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A systematic review of the neuroprotective effects of conditioned media in preclinical models of neurological disorders

Alireza Abdollahi [1], Zahra Tayarani-Najaran [2, 3]*

[1] Medical Toxicology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[2] Targeted Drug Delivery Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[3] Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

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ABSTRACT

Conditioned media (CM) derived from cultured cells, particularly mesenchymal stem cells (MSCs), astrocytes, and glial cells, contain bioactive factors including growth factors, cytokines, and extracellular vesicles (EVs) that exert paracrine neuroprotective effects in preclinical models of neurological disorders.This systematic review, conducted in accordance with PRISMA guidelines and encompassing studies published in PubMed, Scopus, and Web of Science from January 2010 to January 2026, synthesizes evidence on the neuroprotective efficacy of CM in preclinical models of Alzheimer's disease, Parkinson's disease, ischemic stroke, traumatic brain injury, Huntington's disease, amyotrophic lateral sclerosis, spinal cord injury, multiple sclerosis, and retinal disorders. Across 58 included studies, CM consistently demonstrated robust neuroprotective effects through multiple mechanisms: reduction of neuroinflammation (e.g., microglial polarization toward anti-inflammatory phenotypes and down-regulation of pro-inflammatory cytokines), inhibition of apoptosis and oxidative stress, promotion of neuronal survival and synaptic plasticity, and enhancement of regenerative processes such as neurogenesis, angiogenesis, and axonal repair. These benefits were mediated primarily by secreted trophic factors (e.g., BDNF, GDNF, VEGF, IGF-2) and EVs, with preconditioning strategies further enriching the secretome for greater potency. Preclinical evidence strongly supports CM as a promising cell-free therapeutic approach with multifactorial neuroprotective mechanisms and advantages over cell-based therapies, including reduced risks of immune rejection and tumorigenicity. Standardization of CM preparation, detailed characterization of bioactive components, and progression to clinical trials are essential to realize its translational potential for neurological disorders. Limitations include the lack of protocol registration and a meta-analysis.

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Introduction

Neurological disorders, most prominently ischemic stroke, Alzheimer's disease, Huntington's disease, spinal cord injury, and traumatic brain injury, are leading causes of morbidity and mortality worldwide (1). These disorders often result in irreversible neuronal injury and functional impairment, and conventional therapeutic agents are insufficient to promote neuronal survival, regeneration, and functional outcomes (2). Recently, conditioned media (CM) has emerged as a strong alternative for neuroprotection (3). CM is the cell-free supernatant collected from cultured cells and contains secreted bioactive factors, including growth factors, cytokines, exosomes, and anti-inflammatory factors. Compared with cell-based therapies, CM reduces the risk of immune rejection and tumorigenicity (4). Emerging evidence further indicates that the primary therapeutic benefits of stem cell therapies are mediated through paracrine mechanisms rather than cell engraftment or differentiation, establishing CM not merely as a surrogate but as an independent, potentially superior modality with advantages in safety, scalability, manufacturing, storage, and administration (5).

Many studies have shown that conditioned medium derived from mesenchymal stem cells (MSC-CM) exerts protective effects in ischemic stroke models by supporting neuronal survival and functional recovery through neurological restoration, reducing infarct size, and promoting angiogenesis (6). Furthermore, MSCs can be preconditioned with hypoxia or chemical agents to enhance the neuroprotective attributes of MSC-CM, as demonstrated in various *in vitro* models of Alzheimer's disease, in which preconditioned MSCs, with enhanced regulation of neurotrophic factors, can reduce beta-amyloid toxicity (7). In studies comparing CM from neuronal progenitor cells and glial progenitor cells, CM exhibited varying neuroprotective and neurotrophic effects against glutamate excitotoxicity, reinforcing the importance of cell type in the formulation of cell-derived CM (8). In spinal cord injury studies, CM derived from neural stem cells have protected neurons and

***Corresponding author:** Zahra Tayarani-Najaran. Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran. Tel: +98-51-31801178, Email: tayaraninz@mums.ac.ir

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reconnected propriospinal relay circuits, promoting axonal regeneration and functional recovery (9). In experiments, CM from melatonin-stimulated stem cells isolated from the apical papilla conferred protection against glutamate-induced neurotoxicity in neuronal cell lines, possibly via antioxidant mechanisms (10). Early studies on the use of CM derived from neural stem cells in models of Huntington's disease have shown neuroprotection by blocking mutant huntingtin-mediated apoptosis and oxidative stress (11).

