Regenerative Biologic Therapies for Neuropathy

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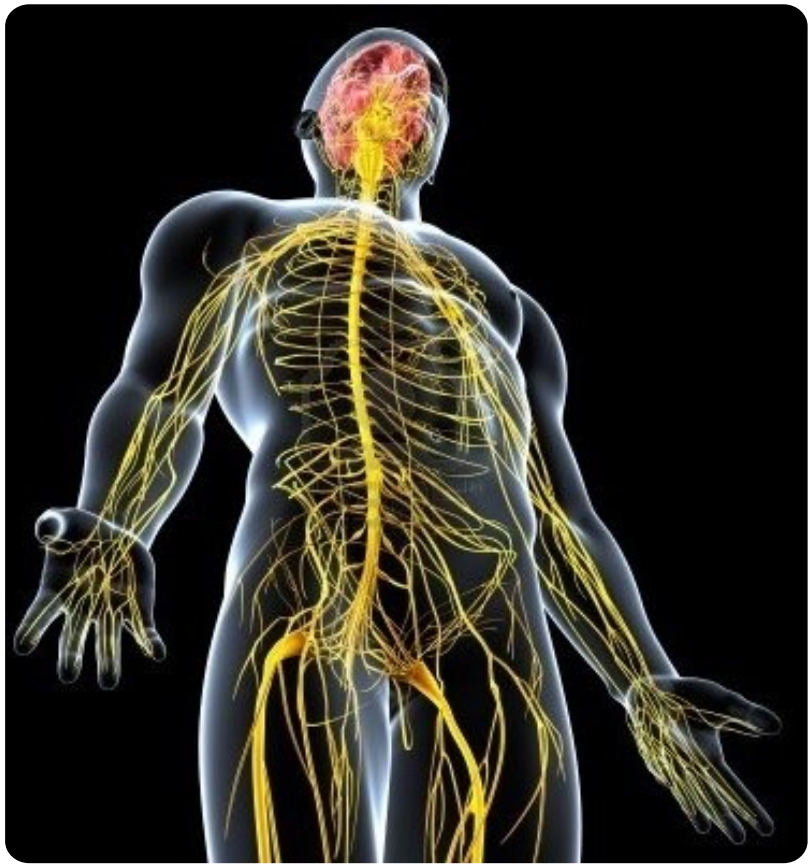
“I was told by my neurologist that I have neuropathy and that there is nothing I can do to fix it"

“I will have to be on gabapentin or Lyrica for the rest of my life”



Can We Treat Neuropathy With Regenerative Medicine?

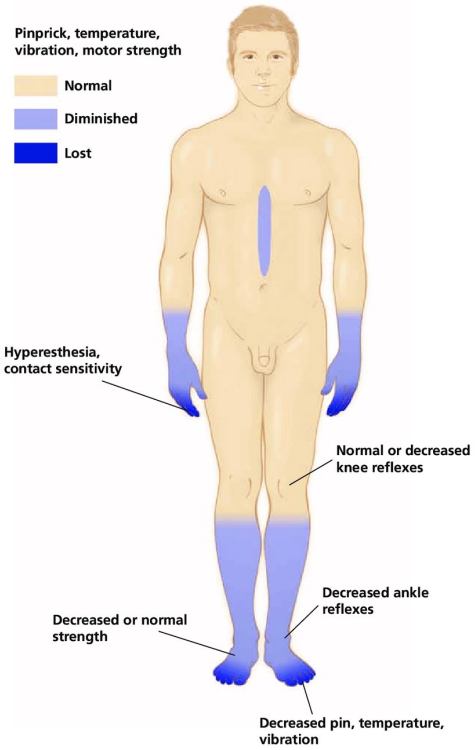
Objectives:

- Brief look at pathophysiology of polyneuropathy
- Where is polyneuropathy?
- Biochemical and mechanical lesions
- Regenerative medicine treatment opportunities

Neuropathy
Disease or dysfunction of peripheral nerve(s)

General causes of neuropathy:

- Metabolic (nutritional, DM, thyroid)
- Toxic (medication, alcohol, chemotherapy, environmental)
- Autoimmune and Infectious
- Mechanical (compression/entrapment, stretch injury, repetitive abrasion, traumatic or surgical - cut/torn)



Pathophysiology in Polyneuropathy

Functional and Structural changes to the nerves and supporting tissue

- Neurogenic inflammation
- Axon and nerve trunk edema (osmotic and inflammatory)
- Endoneural vasculopathy - ischemia
- Schwann cell dysfunction
- Impaired axonal transport
- Breakdown of axons and myelin
- Mitochondrial dysfunction

Gonçalves, Nádia P., et al. "Schwann cell interactions with axons and microvessels in diabetic neuropathy." *Nature Reviews Neurology* 13.3 (2017): 135-147.

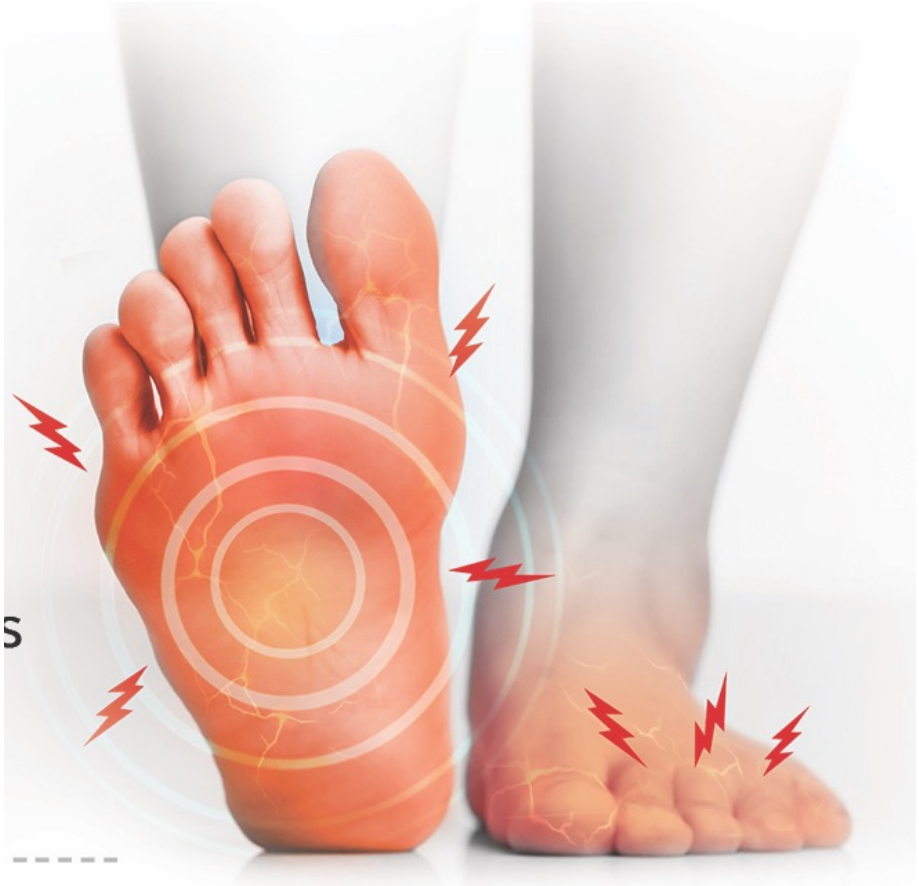
Neuropathy Symptoms

Positive Symptoms

- Pain (allodynia, hyperalgesia)
- Burning
- Cramps
- Cold sensation

Negative Symptoms (what is missing)

- Numbness
- Weakness
- Lack of proprioception (poor balance)



Dorsal Root Ganglia – Affected Neuropathy

- Cell bodies of sensory neurons found in the neuroforamina of the lumbar spine
- Involved in maintenance of neuropathic pain
- In Neuropathy - increase in inflammatory and neuroexcitatory cytokines in the DRG
- Severity of changes correlate with symptoms scores in DPN

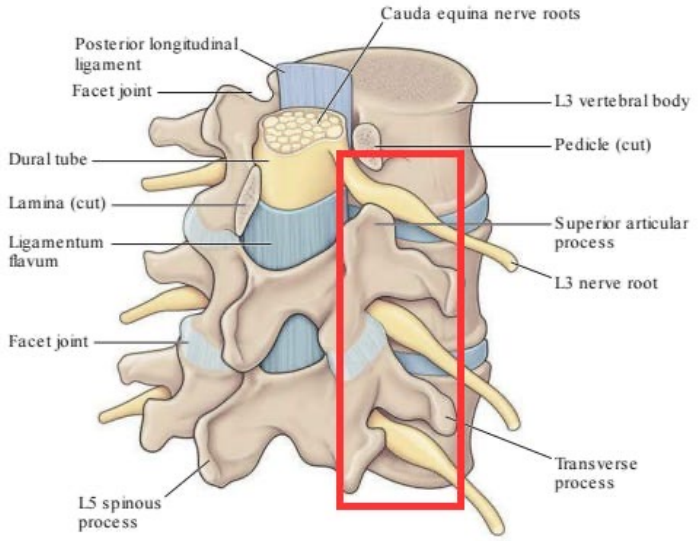


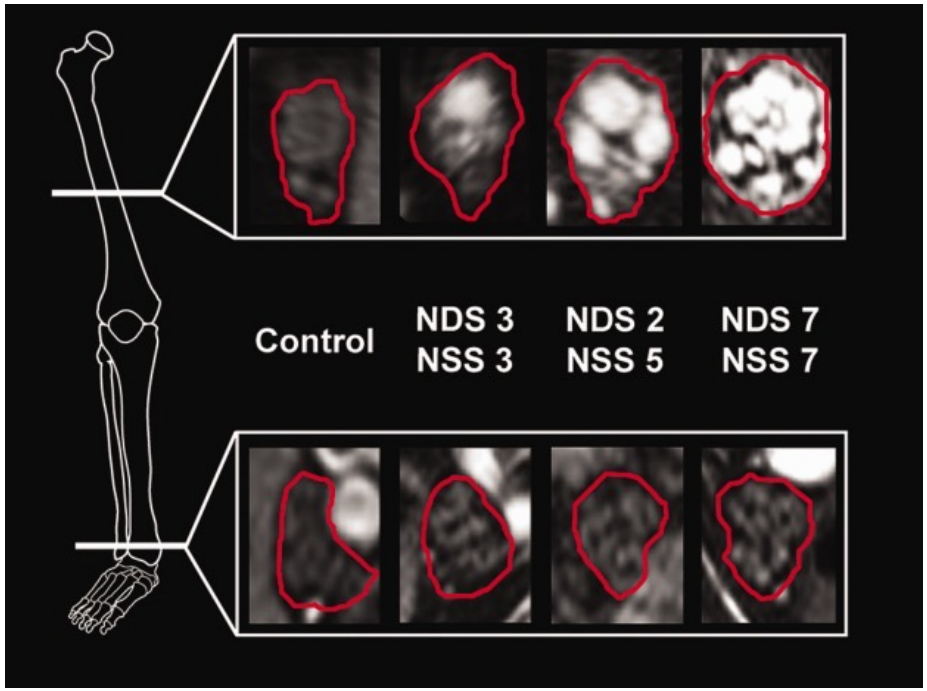
FIGURE 28-1 Structures of the lumbar spine.

