INVITED REVIEW

The role of undifferentiated adipose-derived stem cells in peripheral nerve repair

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Abstract

Peripheral nerve injuries impose significant health and economic consequences, yet no surgical repair can deliver a complete recovery of sensory or motor function. Traditional methods of repair are less than ideal: direct coaptation can only be performed when tension-free repair is possible, and transplantation of nerve autograft can cause donor-site morbidity and neuroma formation. Cell-based therapy delivered *via* nerve conduits has thus been explored as an alternative method of nerve repair in recent years. Stem cells are promising sources of the regenerative core material in a nerve conduit because stem cells are multipotent in function, abundant in supply, and more accessible than the myelinating Schwann cells. Among different types of stem cells, undifferentiated adipose-derived stem cell (uASC), which can be processed from adipose tissue in less than two hours, is a promising yet underexplored cell type. Studies of uASC have emerged in the past decade and have shown that autologous uASCs are non-immunogenic, easy to access, abundant in supply, and efficacious at promoting nerve regeneration. Two theories have been proposed as the primary regenerative mechanisms of uASC: *in situ* trans-differentiation towards Schwann cells, and secretion of trophic and anti-inflammatory factors. Future studies need to fully elucidate the mechanisms, side effects, and efficacy of uASC-based nerve regeneration so that uASCs can be utilized in clinical settings.

Key Words: *peripheral nerve injury; adipose-derived stem cells; Schwann cells; cell therapy; nerve conduits; axonal regeneration; stem cell differentiation; neurotrophic factors; anti-apoptosis; immunosuppression*

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Review Structure

The research of undifferentiated adipose-derived stem cells (uASCs) has emerged in the past decade, showing that these stem cells are efficacious at aiding peripheral nerve regeneration in a timely manner. uASCs have promising clinical utility because they can be accessed, processed, and ready-to-be deployed in a matter of hours. However, their efficacy and mechanisms in regenerating peripheral nerves are not fully understood. We therefore intended this review to serve a progress update of the field's current understanding of uASCs in peripheral nerve repair.

After illustrating the Seddon and Sunderland classification of peripheral nerve injury, we reviewed pathophysiology of peripheral nerve injury at different anatomical locations: injury site, distal stump, proximal stump, neuronal cell body, and end organ. What follows is a short introduction of stem cell-based therapy for peripheral nerve repair. Then, we summarized, both with a table and with narration, the findings from 39 original studies published in the past decade on the efficacy of uASCs. Lastly, we reviewed several possible mechanisms through which uASCs promote peripheral nerve repair.

By reviewing the recent studies, we concluded that uASCs were efficacious at aiding peripheral nerve repair through mechanisms that were still unclear. Among the different theories, the secretion of neurotrophic, neuroprotective, and anti-inflammatory factors appears to be the most likely mechanism through which uASCs exerted their impact. We suggest future research fully elucidate the mechanisms, side effects, and efficacy of uASC-based nerve regeneration.

Introduction

Nerve fibers in the peripheral nervous system, which relay sensory and motor information between the brain, the spinal cord and the rest of the body, regenerate more readily than nerve fibers in the central nervous system (Ide, 1996). However, surgical intervention is often if not always needed after severe peripheral nerve injuries secondary to motor vehicle accidents, penetrating traumas, gunshot wounds, and failing injuries (Kouyoumdjian, 2006; Campbell, 2008). Traditional methods of repair include direct coaptation of the proximal and distal stumps of a severed nerve and transplantation of nerve autografts using patient's own cutaneous nerves (Lee and Wolfe, 2000). However, these techniques are less than ideal: direct coaptation is only beneficial when tension-free repair is achievable while nerve autografts run the risk of creating sensory deficits and even neuroma at the donor site (Grinsell et al., 2014). Therefore, nerve conduits, which are made up of biological, synthetic, or tissue-engineered materials, have been explored as an alternative (Carriel et al., 2014). Moreover, nerve conduits that have been pre-seeded with aligned structures, growth factors, or viable cells render better clinical outcome than empty conduits (Gu et al., 2011; Daly et al., 2012; Lin and Marra, 2012; Carriel et al., 2013). Autologous adipose-derived stem cell (ASC), which is a type of precursor cell obtained and processed from patient's own adipose tissue, is a promising source of core material in nerve conduits because autologous ASCs are easily accessible, multipotent, and non-immunogenic (Mizuno, 2009; Klein et al., 2016). These ASCs can be differentiated towards