In addition to considerations regarding neural stem cells, research has shown that Wharton's jelly-derived MSC-CM has neuroprotective benefits, including attenuating diabetes-associated cognitive impairment in animal models by alleviating oxidative stress, neuroinflammation, and neuronal apoptosis (12). In the case of human gingival MSC-CM, researchers found that it protected human motor neuron-like cells from mechanical injury-induced cell death and provided evidence of its anti-apoptotic and regenerative capacity in a neurodegenerative context (13). Unmodified CM from adipose-derived stem cells has resulted in substantial indices of improvement in animal stroke models with regard to neuronal loss and improved behavioral outcomes after administration of murine and human adipose-derived stem cell CM (14). Inflammatory processes associated with spinal cord injury have been partially mitigated by rat adipose tissue-derived MSC-CM, which modulated the inflammatory response and neuronal survival in cortical neuronal cultures (15). When exposed to CM derived from nerve progenitor cells, it induced a long-term post-ischemic neuroprotective effect, prolonging neurological recovery outcomes, possibly by increased neurogenesis and angiogenesis that extended well beyond the original TBI 30 days later (16). In a combinatorial approach using coupled acetylsalicylic acid with MSC-CM, it was found to have synergistic effects on cognitive function with hippocampal injury by preserving histology and oxidant-antioxidant balance (17).

Further evidence supports the widespread use of CM: neural stem cell CM ameliorates cerebral ischemia-reperfusion injury in rats, reduces edema and infarct volume, and promotes tissue healing in the injured area (18). Periodontal ligament MSC-CM shows anti-inflammatory effects in lipopolysaccharide-activated motoneurons, likely via cytokines (19). Notably, neuroglobin blockade reduces the protective effects of human MSC-CM in astrocyte scratch assays, suggesting that key molecular mediators are involved in CM-mediated injury management (20). Endothelial progenitor cell-CM promotes angiogenesis and confers neuroprotection following spinal cord injury (21). Concentrated adipose tissue-derived MSC-CM alters glutamate regulatory proteins and aquaporin-4 expression in the retina after mild traumatic brain injury, reducing excitotoxic damage (22). While predominantly studied in vascular calcification, choroid plexus epithelium cell CM decreases reactive oxygen species-mediated pathways, potentially contributing to neuroprotective signaling (23). Granulocyte-colony stimulating factor increases the neuroprotectiveness of dental pulp stem cell CM against hypoxic neural injury in neuroblastoma cells (24). Neural stem cell CM decreases amyloid-beta-induced neuronal damage via PCMT1/MST1, indicating relevance to Alzheimer's pathology (25). While a few articles extend the discussion to non-neurological contexts, e.g., the suppressive effects of dental pulp stem cell CM on colorectal cancer cell migration via MAPK pathways, these studies highlight the pleiotropic potential of CM bioactive factors, which could allow us to build on neuroprotective evidence (26).

This systematic review evaluates and synthesizes preclinical evidence for the neuroprotective effects of CM across a range of neurological models, examining methodological quality, effect sizes, and translation barriers to inform future research and clinical applications.

Materials and Methods

Protocol and registration

This review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The protocol was not prospectively registered (e.g., in PROSPERO) but still adheres to systematic review methodological standards.

Eligibility criteria

Included studies met these criteria

· Participants: Preclinical models (*in vitro* or *in vivo*) of AD, PD, HD, ALS, ischemic stroke, TBI, SCI, retinal disorders and MS.

· Intervention: Conditioned media from any cell type, such as stem cells, glial cells, or others.

· Comparisons: Controls using vehicle, non-conditioned media, or equivalent.

· Outcomes: Neuroprotective measures like neuronal survival, apoptosis reduction, or functional recovery. Excluded: Clinical trials, non-quantitative outcomes, non-English publications.

Information sources and search

PubMed, Scopus, and Web of Science were searched from January 2010 to January 2026 using terms such as "conditioned media" AND "neuroprotection" AND specific disorders (e.g., "Alzheimer's," "stroke"). Boolean operators refined searches. Example: ("conditioned media"[Title/Abstract] OR "conditioned medium"[Title/Abstract]) AND (neuroprotection [Title/Abstract] OR neurodegenerative[Title/Abstract]) AND (2010/01/01:2026/01/02[Date - Publication]). References from included studies were manually checked.