Ahimsadasan, Nilah, et al. "Neuroanatomy, Dorsal Root Ganglion." *StatPearls [Internet]* (2024).

Jende, Johann ME, et al. "Diabetic polyneuropathy is associated with pathomorphological changes in human dorsal root ganglia: a study using 3T MR neurography." *Frontiers in Neuroscience* 14 (2020): 570744.

Proximal Lesions in DPN & ALN Neuropathy

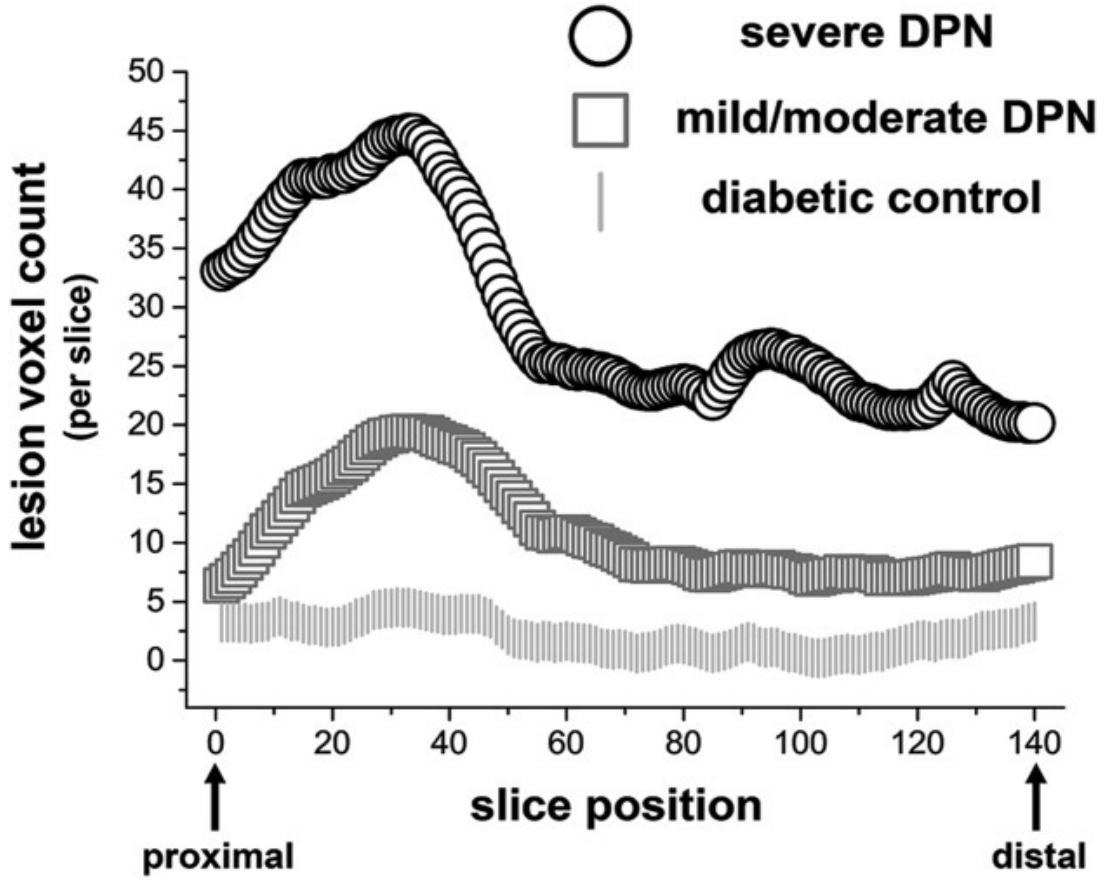
- MR neurography of the whole leg in DPN and ALN
- Microstructural nerve alterations predominent at thigh level
- CSA higher in DPN/ALN vs normal
- MRN findings + correlation to severity of symptoms



Pham, Mirko, et al. "Magnetic resonance neurography detects diabetic neuropathy early and with proximal predominance." *Annals of neurology* 78.6 (2015): 939-948.

Rother, Christian, et al. "Characterization and quantification of alcohol-related polyneuropathy by magnetic resonance neurography." *European Journal of Neurology* 29.2 (2022): 573-582.

Proximal Lesions in DPN & ALN Neuropathy



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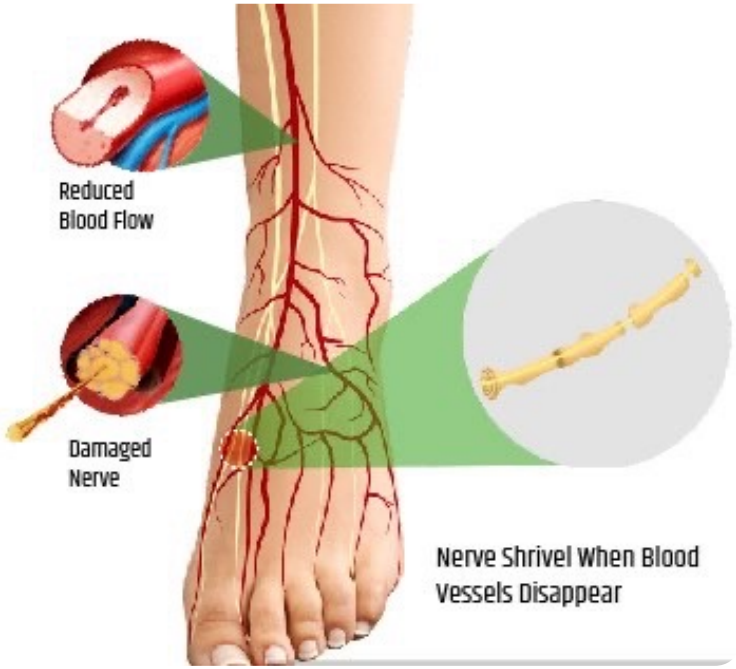


Distal Damage to Peripheral Nerves

- Endoneurial and perineural microangiopathy
- Vasculopathy of small nerves at the site of epidermis
- Hypoxic damage to the axons/myeline
- Altered axonalplasmic transport = distal nerve malnutrition
- Buildup of oxidative stress/inflammatory molecules = directly toxic to the axons, myeline and mitochondria
- Decreased Schwann cell activity
- Distal entrapments ??

Yagihashi, Soroku, Hiroki Mizukami, and Kazuhiro Sugimoto. "Mechanism of diabetic neuropathy: where are we now and where to go?." *Journal of diabetes investigation* 2.1 (2011): 18-32.

Biologics Used For Neuropathy

Autologous

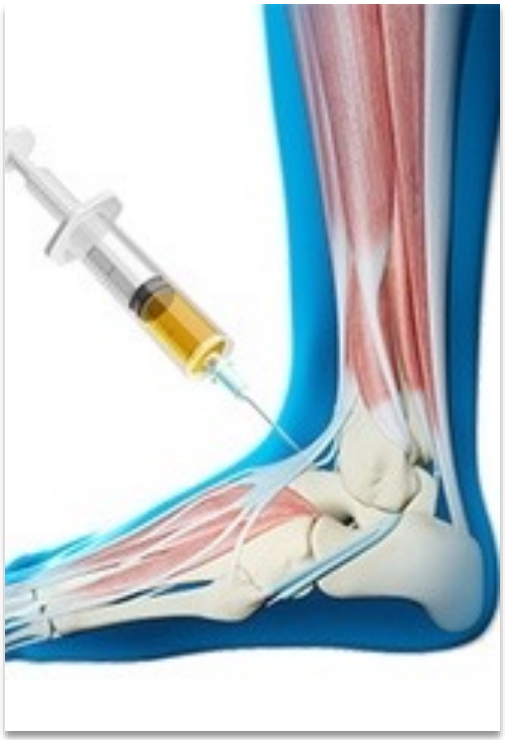
- Blood Products: PRP
- Bone Marrow Stem Cells
- Adipose Derived Stem Cells

Allogenic

- Birth products (Umbilical Cord Stem Cells/ Amnionic Fluid)
- Exosomes

PRP: The Perfect Agent to Heal Nerves

- Decreases nerve inflammation
- Decreases nerve swelling (decreases CSA)
- Stimulates Schwan Cells (migration, activation to release NGF)
- Stimulates Axon and Myelin Regeneration
- Stimulates Angiogenesis (Neural and Perineural)



- Sánchez, Mikel, et al. "Platelet-rich plasma, a source of autologous growth factors and biomimetic scaffold for peripheral nerve regeneration." *Expert opinion on biological therapy* 17.2 (2017): 197-212.
- - Zheng, Canbin, et al. "Effect of platelet-rich plasma (PRP) concentration on proliferation, neurotrophic function and migration of Schwann cells in vitro." *Journal of Tissue Engineering and Regenerative Medicine* 10.5 (2016): 428-436-Bir,
- Shyamal Chandra, et al. "Therapeutic treatment with sustained-release platelet-rich plasma restores blood perfusion by augmenting ischemia-induced angiogenesis and arteriogenesis in diabetic mice." *Journal of vascular research*48.3 (2011): 195-205.