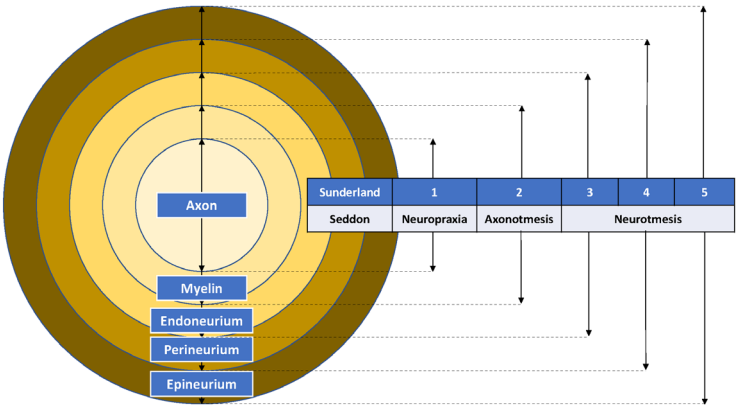

Figure 1 Seddon and Sunderland classification of peripheral nerve injury.

a Schwann cell-like phenotype to promote regeneration (Jiang et al., 2008). However, such differentiation is time- and cost-intensive, yielding suboptimal outcomes when urgent nerve repair is called for. Here we review the efficacy and mechanism of autologous undifferentiated ASCs (uASCs) in promoting peripheral nerve regeneration.

Classification of Peripheral Nerve Injury

Seddon (Seddon, 1943) and Sunderland (1990) have developed the widely accepted classification schemes for peripheral nerve injuries (Rosen, 1981; Burnett and Zager, 2004). Neuropraxia (Seddon) or first-degree injury (Sunderland) is the mildest form of nerve injury, which disrupts the surrounding myelin and causes transient functional block but preserves nerve continuity. Axonotmesis (Seddon) or second-degree injury (Sunderland) crushes both the axon and the myelin but spares the surrounding mesenchymal structures including the endoneurium, perineurium, and epineurium. Axonotmesis has a good prognosis because axons can regenerate along the uninjured mesenchymal structures and reinnervate the target organ (Burnett and Zager, 2004). Neurotmesis (Seddon) is the complete severance of nervous continuity, cutting the nerve into a proximal and a distal stump. This is the most severe form of nerve injury and its prognosis is poor without surgical intervention.(Burnett and Zager, 2004) Sunderland further stratified neurotmesis into third, fourth, and fifth-degree injuries, based on the involvement of mesenchymal structures (**Figure 1**). Most research models are based on neurotmesis or fifth-degree injury, because complete laceration of a nerve is more reproducible than lesser degrees of injury (Derby et al., 1993; Fine et al., 2002; Burnett and Zager, 2004).

Pathophysiology of Peripheral Nerve Injury

Here we review the site-specific degenerative and regenerative processes following a neurotmesis or fifth-degree injury in the following order: injury site, distal stump, proximal stump, cell body, and end organ. Similar processes are reviewed by Terenghi (Terenghi, 1999) and Burnett and Zager (Burnett and Zager, 2004).

Injury site

After a complete severance of a nerve, the two cut ends will retract and capillary permeability will increase (Madura, 2012). The cellular environment at the injury site is edematous and messy, featuring capillaries, fibroblasts, collagen fibers, macrophages, and Schwann cells (Burnett and Zager, 2004). Burnett noted that degeneration has to occur before regeneration because such an environment is not conducive to healing (Burnett and Zager, 2004).

Distal stump

Within the first few days of injury, Wallerian degeneration takes place at the distal stump where axons degenerate and the myelin sheath detaches and degrades. Macrophages are recruited to phagocytose debris and to activate Schwann cells, which have two roles – assisting phagocytosis and, later on, guiding regenerating axons (Jessen and Mirsky, 2016). In regeneration, Schwann cells proliferate to form the bands of Büngner and secrete neurotrophic factors that travel retrogradely to guide regenerating axons (Frostick et al., 1998)

Proximal stump

After the injury, Wallerian degeneration also takes place in a retrograde fashion up to the first node of Ranvier at the proximal stump (Burnett and Zager, 2004). Within a few hours, however, neuronal sprouts are formed with terminal growth cones searching for neurotrophic factors secreted from the distal stump (Li et al., 2005). When the regenerating axons successfully reach the matrix of the distal stump, they will grow within the bands of Büngner formed by Schwann cells (Bunge, 1994).

Cell body

In a severe nerve injury, the cell body might be damaged even though the injury is distal in the axon. Histologically, the stressed cell body will undergo a characteristic process called chromatolysis, in which the cell body swells, its nucleus migrates to the periphery, and the Nissl bodies, the neuronal protein production sites, break up and disperse (Evans, 2001; Burnett and Zager, 2004). Approximately 40% of involved dorsal root ganglions undergo retrograde cell death after peripheral nerve injuries, primarily when there is deficient target-derived neurotrophic support (Schmidt and Leach, 2003; Hart et al., 2004; Hall, 2005).