Study selection

Titles/abstracts, then full texts, were screened by two reviewers, with disagreements resolved by consensus. PRISMA flow: 2932 articles identified, 1489 duplicates removed, 1443 screened, 216 full-text assessed, 58 included as shown in Figure 1.

Study characteristics

The included studies were categorized into Alzheimer's disease (AD), Parkinson's disease (PD) ischemic stroke, traumatic brain injury (TBI), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), spinal cord injury (SCI), multiple sclerosis (MS), retinal disturbances, and hypoxic neural injury. Characteristics of included studies are summarized in Table 1; Additionally Figure 2 illustrates the signaling pathway and mechanistic effects of CM in the treatment of neurological disorders.

Data collection

Data extracted: study design, cell type, model, neuroprotective effects, bioactive factors. Performed by two reviewers, consensus-resolved.

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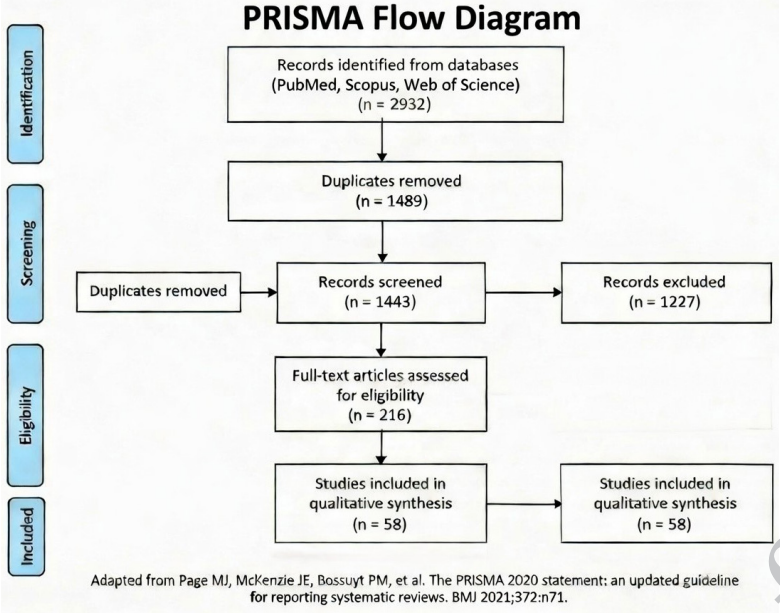


Figure 1. PRISMA flow diagram for screening of articled published between 2010 and July 2026

Table 1. Characteristics of Included Studies published between 2010 and July 2026

Disease	Model/Participant	Intervention	Dosage	Outcome	Ref.
Alzheimer's disease (AD)					
AD	*In vitro*: Mouse hippocampal neurons/astrocytes exposed to Aβ oligomers	Astrocyte conditioned medium (CM) or TGF-β1	CM (murine/human); 10 ng/ml TGF-β1	Protects synapses against Aβ via TGF-β1 from astrocytes; reduces Aβ binding and synapse loss	27
AD	*In vivo*: Mouse model with β-amyloid plaques	Conditioned medium (CM) of stem cells of human exfoliated deciduous teeth (SHED-CM)	Intranasaladministration (specific volume/ concentration not specified)	Improved cognitive function; attenuated pro-inflammatory responses induced by β-amyloid plaques; generated anti-inflammatory/tissue-regenerating environment; induced anti-inflammatory M2-like microglia; neuroprotection via factors promoting axonal elongation, neurotransmission, and suppression of inflammation	28
AD	*In vivo*: Rat model with intra-hippocampal Aβ1-40 injection	Hypoxic-conditioned medium from adipose tissue mesenchymal stem cells (ATSC-CM)	200 µl intraperitoneally once a day for 8 days	mproved spatial and recognition memory; decreased beta-amyloid plaques; enhanced neuronal survival; reduced TLR2 and TLR4 expression; decreased inflammation markers (IL-1β, TNF-α)	29
AD	*In vitro*: PC12 neuron-like cells (oxidative stress with H_2O_2) and THP-1 microglia cells (inflammation with LPS)	Conditioned medium from preconditioned MSCs: pCM (TLR3 ligand-activated) or hCM (CoCl2 hypoxia mimetic)	Not specified (applied to cell cultures post-preconditioning of MSCs)	Immunosuppressive effects on M1 macrophages: reduced pro-inflammatory cytokines (IL-1β, IL-6, TNF-α), increased IL-4, IL-10, TGF-β; enhanced PC12 cell viability under oxidative stress; increased growth factors (e.g., VEGF); activated Nrf2/ARE pathway, reducing ROS and apoptosis	7
AD	*In vivo*: Rat model induced by trimethyltin (TMT)	Bovine umbilical mesenchymal stem cell-conditioned medium (BUMSC-CM)	0.2 ml/kg BW or 0.5 ml/kg BW intraperitoneally, 14 days post-induction	Increased BDNF and NGF expression; down-regulated IL-1β and TNF-α; increased neuron density in hippocampus (CA1, CA3, DG regions); binding of CM amino acids (isoleucine, leucine, valine) to amyloid beta protein via hydrophobic/hydrogen-bonded interactions; promoted neuron proliferation	30