Proliferation and Neurotrophic Expression of Schwann Cells Treated w PRP

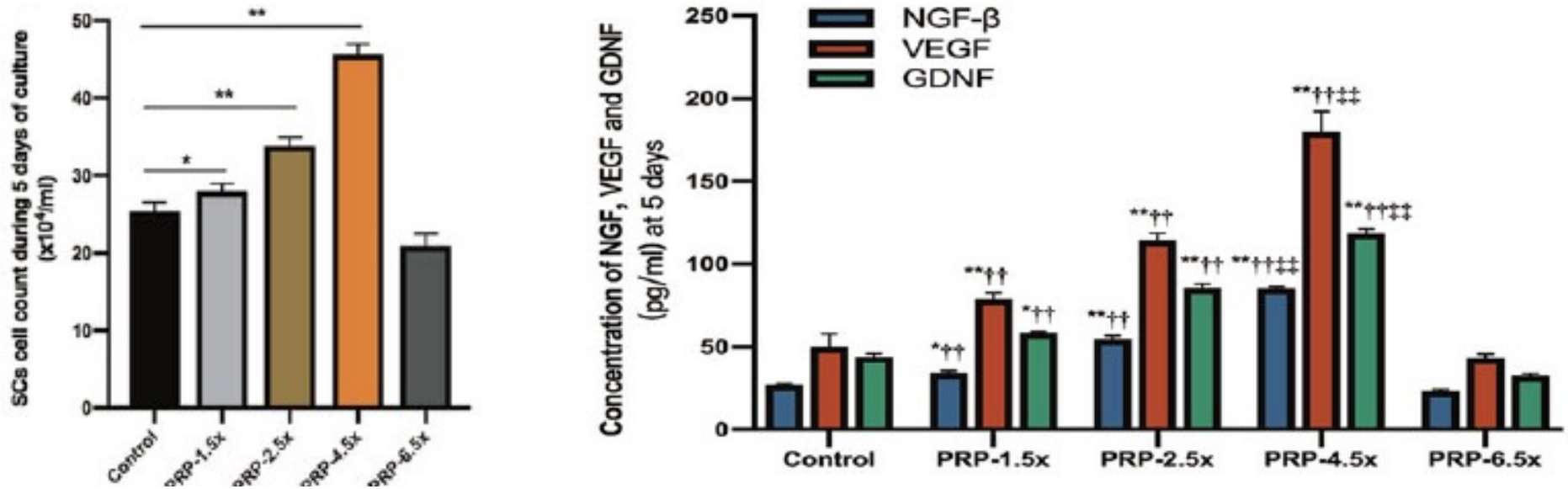
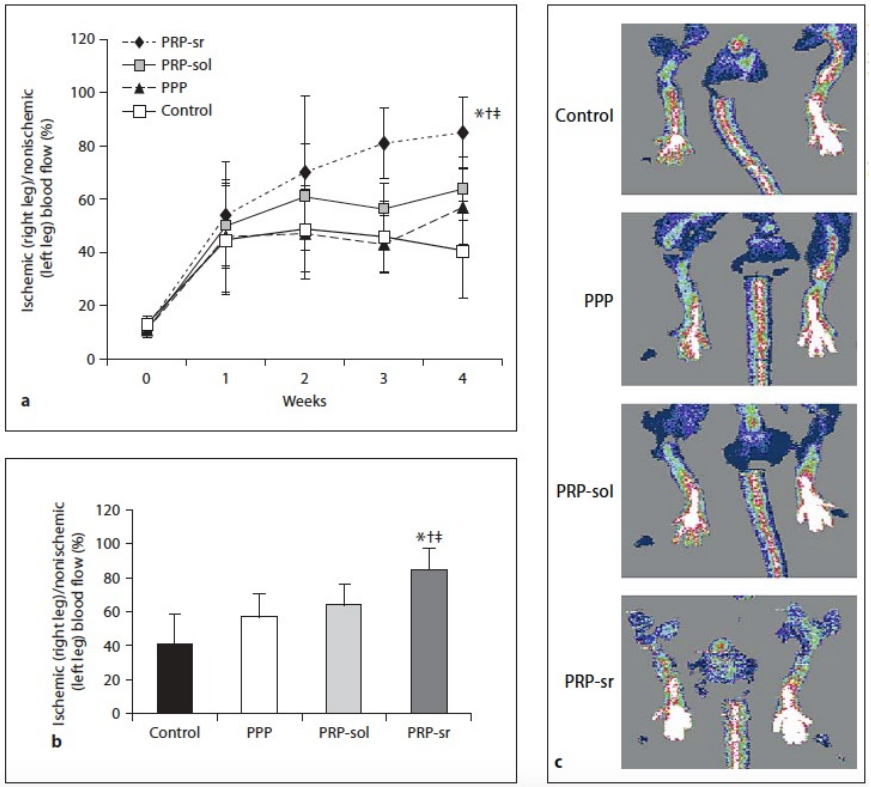


Fig. 1 The proliferation and neurotrophin expression of SCs treated with PRP. a Immunostaining of harvested SCs. **b** SCs exposed to various PRP concentrations during 5 days of culture. **c, d** displayed the SCs count during 3 days and 5 days of culture ($^{*}P < 0.05$, $^{**}P < 0.01$). **e** CCK-8 colorimetric assay was performed to evaluate the effect of PRP in various concentration on the proliferation of SCs ($^{**}P < 0.01$ vs. control group; $^{\dagger\dagger}P < 0.01$ vs. PRP-6.5× group; $^{\ddagger\ddagger}P < 0.05$ vs. other five groups). **f** The NGF-β, VEGF and GDNF secreted by SCs that cultured with PRP at different concentrations were measured by ELISA at 5 days of culture ($^{*}P < 0.05$ $^{**}P < 0.01$ vs. control group; $^{\dagger\dagger}P < 0.01$ vs. PRP-6.5× group; $^{\ddagger\ddagger}P < 0.05$ vs. other five groups). Error bars = SD, $n = 3$. SCs Schwann cells, PRP platelet-rich plasma, CCK-8 Cell Counting Kit-8.

Zhu, Yaqiong, et al. "Ultrasound-guided platelet-rich plasma injection and multimodality ultrasound examination of peripheral nerve crush injury." *Npj Regenerative Medicine* 5.1 (2020): 21.

PRP Stimulates Angiogenesis



DM mice with induced severe limb ischemia (removal of iliac, saphenous and femoral arteries/veins)

a. Time course of the ischemic /nonischemic blood perfusion ratio (%)

b. Ischemic/nonischemic blood perfusion ratio in the different
groups at 4 weeks

c. Laser Doppler Perfusion Imaging in the different groups.

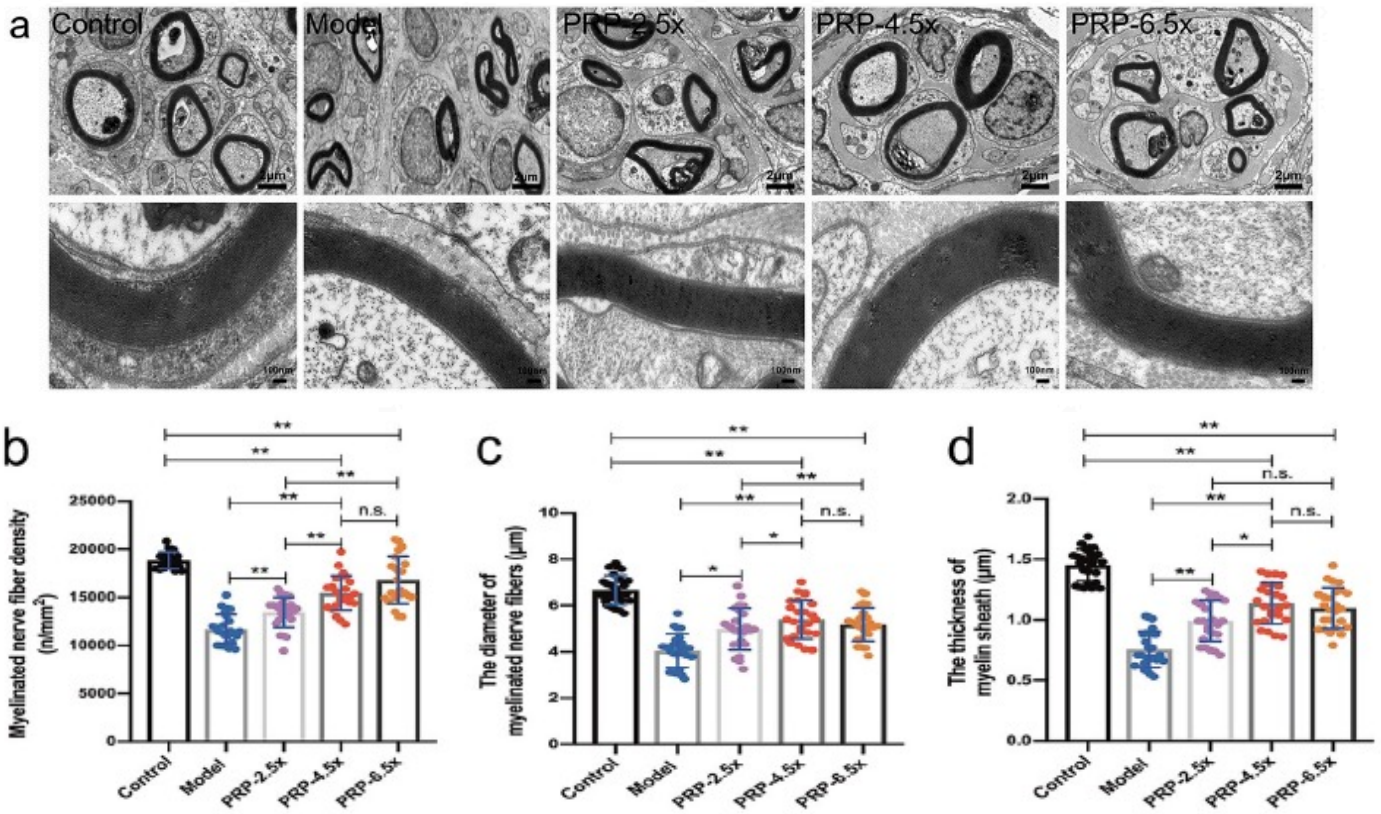
PRP increased capillary density and mature vessel density

Bir, Shyamal Chandra, et al. "Therapeutic treatment with sustained-release platelet-rich plasma restores blood perfusion by augmenting ischemia-induced angiogenesis and arteriogenesis in diabetic mice." *Journal of vascular research*48.3 (2011): 195-205.