End organ

The end organ involved in a peripheral nerve injury is often somatic muscle of the upper or the lower extremities. In a retrospective study of 456 peripheral nerve injuries, Kouyoumdjian (2006) reported that 73.5% injuries happened to

the upper limbs while 21.5% to the lower limbs, leaving 5% injuries to the face. After prolonged denervation, muscles fibers will decrease in numbers, cross-sectional area, and force, only recovering partially after reinnervation (Gutmann and Young, 1944; Fu and Gordon, 1995; Rosen, 1981; Burnett and Zager, 2004).

Cell-Based Therapy for Peripheral Nerve Repair

As noted above, Schwann cells play important roles in peripheral nerve regeneration by clearing injury debris, secreting trophic factors, and guiding regenerating axons. Additionally, transplanted Schwann cells have been widely shown to enhance axonal regeneration after peripheral nerve injury (Guénard et al., 1992; Hadlock et al., 2000; Evans et al., 2002). However, Schwann cells are the less-than-ideal cell-therapy to repair peripheral nerves because they are difficult to harvest and time-consuming to expand in culture (Tohill and Terenghi, 2004). In search of a more suitable cell-therapy, researchers and clinicians have turned to stem cells, which have already been explored in many disease models, such as sickle cell anemia, Parkinson's-like syndrome, and graft-versus-host disease (Daley and Scadden, 2008). Among different types of stem cells, autologous ASC is the most clinically promising option for the following reasons (Mizuno, 2009):

1.ASCs are processed from patients' own adipose tissue, therefore they do not engender the ethical concerns often associated with embryonic stem cells (Lo and Parham, 2009);

2.Compared to the painful procurement of bone marrow-derived stem cells, ASCs can be harvested from adipose tissue obtained from the minimally invasive liposuction procedure under local anesthesia;

3.Adipose tissue has a higher stem cell yield than bone marrow does (Kern et al., 2006). One gram of adipose tissue can yield 3.5×10^5 to 1×10^6 stem cells, while one gram of bone marrow can only yield 500 to 5×10^4 stem cells (Tsuji, 2014).

ASCs are self-renewal and multipotent, capable of differentiating into mesodermal lineages such as bone, fat, cartilage, and muscle (Zuk, 2013). In peripheral nerve repair, Di Summa et al. (2010) demonstrated that fibrin conduits seeded with ASCs previously differentiated towards a Schwann cell-like phenotype induced greater axonal regeneration than empty conduits did. Although differentiated ASCs (dASCs) yield promising clinical outcome, the process of differentiation can take more than 2.5 weeks (Kingham et al., 2007; Di Summa et al., 2010) which would prolong denervation of the injured nerve and worsen functional recovery.

Efficacy of Autologous uASCs

In recent years, uASC has been explored as an accessible, abundant, multipotent, and efficient source of stem cells. The process of obtaining these stem cells is well documented in both animal experiments and clinical applications (Yoshimura et al., 2008; Sterodimas et al., 2010; Klein et al., 2016; Zhou et al., 2016) Adipose tissue is first harvested either by dissection (in animal models) or by liposuction (in humans) and is then enzymatically digested by collagenase in a buffered solution for 30–60 minutes at 37°C. The solution is filtered, and the infranatant is centrifuged to separate the stem cells from adipocytes and fluids. The cellular pellet is then rinsed and resuspended in a minimal essential medium with fetal bovine serum and antibiotics and is then passed through a mesh to remove debris. The hence obtained stromal vascular fraction, which consists of heterogeneous mesenchymal cells including progenitor cells (Bourin et al., 2013), can either be further expanded in culture for another seven days or can be directly mixed with a previously saved lipoaspirate fat graft and transplanted to the injury site. In urgent situations, the method that combines stromal vascular fraction with fat graft, or otherwise known as cell-assisted lipotransfer, takes no more than 90 minutes for cellular processing and 15 minutes for mixing (Yoshimura et al., 2008).

After searching PubMed (**Additional file 1**) for studies from 2008 to 2018, we identified 39 original articles that directly examined the efficacy of uASCs in aiding peripheral nerve repair (**Additional Table 1**). We summarize the key findings here.