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Continued Table 1.

Parkinson's disease (PD)					
PD	*In vitro*: SH-SY5Y with 6-OHDA	ASC secretome from hydrogel	CM (equivalent to 1×10^6 cells)	Protects against 6-OHDA toxicity via secretome	31
PD	*In vitro*: Rotenone DA neurons	Human iPSC-derived astrocytes	Co-culture	Rescues mitochondrial dysfunction via mitochondrial donation	32
PD	*In vivo*: Rotenone rats	Neural-induced hADSC secretome	2 mg/kg IV	Neuroprotection against rotenone-induced PD	33
PD	*In vitro*: VM NPC with rotenone	EPC-conditioned medium	CM	Promotes DA neurons; exerts neuroprotection	34
PD	*In vivo*: 6-OHDA rats	ASC-CM	Intrastriatal (20 µl)	Protects DA neurons in parkinsonian rats	35
PD	*In vitro*: SH-SY5Y with MPP+	hMBSC-conditioned medium	CM	Protects against MPP[illegible]-induced cytotoxicity	36
PD	*In vitro*: 6-hydroxydopamine-induced SH-SY5Y cells; *In vivo*: 6-hydroxydopamine-induced mouse models	Insulin-like growth factor-2 (IGF-2)	Not specified (different concentrations: 5, 25, 50, 100 ng/ml *in vitro*)	Activated the PI3K/A[illegible] pathway; alleviated down-regula[illegible]n of IGF-2 rec[illegible]ptor; d[illegible]eased α-synuclein aggregation; r[illegible]ored ty[illegible]osine hydroxylase le[illegible]els; redu[illegible]d oxidative stress; shifted micr[illegible]glia to M2 subtype	37
PD	*In vitro* and *in vivo* (mouse model)	N/A (focus on STAT4/KISS1 interaction, no CM)	N/A (no CM [illegible]ed)	In[illegible]bits oxidative damage, inflammation, and neuronal apoptosis; improves dopaminergic neuron survival	38
PD	*In vitro* (rotenone-induced toxicity in SH-SY5Y cells)	Neural-induce[illegible] human adipose tissue-[illegible]erive[illegible] stem [illegible]lls	Direct addition to culture medium	Counteracted rotenone-induced autophagy dysregulation and toxicity; alleviated alpha-synuclein aggregation Comprehensive restoration of mTOR-dependent autophagic flux (↑ p-mTORC1/mTORC2, p-ULK1, ATG13, beclin-1; ↓ p-AMPK, DEP-TOR); enhanced p-Akt/p-ERK1/2 signaling with reduced GSK3β activity; normalized LC3B lipidation, p62 accumulation, ATG3/5/7/12 expression, and lysosomal markers LAMP1/LAMP2/TFEB	39
PD	*In vivo* (rotenone-induced rat)	Stem cells from human exfoliated deciduous teeth (SHED)	Standardized collection of supernatant; concentrated	Improved motor function (Rotarod); ↑ TH expression; ↓ α-synuclein; ↓ neuroinflammation (Iba-1, CD4) Anti-inflammatory; neuro regeneration; IGFBP-6, TIMP-1, TIMP-2, TGF-β1; cholinergic/calcium/serotoninergic synapses, axon guidance	40
Hypoxic neural injury					
Hypoxic neural injury	*In vitro*: SH-SY5Y cells exposed to CoCl2 (hypoxia model)	Treatment with dental pulp stem cell-conditioned medium (DPSCs-CM), G-CSF, or DPSCs-CM from G-CSF-transfected cells (DPSC-CMT)	Not specified (treatment post-hypoxia induction)	Protected against neural damage; reduced apoptotic cells; decreased IL-6/IL-10 ratio; suppression of inflammatory mediators; modulation of β-catenin, cleaved PARP, caspase-3, and surviving expression	24
Ischemic stroke					
Ischemic stroke	*In vitro*: OGD neurons with microglia	BM MNCs	Co-culture (1:1 ratio)	Protect neurons; modulate microglia	41
Stroke	*In vivo*: MCAO rats	Astrocyte PTX3	Endogenous	Supports BBB integrity via VEGF	42
Ischemic stroke	*In vivo*: MCAO mice	hNPC-conditioned medium	IV (100 µg)	Neurorestorative effects	43