PRP Improves Re-Myelination of The Injured Nerve

At 12 weeks, regenerated nerves were evaluated with an electron microscope

a. Electron micrograph of regenerated nerves
b. Mean myelinated nerve fiber density
c. Mean diameter of myelinated nerve fiber
d. Mean thickness of the myeline sheath

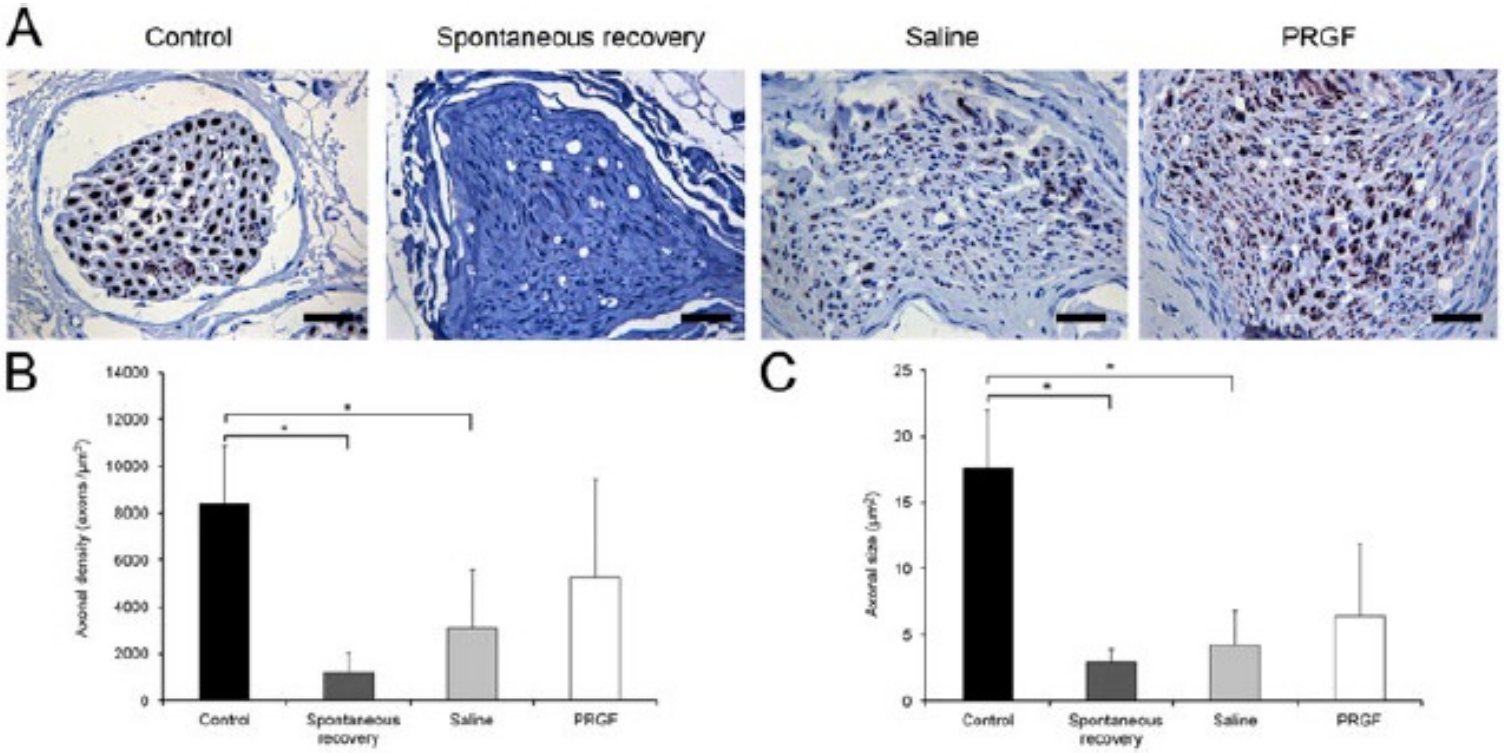


Zhu, Yaqiong, et al. "Ultrasound-guided platelet-rich plasma injection and multimodality ultrasound examination of peripheral nerve crush injury." *Npj Regenerative Medicine* 5.1 (2020): 21.

PRGF Improves Axonal Regeneration

In the distal portion of the common peroneal nerve

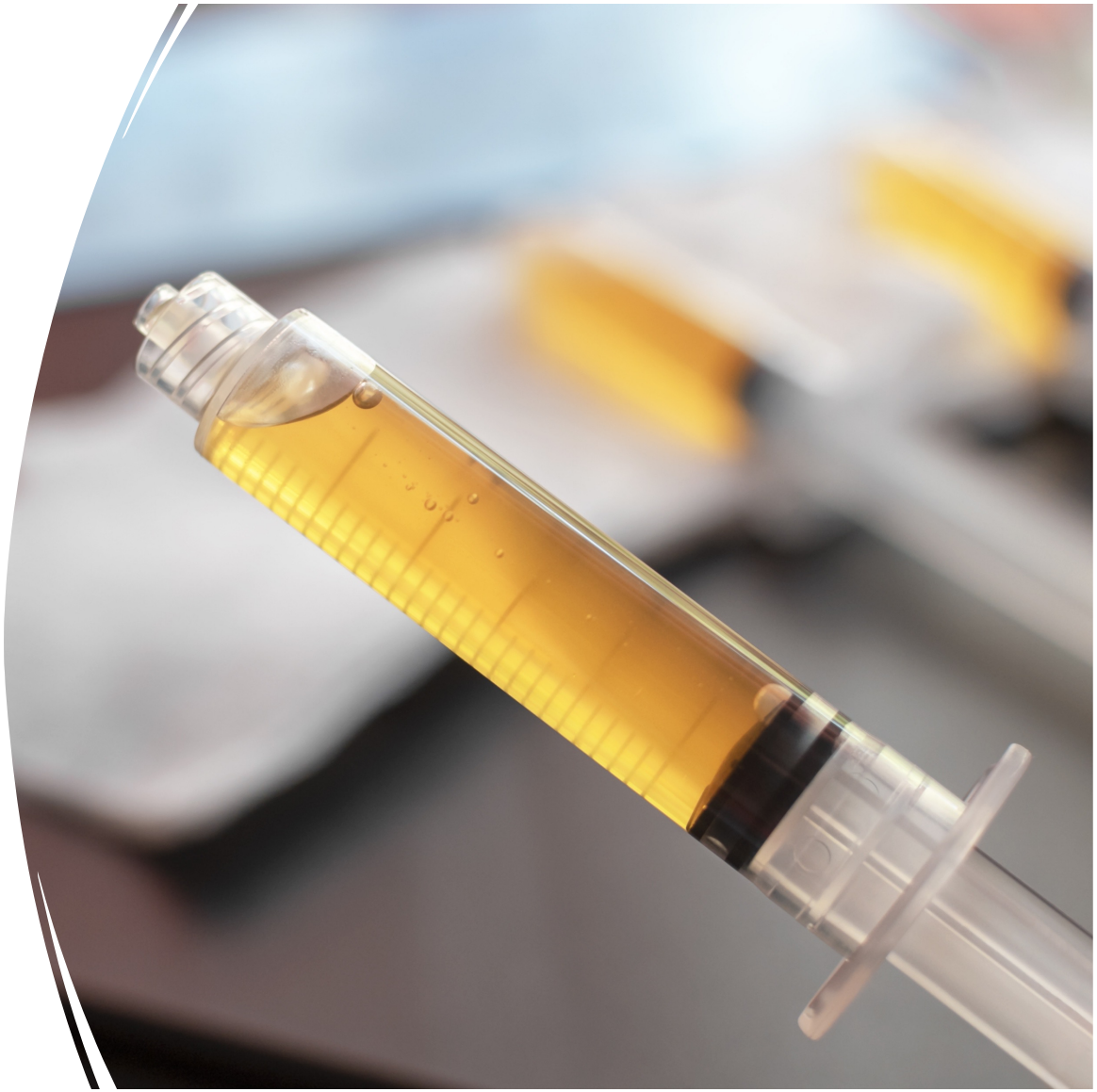
PRGF improved:
- Axon density (B)
- Axon size (C)



Sánchez, Mikel, et al. "Ultrasound-guided plasma rich in growth factors injections and scaffolds hasten motor nerve functional recovery in an ovine model of nerve crush injury." *Journal of tissue engineering and regenerative medicine* 11.5 (2017): 1619-1629.

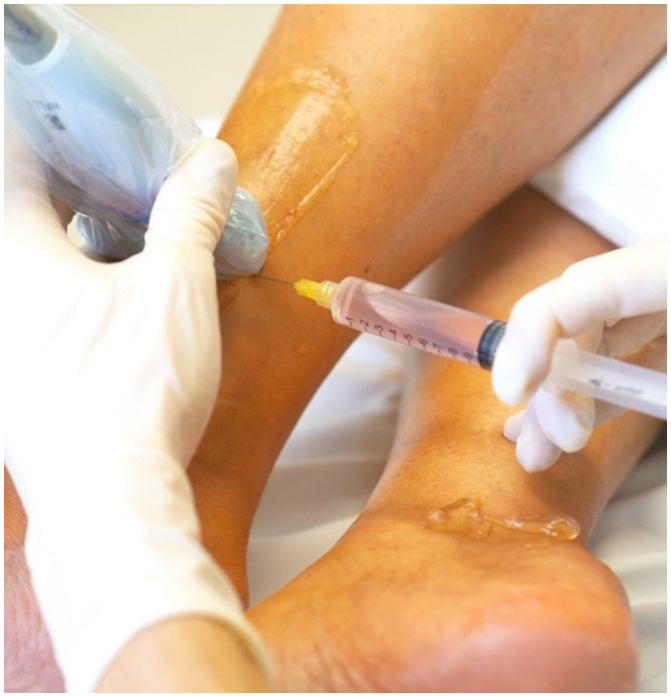
PRP Can Improve Nerve Health

- Decrease edema and inflammation of the nerve
- Improve blood supply
- Stimulate cells – Schwann Cells
- Stimulate Axon and myelin regeneration
- Improves nerve function