1. Utilization of uASCs delivered significantly better results than the control groups, such as empty conduits, at promoting peripheral nerve regeneration. Such improvement was shown in different experiment models, such as sciatic nerve defect (Bloancă et al., 2017), facial nerve defect (Abbas et al., 2016), and cavernous nerve injury (Fandel et al., 2012). The only exception to this pattern of positive effect of uASCs was shown by Tomita et al. (2013) who demonstrated that uASCs did not significantly promote neurite outgrowth compared to the control NG108-15 neuronal cells.

2. Compared to Schwann cells or ASCs differentiated towards the Schwann cell phenotype, uASCs have been found to achieve either similar (Orbay et al., 2012; Watanabe et al., 2014; Sowa et al., 2016) or worse clinical outcomes (Tomita et al., 2013; Kappos et al., 2015).

Hundepool et al. (2014) and Mohammadi et al. (2011) have separately shown that uASCs have similar regenerative efficacy as do bone marrow stromal cells.

3. There still is a debate on the primary regenerative mechanism of uASCs. The two competing hypotheses are *in-situ* trans-differentiation (Kingham et al., 2007; Orbay et al., 2012; Abbas et al., 2016) and secretion of trophic factors (Santiago et al., 2009; Erba et al., 2010; Carlson et al., 2011; Marconi et al., 2012; Suganuma et al., 2013; Hsieh et al., 2016).

4. Farinazzo et al. (2015), Mohammadi et al. (2016), and Qiu et al. (2012) separately suggest that stromal vascular fraction, which is the rapid acquisition of uASCs from adipose tissue, has therapeutic potential in treatment settings.

Mechanism of uASCs in Aiding Peripheral Nerve Regeneration

Proximal and distal stumps: axonal regeneration

Erba et al. (2010) showed that uASCs could stimulate axonal growth from the proximal stump and even greater Schwann cell proliferation from the distal stump of an injured peripheral nerve. Schwann cells and stem cells that have differentiated towards a Schwann cell phenotype have been shown to promote nerve regeneration (Guénard et al., 1992; Dezawa et al., 2001), but how do the naïve, undifferentiated stem cells freshly harvested from adipose tissue promote axon regrowth? Having found no significant regenerative benefits uASCs, Tomita et al. (2013) argues that these stem cells need to trans-differentiate *in situ* towards a downstream

cell type, most likely Schwann cells, in order to promote axonal regeneration. Wei et al. (2010) showed that, after co-cultured with Schwann cells, ASCs could differentiate into Schwann-like cells, which suggests that Schwann cells at an injury site could induce trans-differentiation of ASCs. However, many studies argue that such trans-differentiation is unlikely. For example, Santiago et al. (2009), Carlson et al. (2011), Suganuma et al. (2013), and Hsieh et al. (2016) and their respective colleagues showed that markers of ASCs and their downstream lineages do not colocalize with markers of Schwann cells, usually S-100 protein, suggesting that those Schwann cells did not belong to the lineage of ASCs.

Besides trans-differentiation, the trophic effect mediated by secreted factors is the other contending explanation for the regenerative ability of uASCs. Salgado et al. (2010) provides an excellent summary of the various soluble factors produced from ASCs in various environments. Among them, glial-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), and insulin-like growth factor-I (IGF-I), nerve growth factor (NGF), and angiopoietin 1 (Ang-1) are the most relevant for nerve regeneration. For example, Shi et al. (2011) showed that intramuscular GDNF gene delivery improved myelination and functional recovery after constriction-induced nerve injury. Lopatina et al. (2011) showed ASCs could not stimulate nervous regrowth if BDNF neutralizing antibodies were introduced. Yamagishi et al. (2003) demonstrated that IGF-I prevented neuronal apoptosis by inhibiting the apoptotic p38-c-Jun pathway. Additionally, Anton et al. (1994) showed that antibodies to NGF strongly inhibited the otherwise robust migration of Schwann cells in denervated models. Besides directly secreting neurotrophic and neuroprotective factors, uASCs have also been shown to recruit and stimulate endogenous Schwann cells to aid regeneration (Hill et al., 2006; Erba et al., 2010; Marconi et al., 2012).

