerve gap, facilitated in the myelination of regenerating axons and also helped to attain functional recovery comparable to autologous nerve grafting [18].

Like BMdSCs, adipose-derived stem cells (ADSCs) can also differentiate into cells with SC-like properties (have the myelin-forming ability), and are reported to proliferate better when mixed with poloxamer hydrogel and aided in reinnervating muscle tissue [8]. In another instance, a neural tissue membrane containing differentiated ADSCs in a NeuraWrap™ prosthesis supported robust neural regeneration of a rat sciatic nerve in terms of axonal density, the diameter of nerve fibers, and myelination compared with NeuraWrap™ without ADSC membranes [6]. The cumulative benefits that ADSCs impart include improved functional recovery and increased conservation of the muscle–mass ratio, nerve conduction velocity, and density of myelinated fibers [39].

Stem cells harvested from human umbilical cords and dental pulp can also promote neurite outgrowth [40,41], are more easily accessible than BMdSCs and ADSCs, and are reported to proliferate better than SCs [42]. Sciatic nerve repairs performed using these cells successfully stimulated regeneration and helped to improve functional recovery in dogs [19]. Differentiated human dental pulp stem cells (d-hDPSCs) in a NeuraWrap™ found to effectively increase neurite growth and the number of myelinated nerve fibers and blood vessels compared with the control group [7]. It has also been reported that amniotic fluid stem cells (AFSCs) possess the properties of mesenchymal and neural stem cells and can help to attain better functional recovery and a higher ratio of regenerated fibers than a control [43].

Mesenchymal stem cells (MSCs), mainly derived from bone marrow, are known to impart a therapeutic effect on tissue regeneration and modulate the immune system. They have attracted increasing research interest due to their possible use as support cells in nerve tissue engineering approaches [44]. In a comparative study, MSCs aligned within a collagen-based gel in a chitosan prosthesis demonstrated 90% regeneration compared to the repair performed using an SC-filled chitosan prosthesis [10]. A chitosan/poly(lactic-co-glycolic acid) (PLGA)-based prosthesis, when seeded with autologous MSCs, helped in bridging a segmental defect in a dog sciatic nerve, with the repair outcome being close to that of nerve autografting and better than that using the chitosan/PLGA-based scaffold alone [21]. Similarly, in another study, a chitosan/PLGA-based prosthesis filled with autologous bone marrow MSCs promoted regeneration of a median nerve in rhesus monkeys, where the recovery of nerve function in the treatment group was more efficient than that in the prosthesis-only group and comparable to autograft repair [20].

These findings suggest that adipose and skin tissues, as well as bone marrow, contain a pool of regenerative stem cells that can differentiate into cells with a Schwann-cell-like phenotype and properties, such as a myelin-forming ability; furthermore, they can be employed for repairing larger defects in peripheral nerves and may be of benefit for the treatment of peripheral nerve injuries. Table 1 reports studies on repairing critical-sized nerve defects ranging from 12 mm to 60 mm, highlights the delivery mode of cells, lists the methods adopted to access nerve regeneration, and presents research findings to show the effect of cell-based therapy on nerve regeneration. Each study in Table 1 was appraised and graded based on the following 11 selected key parameters;

a. Count of (i) regenerated axons, (ii) blood vessels, and (iii) FG-labeled motoneurons;
b. Measurement of (iv) axonal diameter, (v) myelin thickness, (vi) muscle weight ratio, (vii) compound muscle action potential, (viii) sciatic function index, (ix) nerve conduction velocity, (x) gap length, and (xi) follow-up time.

Each parameter was assigned a single point, except for gap length and follow-up time, which were given three points each, for a maximum possible total of fifteen points. A study was given one point if the repaired gap was 11 to 20 mm, two points for a gap of 21 to 40 mm, or three points for a gap greater than 40 mm. Similarly, a study was given one point if the follow-up time was up to four months, two points if the follow-up time was up to eight months, and three points if the follow-up time was greater than eight months. Studies with eight points or greater are marked with an asterisk sign in Table 1 and are of special interest because of their decisive role in crafting the conclusion of this manuscript.

4. Discussion and Perspective

Nerve prostheses made up of a variety of biomaterials and aided with either Schwann or stem cells derived from neural and non-neural tissues are becoming popular for repairing segmental defects in peripheral nerves. Table 1 highlights the use of a variety of cells in nerve prostheses for bridging critical-sized defects in peripheral nerves, along with the histological, electrophysiological, and functional assessment of nerve regeneration. The research findings confirmed that cell transplantation in a bioartificial prosthesis creates a favorable environment for achieving sustainable nerve regeneration and functional recovery across larger nerve defects, and has emerged as a novel therapeutic approach. Stem cells that are differentiated into cells having a Schwann-cell-like phenotype present a therapeutic modality and could be a potential replacement for nerve grafting. Axonal regeneration in the absence of a nerve prosthesis fails to completely bridge a nerve gap; however, a nerve may partially regenerate if a nerve prosthesis is used without any filler material (i.e., absence of stem cells or hydrogels). Conversely, cell transplantation in a prosthesis not only results in the complete bridging of a nerve gap but also helps to promote functional recovery.