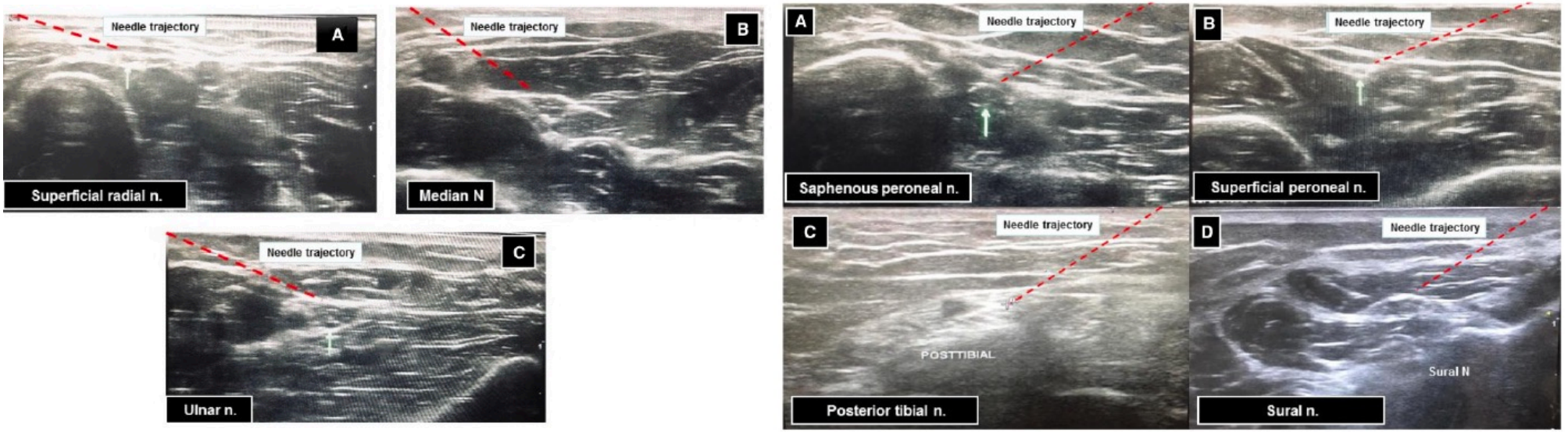
Perineural PRP for Diabetic Neuropathy – Clinical Trial

- Randomized prospective clinical trial
- 60 adults w DM2 neuropathy divided into medical vs medical + PRP
- Medical treatment: glycemic control, Vit B complex, alphalipoic acid, pregabalin
- PRP: ultrasound guided PRP injection of distal UE and LE nerves
- Evaluation: VAS (Pain & Numbness), NCS and Toronto Clinical Neuropathy Score (TCNS) at 0,1,2,3,6 months



Hassanien, Manal, et al. "Perineural Platelet-Rich Plasma for Diabetic Neuropathic Pain, Could It Make a Difference?." *Pain Medicine* 21.4 (2020): 757-765.

Perineural PRP for Diabetic Neuropathy – Clinical Trial



1.5ml of PRP (+ CaCl) was injected around UE/LE nerves distally under ultrasound guidance (small volume, no hydrodissection, not at compression sites?)

Hassanien, Manal, et al. "Perineural Platelet-Rich Plasma for Diabetic Neuropathic Pain, Could It Make a Difference?." *Pain Medicine* 21.4 (2020): 757-765.

Perineural PRP for Diabetic Neuropathy: Results

Table 2. Pain and numbness assessment VAS scales

Variables	Group I (PRP) (N = 31)	Group II (Medical) (N = 29)	*P* Value
Pain			
Baseline VAS	7 (2.5)	6 (2.5)	0.62
1st mo VAS	1 (0.3)*	5 (2)	<0.001
3rd mo VAS	1 (0.3)*	6 (1.3)	<0.001
6th mo VAS	1 (0)*	7 (2)*	<0.001
Numbness			
Baseline VAS	6.5 (3.3)	6 (3.3)	0.39
1st mo VAS	0 (1)*	5 (3)*	<0.001
3rd mo VAS	1 (0.3)*	5 (2)	<0.001
6th mo VAS	1 (0)*	7 (2)*	<0.001

Data are expressed as median and interquartile range. $P < 0.05$ is considered statistically significant.

VAS = visual analog scale.

*Significant change from the baseline value within the same group.

Table 5. Nerve conduction study in the lower limb

Variables	Group I (PRP) (N = 31)	Group II (Medical) (N = 29)	*P* Value
Mean motor NCV (posterior tibial n. medial planter branch) (normally 41+ m/s)			
Baseline	34.5 ± 8.3	33.5 ± 9.2	0.1
6 mo	48.2 ± 4.7*	40.7 ± 6.5*	0.05
Mean motor NCV (deep peroneal n.) (normally 44+ m/s)			
Baseline	35.4 ± 9.3	35.7 ± 10.3	0.5
6 mo	49.1 ± 3.5*	40.9 ± 6.3*	0.01
Mean DML (posterior tibial n. medial planter branch) (normally 6.1 m/s)			
Baseline	6.3 ± 0.22	6.2 ± 1.06	0.5
6 mo	5.6 ± 1.2*	5.8 ± 0.7*	0.07
Mean DML (deep peroneal n.) (normally 6.5 m/s)			
Baseline	6.2 ± 1.02	6.2 ± 1.25	0.8
6 mo	5.7 ± 1.0*	5.9 ± 0.9*	0.1
Mean sensory NCV (superficial peroneal n.) (normally 40+ m/s)			
Baseline	17.3 ± 9.8	17.6 ± 10.6	0.7
6 mo	37.05 ± 1.2*	18.6 ± 9.7	0.001
Mean sensory NCV (saphenous n.) (normally 40+ m/s)			
Baseline	20.4 ± 8.8	20.6 ± 8.6	0.6
6 mo	35.5 ± 2.2*	21.9 ± 8.2	0.001
Mean sensory NCV (posterior tibial n. medial planter branch) (normally 35+ m/s)			
Baseline	19.7 ± 9.1	19.6 ± 9.6	0.5
6 mo	37.4 ± 1.04*	20.2 ± 7.2	0.001
Sural n. NCV (normally 46–64 m/s)			
Baseline	17.00 ± 10.86	17.38 ± 12.36	0.7
6 mo	36.18 ± 3.21*	19.42 ± 11.34	≤0.001

Hassanien, Manal, et al. "Perineural Platelet-Rich Plasma for Diabetic Neuropathic Pain, Could It Make a Difference?." *Pain Medicine* 21.4 (2020):

Perineural PRP for Diabetic Neuropathy

Changes in Toronto Clinical Neuropathy Score

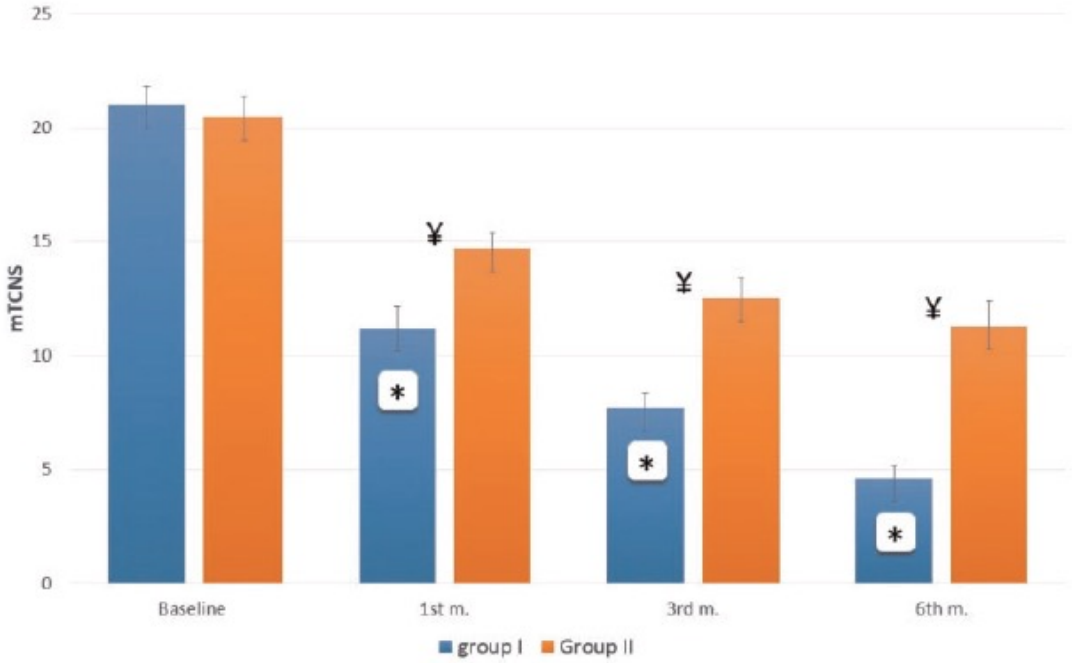


Table 3. The components of the modified Toronto Clinical Neuropathy Score

Symptom Scores	Sensory Test Scores
• Foot pain • Numbness • Tingling • Weakness • Ataxia • Upper limb symptoms	• Pinprick • Temperature • Light touch • Vibration • Position sense
Symptom scores graded as	Sensory test scores graded as
0 = absent	0 = normal
1 = present but no interference with sense of well-being or activities of daily living	1 = reduced at the toes only
2 = present, interferes with sense of well-being but not with activities of daily living	2 = reduced to a level above the toes, but only up to the ankles
3 = present and interferes with both sense of well-being and activities of daily living	3 = reduced to a level above the ankles and/or absent at the toes
Maximum mTCNS is 33.	
Symptoms and signs (sensory tests) are considered present if as a result of diabetic sensorimotor polyneuropathy in the opinion of the investigator.	

Hassanien, Manal, et al. "Perineural Platelet-Rich Plasma for Diabetic Neuropathic Pain, Could It Make a Difference?." *Pain Medicine* 21.4 (2020): 757-765.

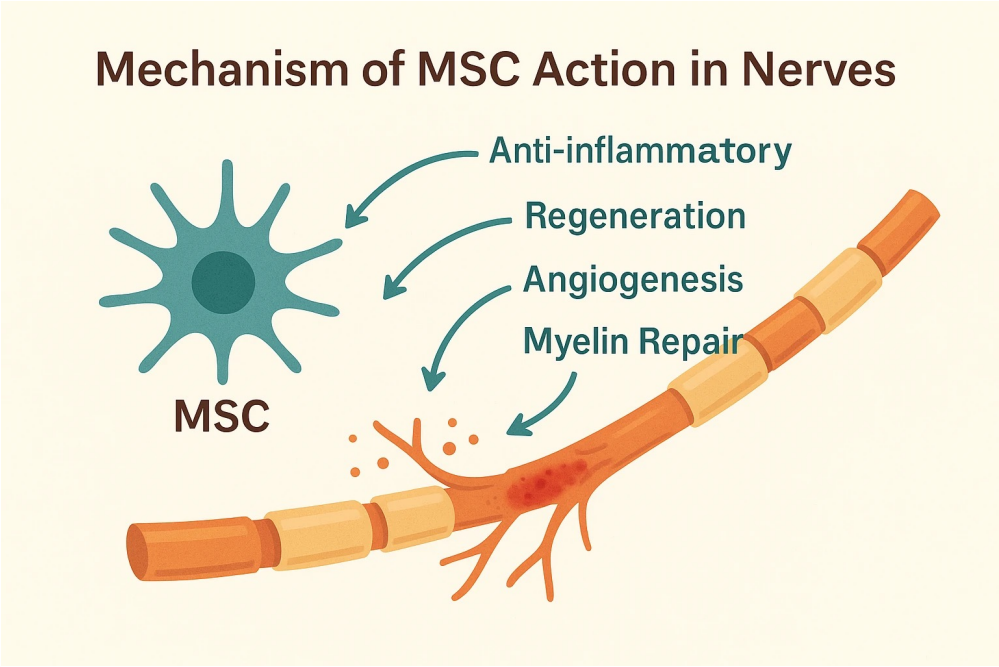
Bone Marrow Cell Therapy For Neuropathy

Improve neuropathy primarily through paracrine signaling

Major mechanisms:

- neurotrophic factor release
- angiogenesis
- immune modulation

These mechanisms repair the peripheral nerve microenvironment.