Neuron and end-organ: anti-apoptosis

Besides promoting axonal regrowth from the proximal and distal stumps, uASCs also have the potential to curb neuronal cell death retrogradely and muscular atrophy anterogradely (Reid et al., 2011; Fandel et al., 2012; Masgutov et al., 2016). Reid et al. (2011) showed that ASCs differentiated towards a Schwann cell phenotype significantly increased anti-apoptotic Bcl-2 mRNA expression and significantly decreased pro-apoptotic Bax and caspase-3 mRNA expressions compared to empty conduits. Furthermore, Reid et al. (2011) suggested that the anti-apoptotic property of dASCs were achieved by retrograde delivery of neurotrophic factors, which were shown to be increased in their study. While uASCs have not been shown to be anti-apoptotic, they do secrete neurotrophic factors similar to, although to a lesser extent than, those secreted by dASCs, such as BDNF, NGF, and GDNF (Tomita et al., 2013). In addition, Wei et al. (2009) demonstrated that uASCs secreted IGF-1 and BDNF, both of which are neuroprotective in brain hypoxic-ischemic injury. Therefore, it is likely that uASCs utilize a similar mechanism to prevent neuronal cell death in peripheral nerve injury.

uASCs also curb atrophy in denervated muscles as shown by Santiago et al. (2009). In a 6-mm sciatic nerve defect model, Santiago et al. (2009) measured the E/C ratio of experimental muscle mass to controlled muscle mass (uninjured leg) in four groups – no treatment (0.192 ± 0.024), nerve autograft (0.666 ± 0.070), conduit alone (0.487 ± 0.151), and conduit with uASCs (0.522 ± 0.108). The E/C ratio that is closer to 1 reflects better preservation of the muscle mass. Although the muscle preservative effect of the stem cells is not significant (P = 0.632), the group that received uASCs suffered less muscle atrophy than the group that received no treatment. Studies in different disease models also demonstrated the ability of ASCs to inhibit muscle atrophy. In a murine ischemic hindlimb model, Kang et al. (2010) showed that endothelial-differentiated ASCs promoted angiogenesis and myogenesis. In a burn injury model, Wu et al. (2015) demonstrated that uASCs significantly inhibited denervation atrophy of the gastrocnemius muscle and attenuated apoptotic death of burn injury-induced spinal cord ventral horn motor neurons. Taken together, it is evident that ASCs can curb denervation-induced muscular atrophy.

Regenerating environment: immunosuppression

The immunomodulatory effect of ASCs has been demonstrated in a wide range of disease models such as rheumatoid arthritis, graft-*versus*-host disease, and tissue repair (Yañez et al., 2006; Hong et al., 2010; Zhang et al., 2017). Similarly, in a sciatic nerve injury model, Marconi et al. (2012) noted that inflammatory infiltrates including both lymphocytes and macrophages have been reduced after uASCs were systemically delivered through intravenous administration. There are two main ways through which uASCs exert their immunomodulatory effects: boosting anti-inflammatory factors and reducing inflammatory ones. To enhance anti-inflammatory effect, ASCs 1) promote regulatory T cells, which trigger the alternative activation of macrophage towards the anti-inflammatory M2 phenotype (Gonzalez-Rey et al., 2010; Kawanishi et al., 2010; Guo et al., 2016; Bowles et al., 2017; Zhang et al., 2017), and 2) reduce the level of interleukin-10 (IL-10), which is a potent immunosuppressant *in vitro* (de Vries, 1995; Franchi et al., 2014; Lee et al., 2015; Zhang et al., 2017). Cui et al. (2007) also demonstrated that prostaglandin E2 (PGE2) might be the principal factor responsible for ASC-mediated immune suppression. When exposed to the pro-inflammatory environment of mixed lymphocyte reactions (MLRs), ASCs expressed significantly higher levels of PGE2, and subsequent PGE2-inhibitor counteracted the immunosuppression, thereby proving the pivotal role of PGE2 in immunomodulation. To downgrade inflammatory tone, ASCs 1) inhibit the proliferation and cytokine production of T cells in response to mitogens (Yañez et al., 2006; Gonzalez-Rey et al., 2010), and 2) decrease the production of inflammatory cytokines and growth factors, such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6) (Premaratne et al., 2011; Franchi et al., 2014; Lee et al., 2015; Guo et al., 2016; Zhang et al., 2017). Melief et al. (2013) also notes that ASCs demonstrate better immunomodulatory potency than bone marrow-derived stem cells, which are considered the prototypical mesenchymal stem cells.

Limitations of uASCs in Peripheral Nerve Regeneration

There are at least three concerns when using uASCs for peripheral nerve regeneration.

First and foremost, uASCs can potentially differentiate into

unwanted cell types of mesenchymal lineage or form teratomas. However, the risk of these processes is low. Differentiation of uASCs into specific mesenchymal lineages requires weeks of culturing using lineage-specific support medium (Banas et al., 2007). Santiago et al. (2009) has also shown that uASCs did not differentiate into Schwann cells after 12 weeks. The risk of teratoma formation is even lower, because uASCs only develop into cells of mesodermal lineages, while the composition of teratoma requires all three germ cell layers, namely ectoderm, mesoderm, and endoderm (Sun et al., 2009). Nonetheless, if uASCs were to be used clinically, future studies would still have to investigate the risk of spontaneous differentiation and teratoma formation.