The information presented in Table 1 was quantitively analyzed using GraphPad Prism 8.4.0 (GraphPad Software, San Diego, CA, USA) and is presented in Figure 2, which shows cell diversity and highlights the potential of various cells in bridging a wide range of nerve gaps. Figure 2a shows that SCs are a popular choice for cell therapy, where 33% of the studies employed them to promote nerve regeneration. Additionally, bone-marrow-derived mesenchymal cells also remained a center of interest, most likely because these cells—passing through pluripotency along neuronal cell lineages—proceed toward an SCs phenotype [4] that is chemically positive to SCs detection markers; they also account for 33% of the total of the studies. MSCs, besides mimicking SCs phenotypes, also contribute to neurogenesis by inducing the secretion of different neurotrophic factors, either directly from local precursors or indirectly from nearby activated astrocytes [45]. ADSCs were used by 10% of the studies examined, and stem cells procured from less popular sources each accounted for less than 5% of the studies examined. Figure 2b highlights the types of cells employed in different gap length repairs. SCs were used for repairing gaps with an average gap length up to 20 mm; interestingly, bone-marrow-derived MSCs have been employed for bridging almost double the nerve gap lengths compared to SCs.

An important aspect of this analysis is that it highlighted the use of stem cells differentiated into SCs in hydrogels for cell transplantation. The harvested cells can be directly implanted in their less differentiated state; however, the findings stress the in vitro treatment of these cells to transform them into SC-like cells before implantation. Although a mere 5% of studies (one study) reported that there is no significant difference in the repair outcome and both types of cells yield similar results, most of the studies, about 62% (13 studies), urged using differentiated stem cells for achieving significantly superior results. Similarly, cell suspension in a hydrogel improves the proliferation of the induced cells since the hydrogel becomes a source of long-time delivery of growth factors and neurotrophic nutrients that these cells secrete [46]. Here, it is also worth noting that cell suspensions with other
biomaterials, such as collagen or gelatine, do not yield positive outcomes [5], and hence, induced cells must be mixed with appropriate hydrogels [16]. The death of transplanted cells in nerve prostheses could severely hamper the therapeutic effect of a repair [47] because the greater the quantity of these cells in the nerve prostheses, the higher the beneficial effect is [12]. However, this statement is contrary to the findings of study [5], (#3 in Table 1) and hence more studies on how the density of stem cells affects the quality of nerve regeneration are required.

Nerve repair using an empty prosthesis alone may assist axonal regeneration to some extent; however, this does not necessarily result in adequate functional outcomes [48]. Medium-sized nerve gaps can heal without cell therapy using hydrogels alone [5], but larger gaps always require cell-based treatment. Based on the studies reported here, cell-mediated nerve repair significantly helps to promote nerve regeneration over considerably longer distances. Cell therapy also provides the neurotrophins and extracellular matrix proteins required for nerve growth and myelination [49], and therefore, it represents a tool for future cell therapy applications in peripheral nerve regeneration by stimulating the proliferation of stem cells into SCs; however, the underlying mechanism remains elusive to date and the synergistic effects of stem cells need further exploration.

The repair outcome and recovery period depend on many factors, such as the repair quality, gap length, prosthesis material, and the quality (health) of the cells. An early sign of axonal regeneration and functional recovery, as measured by axonal counting and nerve conduction velocity, can be observed around the eighth week post repair; however, a period of twelve months is required to achieve functional recovery comparable to an autograft repair. This implies that a nerve prosthesis must be designed in such a way that the mechanical stiffness of its structural framework must support nerve regeneration up to twelve months without degrading or degenerating. A nerve prosthesis can be designed using any combination of synthetic biocompatible materials; however, the analysis advocates the use of collagen and chitosan as both materials significantly dwarf the competition. Thirty percent of the total number of studies reportedly used collagen for fabricating neural prostheses, and almost equal numbers reported using chitosan reinforced with PLGA and silk fibers.

5. Conclusions

The quality of the nerve repair performed using SCs can be approximated by using differentiated stem cells, which can boost nerve regeneration compared to the repair performed using a prosthesis

![Figure 2.](image)
alone. Mesenchymal stem cells, especially bone marrow-derived stem cells, are speculated to yield the most promising results and hence should be used in the treatment of larger defects in peripheral nerves. However, the results are less successful than autologous nerve grafting, the gold standard of nerve repair, which implies that cell therapy can assist nerve regeneration but it cannot replace nerve grafting altogether. It is noteworthy that a few studies have also reported that cell therapy is beneficial only for boosting nerve regeneration at the very beginning of the repair process and it does not necessarily make any difference afterward [50]. Hence, the possibility of stem cells inducing a transient positive effect on nerve regeneration should not be overlooked. However, Brown et al. argued that this occurs only for a gap of less than 30 mm, and the gap length should be greater than 30 mm to observe any significant difference compared to a control [16].

An evident drawback of the studies reported herein is that 52% of the studies employed a rat nerve injury model to observe cells in promoting nerve regeneration, even though a rat model is generally a poor model for the repair of human critical gap defects due to its small size and species-specific neurobiological regenerative profile [51]. In contrast, the trend of using larger mammals remained consistently low, possibly due to operational difficulties, post-operative housing/care, and financial and ethical restrictions. To this end, 38% of the studies reported the use of: dogs (five studies), monkeys (two studies), or goats (one study). Furthermore, it should also be acknowledged that the outcomes of the reported studies were never translated into clinical trials, which unfortunately puts a stern question mark on the current efficacy of this repair approach.

Nonetheless, cell-based therapy possesses tremendous potential for improving nerve regeneration; however, the limited availability of neural-based stem cells makes it highly challenging. Stem cells procured from other sources, such as bone marrow and adipose tissues, require a complex process of cell harvesting and adaption, which limits the reliability of this technique. Cell death soon after transplantation or their limited life span may also limit their efficacy; however, as this work is still in its infancy, the future of cell-based therapy on neural regeneration is promising.

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