Neurotrophic Paracrine Signaling

BMMNCs release growth factors that support nerve repair:

- NGF (Nerve Growth Factor)
- BDNF (Brain-Derived Neurotrophic Factor)
- GDNF (Glial-Derived Neurotrophic Factor)

Effects:

- neuronal survival
- axonal regeneration
- Schwann cell activation
- remyelination

Angiogenesis and Microvascular Repair

Peripheral nerves rely on blood supply from the **vasa nervorum**.

BMMNCs release angiogenic factors:

- **VEGF**
- **bFGF**
- **angiopoietins**

Results:

- increased capillary density
- improved nerve perfusion
- reversal of ischemic nerve injury

Immunomodulation

Diabetic neuropathy is associated with chronic inflammation.
BMMNC therapy shifts the immune environment toward repair.

Decreases:

- TNF-α
- IL-1β
- IL-6

Increases:

- IL-10
- TGF-β

Result: Reduced neuroinflammation & improved nerve regeneration

Bone Marrow MSCs for Diabetic Neuropathy- Animal Model

Animal Model

- Streptozotocin-induced diabetic rodents - with diabetic neuropathy.
- BM-derived MSCs isolated and expanded

Intramuscular MSC injections - Into the gastrocnemius muscles.

Outcome Measures

- Nerve conduction velocity
- Peripheral nerve blood flow
- Capillary density
- Myelin structure
- Expression of angiogenic factors

Han, Ji Woong, et al. "Bone marrow-derived mesenchymal stem cells improve diabetic neuropathy by direct modulation of both angiogenesis and myelination in peripheral nerves." *Cell transplantation* 25.2 (2016): 313-326.

Bone Marrow MSCs for Diabetic Neuropathy

Functional Improvements

- ↑ Nerve conduction velocity
- ↑ Peripheral nerve blood flow

Structural Improvements

- ↑ Capillary density around nerves
- ↑ Schwann cell activity
- ↑ Myelin regeneration

Mechanism

- BM-MSCs improve neuropathy through:
- **Angiogenesis** (VEGF, FGF)
- **Remyelination**
- **Paracrine signaling**

Conclusion:
MSC therapy addresses both **vascular and neural pathology** in diabetic neuropathy

Migration of MSCs to the Sciatic Nerve

Fluorescent labeling of injected MSCs showed:

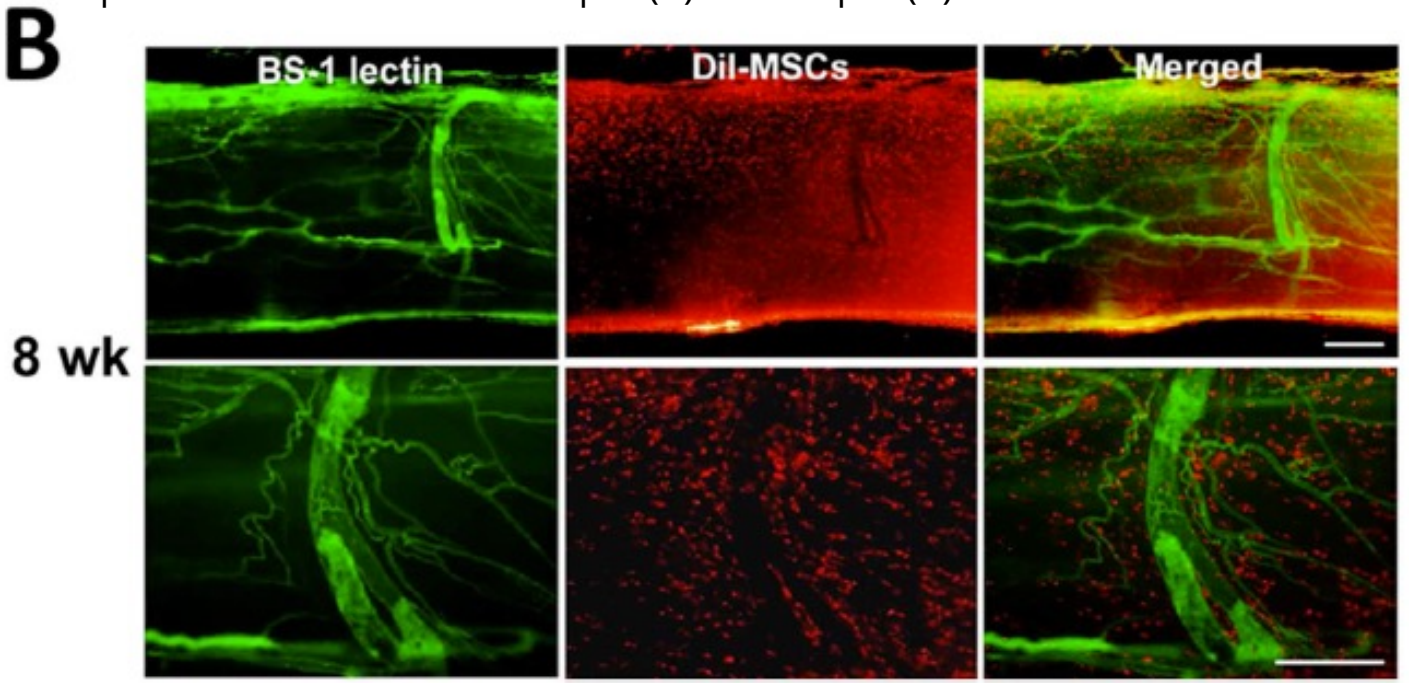
- Cells migrated from the gastrocnemius muscle to the sciatic nerve
- MSCs were durably engrafted within the sciatic nerve
- Many cells localized near the vasa nervorum

Implication

- MSC therapy demonstrates tropism toward injured nerves, suggesting that peripheral injections may allow stem cells to home to neuropathic nerves.

Migration of MSCs to the Sciatic Nerve

Figure 3. Intramuscularly injected MSCs migrated and engrafted in nerves. (A) A representative whole-mount image of a sciatic nerve from a diabetic rat after perfusion with FITC-conjugated BS-1 lectin (green) demonstrated preferential and sustained engraftment of DiI-labeled MSCs (red) into sciatic nerves at 4 and 8 weeks after MSC transplantation. (B) Higher magnification images showed alignment of MSCs mainly along the vasa nervosa at 8 weeks after MSC transplantation. Scale bars: 100 μm (A) and 50 μm (B).



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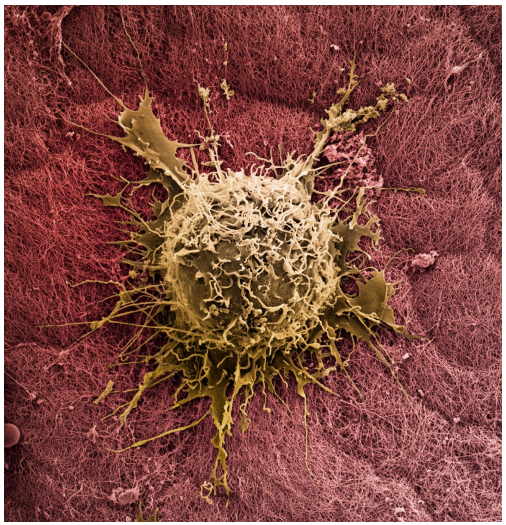
Autologous Bone Marrow Mononuclear Cell Therapy for Refractory Diabetic Neuropathy

- 168 pts with refractory DPN (DPN > 2 years, insufficient relief with conventional drugs)
- 200ml of BMA (cells mobilized w subQ granulocyte colony-stimulating factor G-CSF for 3 days, BMA centrifuge, mononuclear cells washed and resuspended with 50 ml saline)
- IM injection into both thighs (50 sites, 2cmx2cm, 1-1.5 cm deep, 1ml BMC)
- Toronto Clinical Scoring System (TCSS)
  - Score: 1-5 no neuropathy, 6-8 mild, 9-11 moderate, 12-19 severe
- Nerve conduction studies
- Followed for 36 months



Mao, Hong, et al. "Efficacy of autologous bone marrow mononuclear cell transplantation therapy in patients with refractory diabetic peripheral neuropathy." *Chinese Medical Journal* 132.01 (2019): 11-16.

Bone Marrow Harvest and Processing

Stem Cell Mobilization

- Patients received G-CSF (Granulocyte Colony-Stimulating Factor), Dose: 5 µg/kg/day for 3 days to increase circulating stem cells and improve bone marrow stem cell yield

Bone Marrow Aspiration

- Aspirated from posterior iliac crest
- Approximately 200 mL marrow collected

Cell Processing:

- Density gradient centrifugation
- Washing with Saline and re-suspension

BMCs for Patient with Refractory DPN

Table 3: Severity of TCSS scores before and after transplantation treatment, *n* (%)

Items	No neuropathy	Moderate neuropathy	Mild neuropathy	Severe neuropathy	χ^2	*P*
Baseline (n=168)	0 (0.0)	6 (3.6)	45 (26.8)	117 (69.6)	–	–
After transplantation						
1 month (n=168)	9 (5.3)	42 (25.0)	69 (41.1)	48 (28.6)	69.907	<0.001
3 months (n=168)	14 (8.3)	76 (45.2)	58 (34.6)	20 (11.9)	144.076	<0.001
6 months (n=168)	26 (15.6)	92 (55.1)	35 (21.0)	14 (8.4)	183.703	<0.001
12 months (n=167)	26 (15.5)	99 (59.3)	33 (19.8)	9 (5.4)	202.788	<0.001
18 months (n=166)	41 (24.7)	92 (55.4)	28 (16.9)	5 (3.0)	223.244	<0.001
24 months (n=163)	54 (33.1)	83 (50.9)	22 (13.5)	4 (2.5)	232.260	<0.001
36 months (n=161)	58 (36.0)	84 (52.2)	18 (11.2)	1 (0.6)	243.202	<0.001