Second, the regenerative potential of uASCs can be limited compared to that of Schwann cells or ASCs differentiated towards the Schwann cell phenotype (Tomita et al., 2013; Kappos et al., 2015). Although uASCs can be delivered in a time-efficient manner through stromal vascular fraction, the comparative efficacy of uASCs still needs to be studied.

Third, it is unclear whether the peripherally transplanted uASCs could affect far organs, such as the brain. Wei et al. (2009) and Marconi et al. (2013) have systemically injected uASCs to separately evaluate the effect of these stem cells on the brain and the peripheral nervous system. In both cases, the systemic delivery of uASCs has had favourable effect on their target organs. However, it is unclear whether the local transplantation of uASCs in conduits would have affected far organs.

Conclusion

Research in the past decade has demonstrated that uASCs are efficacious at promoting peripheral nerve repair, although the principal mechanism of repair is still under debate. Several possible mechanisms have been proposed, such as *in-situ* trans-differentiation towards Schwann cells, secretion of neurotrophic and neuroprotective factors, and immunosuppression. *In-situ* trans-differentiation seems the least likely explanation as several studies did not observe co-localization between Schwann cells and cells of ASC cell lineage. Secretion of soluble factors, whether anti-inflammatory, neurotrophic, or neuroprotective, seems to account for most of the regenerative ability of uASC. It is important to note that these stem cells are often transplanted at the injury site, therefore secreted factors must have been transported both retrogradely and anterogradely to deliver therapeutic benefits. However, future experiments should confirm the traveling course of these factors. From a clinical standpoint, the overwhelming advantage of autologous uASC lies in the fact that it can be harvested, processed, and ready-to-be-deployed in less than 2 hours through cell-assisted lipotransfer. In order to safely and effectively utilize uASCs in urgent peripheral nerve repair, future studies need to fully elucidate the mechanisms, side effects, and efficacy of uASC-based nerve regeneration.

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Conflicts of interest: *JMR is a board-certified plastic surgeon who specializes in reconstructive surgery, peripheral nerve surgery, microsurgery, and many other areas of plastic surgery. He has published and presented extensively on peripheral nerve repair, regenerative medicine, and telemedicine and cybercare. JMR holds a United States patent on a microelectronic axon processor that restores nerve function after severance.*
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Additional files:
Additional file 1: *PubMed search strategy.*
Additional Table 1: *In vitro and in vivo experiments that examined the efficacy of undifferentiated adipose-derived stem cells.*

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Additional Table 1 *In vitro* and *in vivo* experiments that examined the efficacy of undifferentiated adipose-derived stem cells.