Table 4: Nerve conduction studies before and after the transplantation treatment

Parameters	Baseline	After transplantation		*F*	*P*
		3 months (n=168)	12 months (n=167)		
mNCV (m/s)	38.21±2.28	40.24±2.80	41.00±2.22	91.298	<0.001
CMAP (μV)	4.67±1.05	5.50±1.20	5.68±1.08	44.537	<0.001
mSCV (m/s)	36.96±2.26	39.15±2.61	40.41±2.22	58.380	<0.001
SNAP (μV)	4.29±0.99	5.14±1.26	5.41±1.14	39.375	<0.001

Motor: tibial and peroneal Sensory: Sural and superficial peroneal

Clinical Results

Neuropathy Scores- TCSS improved significantly:

- Baseline → **12.55**
- 1 month → **9.68**
- 3 months → **8.47**

Nerve Conduction- Motor NCV improved:

- Baseline → **38.21 m/s**
- 3 months → **40.24 m/s**
- 12 months → **41.00 m/s**

High vs Low Cell Count Outcomes

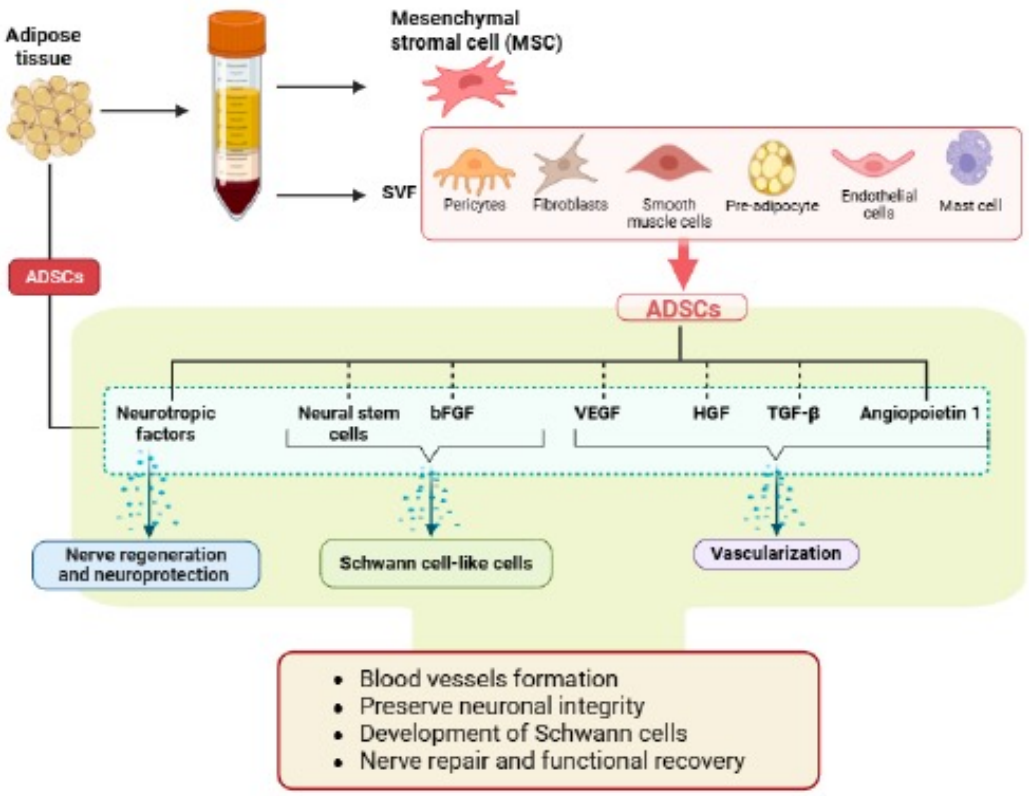
High Cell Count Patients

- • better stem cell mobilization
  • higher mononuclear cell yield
- Results:
- • larger improvements in TCSS scores
  • greater increases in nerve conduction velocity
  • stronger nerve regeneration

Low Cell Count Patients

- • impaired stem cell mobilization
- Results:
- • smaller improvements in neuropathy symptoms
  • less improvement in nerve conduction

Adipose Stem Cell For Neuropathy



Adipose for neuropathy

- Stromal vascular fraction is rich in regenerative cells (pericytes, fibroblasts, Endothelial cells, smooth muscle cells mast cells and peri adipocytes
- Higher yield of cells per gram of product than bone marrow.
- Tendency for Neurogenic Lineage
- Paracrine signaling: release Neurotropic growth factors: BDNF, NGF and GDNF- stimulate axonal and Myelin regeneration
- Release angiogenic growth factors : VEGF (reference back to sciaence of nerve regeberation) - Key Regulator of Angiogenesis
- Adipose stem cells secrete a high level of VEGF.

Adipose Stem Cells for DM Neuropathy - Animal Studies

Model: Streptozotocin-induced diabetic mice
Intervention: Human adipose-derived stem cells (hASCs)

Results:

- Reduced neuropathic pain behavior
- Improved thermal and mechanical sensitivity
- Reduced neuroinflammation
- Reduced apoptosis of neurons and Schwann cells
- Decreased oxidative stress
- Increased neurite outgrowth
- Enhanced axonal regeneration
- Improved functional recovery

Brini, Anna T., et al. "Therapeutic effect of human adipose-derived stem cells and their secretome in experimental diabetic pain." *Scientific Reports* 7.1 (2017): 9904.

De Gregorio, Cristian, et al. "Human adipose-derived mesenchymal stem cell-conditioned medium ameliorates polyneuropathy and foot ulceration in diabetic BKS db/db mice." *Stem Cell Research & Therapy* 11.1 (2020): 168.

The neuro-restorative effect of adipose-derived mesenchymal stem cell transplantation on a mouse model of diabetic neuropathy

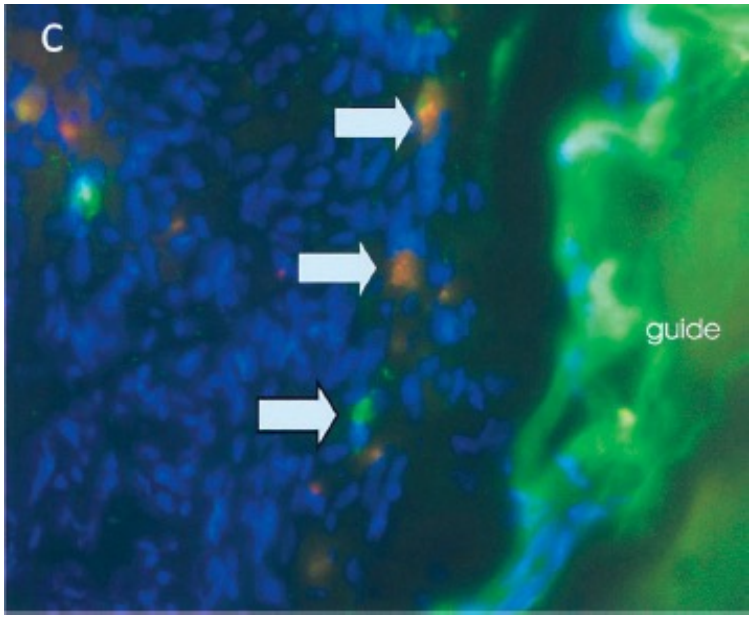
- ASC used for DM neuropathy
- Cells survived for 4 weeks in this current study

- This is a small weak study - But it shows that the cells survived there for a while. it also shows increased nerve growth factor production while compared to diabetic nerves And it showed electro-diagnostic studies that are improved.

Yigitturk, Gurkan, et al. "The neuro-restorative effect of adipose-derived mesenchymal stem cell transplantation on a mouse model of diabetic neuropathy." *Neurological Research* 44.2 (2022): 156-164.

Survival of Adipose Stem Cells After Transplantation Into Nerve Injury

- ASCs survived transplantation for up to 12 weeks in the injured peripheral nerve
- The ASCs did not differentiate into Schwann Cells



Santiago LY, Clavijo-Alvarez J, Brayfield C, Rubin JP, Marra KG (2009) Delivery of adipose-derived precursor cells for peripheral nerve repair. Cell Transplant 18:145–158.

Improved Neurotropic Function of Adipose Stem Cells with Platelet Lysate.

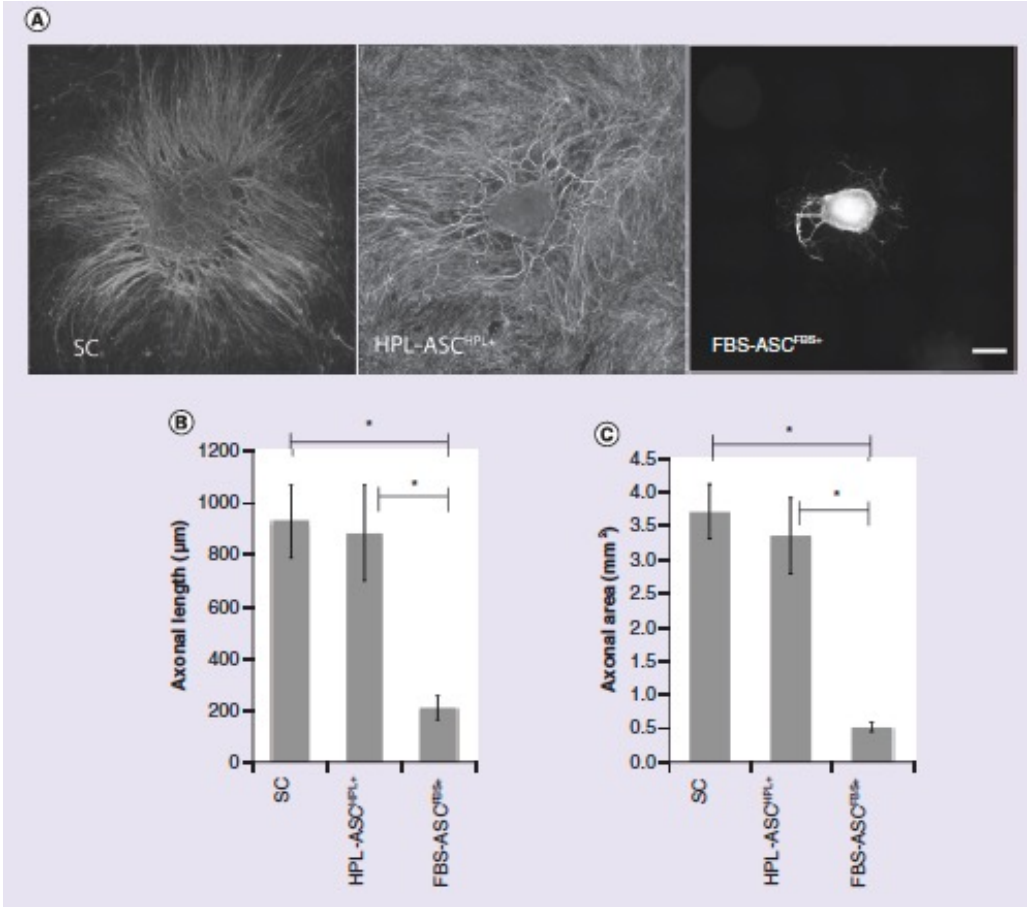


Figure 3. Human platelet lysate stimulated adipose stem cell promote robust axonal outgrowth. Axonal

regeneration from DRG explants in vitro. (A) Images of DRG explant co-cultures involving various cell types, in other

words, DRG co-cultures involving SC that were cultured with standard growth medium for 4 days; DRG co-cultures

involving HPL-stimulated adipose stem cells (ASCHPL+) that were cultured with HPL-supplemented growth medium

(HPL-ASCHPL+) for 2 days and DRG co-cultures involving FBS-stimulated adipose stem cells (ASCFBS+) that were cultured

with FBS-supplemented growth medium (FBS-ASCFBS+) for 2 days. (B) Quantitative measurements of axonal length

and (C) quantitative measurements of axonal area. Axonal length and axonal area resulting from 4 days co-cultures

were normalized to the other cultures of 2 days.

Lischer, M.; di Summa, P.G.; Oranges, C.M.; Schaefer, D.J.; Kalbermatten, D.F.; Guzman, R.; Madduri, S. Human Platelet Lysate Stimulated Adipose Stem Cells Exhibit Strong Neurotrophic Potency for Nerve Tissue Engineering Applications. Regen. Med. 2020, 15, 1399–1408.

PRP improves neurotropic properties of adipose stem cells.

Khaled, Mona M., et al. "Efficacy of using adipose-derived stem cells and PRP on regeneration of 40-mm long sciatic nerve defect bridged by polyglycolic-polypropylene mesh in canine model." *Stem Cell Research & Therapy* 15.1 (2024): 212.

Allogenic Biologics For Neuropathy:

Birth Tissue Products:

- Umbilical cord blood
- Wharton's jelly (umbilical cord matrix)
- Amniotic membrane
- Amniotic fluid

These tissues contain mesenchymal stem cells, cytokines, and extracellular vesicles.

Cellular-Derived Vessicles

- Exosomes

Umbilical Cord Mesenchymal Stem Cells

- Highly proliferative stem cell population
- Low immunogenicity compared with other MSC sources
- High secretory activity (growth factors and cytokines)
- Most regenerative effects occur through the stem cell secretome.

- Secreted molecules include:
  - BDNF, NGF, GDNF, NT-3
  - VEGF and fibroblast growth factor
  - Anti-inflammatory cytokines and antioxidant proteins.

Birth Tissue Products:

- Increased Schwann cell proliferation
- Improved survival under oxidative stress
- Enhanced remyelination of regenerating axons
- Neurotrophic factors stimulate growth cone formation and axonal elongation.
- Extracellular vesicles activate intracellular signaling pathways including PI3K-Akt.
- These pathways support neuronal survival and axonal growth

Ultrasound-Guided Amniotic Membrane / Umbilical Cord Injection for Peripheral Neuropathy

- Retrospective clinical study
- 17 patients with chronic peripheral neuropathy

- Etiology of neuropathy:
- - Diabetic neuropathy (8 patients)
- - Idiopathic neuropathy (7 patients)
- - Chemotherapy-induced neuropathy (2 patients)

- Treatment:
- Ultrasound-guided perineural injection of cryopreserved amniotic membrane / umbilical cord particulate
- Average 2.7 injections per extremity

Buksh, Ahmed Bilal. "Ultrasound-guided injections of amniotic membrane/umbilical cord particulate for painful neuropathy of the lower extremity." *Cogent Medicine* 7.1 (2020): 1724067.

Clinical Outcomes

- Symptom improvement over time:
- 1 week: 30% improvement
- 1 month: 46.6% improvement
- 2 months: 70.7% improvement
- 3 months: 72.3% improvement
- 5–6 months: 61% improvement

- Additional findings:
- - Reduction in neuropathic pain
- - Improved sensation and function
- - No procedure-related complications reported

Intramuscular Injection of Amniotic Fluid–Derived Stem Cells for Nerve Injury

- Animal model:
  Sciatic nerve transection in **72 Sprague-Dawley rats**
- Experimental groups:
- • Saline injection (control)
  • Electrical stimulation (positive control)
  • Amniotic fluid stem cell injection
- Stem cell preparation:
- • Cultured in β-MEM with fetal bovine serum
  • Supplemented with basic fibroblast growth factor
- Injection protocol:
- • **5×10^6 stem cells**
  • **100 µL PBS**
  • Injected into gastrocnemius muscle
  • **3 injection sites**

Chen CJ, Cheng FC, Su HL, Sheu ML, Lu ZH, Chiang CY, Yang DY, Sheehan J, Pan HC. Improved neurological outcome by intramuscular injection of human amniotic fluid derived stem cells in a muscle denervation model. PLoS One. 2015 May 6;10(5):e0124624. doi: 10.1371/journal.pone.0124624. PMID: 25945496; PMCID: PMC4422615.

Key Findings

- Stem cell therapy increased expression of neurotrophic factors:
- • BDNF
  • NT-3
  • CNTF
  • GDNF
- Additional biological effects:
- • Reduced apoptosis in denervated muscle
  • Increased anti-apoptotic signaling (Bcl-2)
  • Preservation of muscle architecture
  • Improved neuromuscular junction integrity
- Functional outcomes:
- • Improved nerve conduction
  • Improved gait function (Sciatic Functional Index)
  • Increased nerve myelination

Umbilical cord stem cells for radial nerve injury: Case report.

Gartit, Mohammed, et al. "Ultrasound (US)-Guided Perineural Amniotic Membrane and Umbilical Cord Particulate Injections for Iatrogenic Radial Nerve Palsy." *Cureus* 16.10 (2024).

Umbilical Cord Stem Cells for Neuropathic Pain (Animal Study)

- Objective
  Evaluate effects of **human umbilical cord blood–derived MSCs** on neuropathic pain.
- Animal models used:
- • Chronic constriction injury (CCI)
  • Spinal nerve ligation (SNL)
  • Spared nerve injury (SNI)
- Treatment:
- • **1 × 10^5 umbilical cord MSCs**
  • Subcutaneous injection
  • Control animals received saline.

Lee MJ, Yoon TG, Kang M, Kim HJ, Kang KS. *Effect of subcutaneous treatment with human umbilical cord blood–derived multipotent stem cells on peripheral neuropathic pain in rats.* **Korean Journal of Physiology & Pharmacology. 2017;21(2):153–160.**

Umbilical cord MSC therapy demonstrated:

- Reduced neuropathic pain behaviors
- Suppression of pain signaling pathways
- Increased anti-inflammatory signaling

Stem cell treatment reduced expression of pain-related markers:

c-fos, CGRP, phosphorylated ERK, phosphorylated p38 MAP kinase, MMP-2 and MMP-9

Additionally:

- Increased **TIMP-2**, an inhibitor of matrix metalloproteinases.

Interpretation:

- Reduced neuroinflammatory signaling and central sensitization.

Exosomes

Cell- Derived Exosome Therapy for Diabetic Peripheral Neuropathy: Pre-Clinical Animal Studies Systemic Review and Meta-Analysis

Models Studied: Primarily **streptozotocin (STZ) diabetic rodents -** peripheral neuropathy models

Sources of Exosomes: Bone marrow MSCs, Adipose-derived MSCs, Umbilical cord MSCs, Schwann cells

Administration: Intravenous injection, Local nerve delivery

Overall Finding- Exosome therapy significantly improved:

- Nerve conduction velocity
- Neuropathic pain behaviors
- Nerve regeneration markers

Lu, Xianying, et al. "Cell-derived exosome therapy for diabetic peripheral neuropathy: a preclinical animal studies systematic review and meta-analysis." *Stem cell research & therapy* 16.1 (2025): 297.

Cell- Derived Exosome Therapy for Diabetic Peripheral Neuropathy

Nerve Function:

- ↑ Motor nerve conduction velocity (MNCV)
- ↑ Sensory nerve conduction velocity (SNCV)

These are objective markers of **peripheral nerve functional recovery**.

Pain and Sensory Outcomes:

- Improved **thermal withdrawal latency**
- Improved **mechanical pain threshold**

Reduction in **hyperalgesia and mechanical allodynia**, two hallmark features of diabetic neuropathy.

Structural Nerve Improvements- (Histologic findings):

- • Increased **axon diameter**
  • Increased **myelin sheath thickness**
  • Increased **nerve fiber density**

This suggests **true regenerative repair rather than symptomatic relief**.

Mechanisms of Exosome-Mediated Nerve Repair

Exosomes function as **biologic signal carriers**, delivering proteins, growth factors, and microRNAs to injured nerves.

Neurotrophic Effects- Exosomes increase expression of:

- **NGF (Nerve Growth Factor)**
- **BDNF (Brain-Derived Neurotrophic Factor)**
- **GDNF (Glial-Derived Neurotrophic Factor)**

These promote **neuronal survival and axonal regeneration**.

Anti-Inflammatory Effects- Exosome therapy decreases:

- **TNF-α**
- **IL-1β**
- **IL-6**

Reducing neuroinflammation is critical in **diabetic nerve injury**.

MicroRNA Delivery- Exosomes deliver regenerative microRNAs such as:

- **miR-21**
- **miR-146a**
- **miR-133b**

These regulate:, Schwann cell activity, Axonal regeneration, Angiogenesis

Microvascular Effects

- Exosomes stimulate:
- ↑ **VEGF signaling**
  ↑ **angiogenesis**
- This improves blood flow to the **vasa nervorum**.

Biologics Used For Neuropathy

Autologous

- PRP
- Bone Marrow Stem Cells
- Adipose Derived Stem Cells

Allogenic

- Birth products (Umbilical Cord Stem Cells/ Amnionic Fluid)
- Exosomes

Common Theme of Biologics for Neuropathy.

Growth factors:

- Stimulate Schwann cells.
- Stimulate axonal and myelin regeneration.
- Stimulate angiogenesis.
- Decreased neurogenic inflammation
- Improve neuromuscular junction function .