Author	Model	Results
Abbas et al. (2016)	Facial nerve transection in rats	Compared to nerve graft alone, undifferentiated adipose-derived stem cells (uASCs) significantly enhanced the axonal regeneration in these aspects: motor performance, electromyographiy, immunohistochemistry, and histology. Differentiation towards a Schwall cell phenotype might be the main regenerative mechanism of uASCs.
Bloancă et al. (2017)	Sciatic nerve transection in rats	Compared to the control group, uASC-treated group stimulated glial fibrillary acidic protein (GFAP)-positive Schwann cell production and improved nerve regeneration. There is a temporal difference in nerve regeneration: Schwann cells are activated in the earlier rather than later stages.
Carlson et al. (2011)	Sciatic nerves of laminin-deficient mice (ablation of laminin γ1 arrests Schwann cell development)	Compared to stem cell growth media, uASC transplantation expressed laminin *in vitro* and *in vivo.* uASC-treatment caused endogenous Schwann cells to differentiate past the point of arrest. uASCs modify sciatic nerve function via trophic effects rather than transdifferentiation.
Carriel et al. (2017)	10-mm sciatic nerve defect in rats	Compared to empty conduit and conduit filled with hydrogels, conduit filled with hydrogels and uASCs had significantly higher expression of growth-associated protein (GAP-43) and neurofilament (NF). GAP-43 and NF were not co-expressed, suggesting their differential expression in newly formed nerve fascicles.
Cherubino et al. (2017)	Burning injury to sciatic nerve in mice	Compared to no treatment, processed adipose tissue (uASC) significantly reduced the formation of scar tissue after burning injuries. uASC performed similar to the carboxymethylcellulose and polyethylene oxide compound. uASCs can be used to prevent perineural adherence after injury.
Erba et al. (2010)	10-mm sciatic nerve defect in rats	Compared to conduit filled with growth factors, conduit filled with uASCs stimulated axonal outgrowth and Schwann cell proliferation. Regenerative effect of uASCs attributed to trophic factors and indirect stimulation of Schwann cells.
Fandel et al. (2012)	Cavernous nerve crush injury in rats	Compared to intracavernous injection of saline, intracavernous injection of uASCs resulted in significantly improved erectile function after cavernous nerve injury. uASCs exerted neuroregenerative effects on the cell bodies of injured nerves.
Farinazzo et al. (2015)	Neuronal cells exposed to oxidative stress; demyelinated cerebellar slice cultures	In both models, uASC-containing vesicles demonstrated *in vitro* neuroprotective and neuroregenerative effects. Stromal vascular factors (containing uASC) might have therapeutic potential.
Ghoreishian et al. (2013)	7-mm facial nerve defect in dogs	Compared to conduit filled with alginate, conduit filled with uASCs improved nerve conduction velocity and action potential, yet reduced nerve diameter. Schwann cell-differentiation may be warranted for better functional and histological results.
Hernández-Cortés et al. (2017)	10-mm sciatic nerve defect in rats	Compared to empty conduit, conduits filled with either ghrelin or uASCs improved the nerve area, the myelin area, and the number of myelinated fibers. However, neither significantly improved functional recovery.
Hsieh et al. (2016)	10-mm sciatic nerve defect in rats	uASC-seeded small conduits provided better functional recovery than did uASC-seeded large conduits. uASCs may have interacted with endogenous Schwann cells and released neurotrophic factors.
Hsueh et al. (2014)	10-mm sciatic nerve defect in rats	Compared to controls, chitosan conduit with uASCs significantly increased axonal density and axonal regeneration.
Hundepool et al. (2014)	Meta-analysis on peripheral nerve reconstruction using conduits	Compared to empty conduits, conduits with either uASC or differentiated adipose-derived stem cells (dASCs; towards a Schwann cell-like phenotype) significantly increased nerve conduction velocity, amplitude, and latency. Further, both ASCs achieved comparable results as bone marrow stem cells.
Kalbermatten et al. (2011)	Superficial and deep abdominal adipose tissue from patients	Superficial adipose-derived stem cells (ASCs) proliferated significantly faster than deep ASCs, but both layers expressed similar levels of neurotrophic factors.
Kappos et al. (2015)	10-mm sciatic nerve defect in rats	Rat dASCs performed significantly better than rat uASCs (less muscle atrophy, superior functional results). Human ASCs from superficial abdominal layer better promoted regeneration than did those from deep abdominal layer.
Klein et al. (2016)	10-mm sciatic nerve defect in rats	Compared to empty nerve conduits, conduits filled with uASCs significantly improved motor and sensory conduction velocity. uASCs also demonstrated more organized axon arrangement in conduit, with increased S100 immunoreactivity.
Lin et al. (2011)	Cavernous nerve injury in rats	Matrix seeded with uASCs provided better erectile functional recovery than did matrix alone or no treatment. However, such difference was not statistically significant ($P = 0.07$).
Luo et al. (2012)	50-mm sciatic nerve defect in dogs	Matrix seeded with both uASCs and transforming growth factor β-1 (TGFβ-1) showed significantly better myelin regeneration than did matrix seeded with uASCs alone or with TGFβ-1 alone. TGFβ-1 prevents ASCs from apoptosis by reducing inflammation and promoting vascular endothelial growth factor (VEGF)-dependent angiogenesis.
Marconi et al. (2012)	Sciatic nerve crush in mice	Compared to systemic injection of saline, systemic injection of human uASCs significantly accelerated functional recovery (significant improvement in fiber sprouting, reduction of inflammatory infiltrates).
Masgutov et al. (2016)	Sciatic nerve injury in rats	Compared to nerve grafting alone, nerve grafting complemented with human uASC transplantation expressed significantly higher levels of neurofilament-stained neurons, significantly higher activity of glial cells, and significantly lower number of caspase-9/apoptosis-prone neurons. Retrograde transport of neurotrophic factors from uASC is a possible mechanism.

Additional Table 1 Continueds.

Author	Model	Results
Mohammadi et al. (2016)	Sciatic nerve defect in rats	Nerve allograft with the addition of stromal vascular fraction (uASC) had better outcomes than did nerve allograft alone. Compared to differentiated and thus manipulated stem cells, stromal vascular fraction might require fewer Food and Drug Administration approval to be utilized in practice.
Mohammadi et al. (2013)	10-mm sciatic nerve defect in rats	Compared to conduit filled with saline, conduit filled with omental uASCs were superior in all aspects of nerve regeneration (recovery speed, muscle mass, and etc).
Mohammadi et al. (2011)	10-mm sciatic nerve defect in rats	Compared to graft filled with bone marrow stromal cells, graft filled with uASCs showed no significant difference in recovery of axons after surgery, showing that the efficacy of uASCs is similar to that of bone marrow stromal cells.
Orbay et al. (2012)	10-mm sciatic nerve defect in rats	Compared to other groups (conduit alone, conduit with collagen, resuturing), conduits with either uASCs or dASCs demonstrated similar, significant regenerative effect (number of vessels, myelin fiber density, number of myelin fibers). *In vitro* differentiation did not exhibit added regenerative benefit, suggesting uASCs might have undergone *in vivo* differentiation in addition to secreting growth factors.
Qiu et al. (2012)	Cavernous nerve crush injury in rats	Compared to saline injection, both immediate and delayed injections of uASCs resulted in significantly increased neuronal nitric oxide synthase, neurofilament, and muscle content. uASC-containing stromal vascular fraction might have therapeutic utility.
Santiago et al. (2009)	6-mm unilateral sciatic nerve defect in rats	Compared to empty conduit, conduit filled with uASCs slowed muscle atrophy (not significantly) but significantly increased nerve cross-sectional area. uASCs survived up to 12 weeks after transplantation and did not differentiate into Schwann cells.
Sowa et al. (2016)	5-mm sciatic nerve defect in rats	Compared to conduits filled with Schwann cells, conduits filled with uASCs achieved comparable outcomes. It is unclear whether ASCs differentiated into Schwann cells at the recipient sites.
Sowa et al. (2012)	Donor Schwann cells and dorsal root ganglion neurons	uASCs promoted peripheral nerve regeneration better than 3T3-L1 cells and comparable to schwann cell- and astrocyte-derived factors. Donor age and anatomic site of origin do not affect the therapeutic potential of uASC.
Sun et al. (2011)	8-mm facial nerve defect in rats	Compared to artery conduit alone, artery conduit filled with uASCs achieved significantly better functional improvement, greater axonal growth, and better reinnervation. However, nerve autografts were resulted in significantly better recovery outcomes than did uASCs.
Suganuma et al. (2013)	10-mm sciatic nerve defects in rats	Compared to conduit filled with saline, conduit filled with uASCs had significantly more axonal regeneration. In uASC, Schwann cell-phenotype not detected in study, while factors such as Neu-1 or VEGFA were, suggesting uASC promotes regeneration through humoral factor.
Tomita et al. (2013)	NG108-15 neuronal cells	dASCs and Schwann cells significantly promoted neurite outgrowth, while uASCs did not.
Tremp et al. (2015)	10-mm sciatic nerve defects in rats	Rat uASCs and rat dASCs significantly accelerated peripheral nerve regeneration. 3T MRI was used to monitor the regeneration axon front over time.
Watanabe et al. (2014)	7-mm facial nerve defect in rats	uASCs, dASCs, and Schwann cells demonstrated similar potential for nerve regeneration. uASCs secreted lower levels of trophic factors than dASCs.
Wei et al. (2011)	10-mm sciatic nerve injury in rats	Compared to scaffoled alone, scaffold loaded with uASCs showed significant reinnervation of target muscle. Compared to scaffold loaded with Schwann cells, scaffold loaded with uASCs similar nerve regeneration by observation.
Yang et al. (2015)	Cavernous nerve injury in rats	Compared to cryoinjury without treatment, cryoinjury with injection of uASC prevented erectile dysfunction, and promoted penile tissue recovery. uASC injection led to the increased level of neuronal nitric oxide synthase and decreased level of apoptotic marker caspase 3.
Ying et al. (2013)	Cavernous nerve crush injury in rats	Compared to the injury-no treatment group, uASC treatment significantly increased ratio of intracavernous pressure/mean arterial pressure, and the quantity of myelinated axons and nerve fibers.
You et al. (2015)	Cavernous nerve injury in rats	uASCs and dASCs are equally effective at recovering penile erection after cavernous injury. uASC was superior to dASC in smooth muscle/collagen ratio and endothelial cell content.
You et al. (2013)	Cavernous nerve injury in rats	Compared to sham surgery, periprostatic and intracavernosal delivery of uASCs are equally effective at recovering penile function. The combination treatment group did not show significant increase in smooth muscle content.
Zhang et al. (2011)	Cavernous nerve injury in rats	Compared to media of penile smooth muscle cell, media of uASC resulted in significantly higher neurite growth in rat major pelvic ganglia. Additionally, the neurotrophic cytokine CXCL5 is secreted 8 times higher in uASC media than in smooth muscle media.