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Continued Table 1.

Ischemic stroke	*In vivo*: Male C57BL/6 mice with middle cerebral artery occlusion	Conditioned medium from IL-1α-primed MSCs (αCM)	Administered at time of reperfusion or 24 hr post-stroke	~30% reduction in lesion volume (at time); improvements in body mass, neurological score, nest building; at 24 hr, improved nest building and neurological score	44
Global cerebral ischemia/reperfusion (IR) injury	*In vivo*: Rat model of global cerebral IR	Intravenous administration of rat adipose-derived MSC-CM (rAD-MSC-CM) or chicken embryo liver-derived MSC-CM (cLD-MSC-CM)	0.5 mg/kg (intravenous, 30 min post-reperfusion)	Improved memory function; reduced anxiety/depression-like behaviors; enhanced exploratory activities; decreased BBB permeability, brain water content, oxidative stress, and hippocampal histopathological changes via antioxidant properties	45
Ischemic stroke	*In vitro*: Primary cortical neurons under oxygen-glucose deprivation (OGD)	Addition of mesenchymal stromal cell-conditioned medium (MSC-CM)	Not specified (added 48 hr or 30 min before OGD, or post-OGD)	Reduced neuronal cell death by 30-35%; protection from apoptosis via Akt-dependent signaling; plasminogen activator inhibitor-1 as key compound	46
In vitro ischemia/reperfusion (oxygen-glucose deprivation, OGD) on astrocytes	*In vitro*	Hair follicle stem cells (HFSCs)	90% HFSC-derived secretome complemented with 10% fetal bovine serum (FBS) for 48 hr	Protects astrocytes from OGD-induced damage, suppresses neuronal apoptosis, reduces markers of cell death	47
Ischemic stroke	*In vivo* (rat 60-min MCAO + 24 hr reperfusion)	Human amniotic mesenchymal stem cells	Intracerebroventricular (30 min post-reperfusion)	Reduced infarct volume, brain edema, BBB permeability, cortical neuronal loss, apoptosis Targeted inhibition of apoptosis (↓ caspase-3, Bax; ↑ Bcl-2)	48
Ischemic stroke	*In vivo* (rat MCAO + reperfusion)	Amniotic mesenchymal stem cells	Stereotactic intracerebral (30 min post-reperfusion)	Reduced neuronal loss and DNA fragmentation; improved neurological function Activation of ERK1/ERK2-BDNF signalling; up-regulation of BDNF, VEGF, NGF; promotion of neurogenesis and angiogenesis	49
Cerebral ischemia	*In vitro* (primary rat cortical neurons + OGD/reperfusion; astrocytes preconditioned with OGD)	Primary rat cortical astrocytes (6 hr OGD preconditioned)	Addition to neuronal culture post-OGD	Enhanced neuronal survival, preserved dendritic architecture and mitochondrial function; reduced cell death Induction of neuronal autophagy (↑ LC3b-II, ↓ p62); secretion of protective soluble factors by preconditioned astrocytes	50
Ischemic stroke	*In vivo* (MCAO rat)	Bone marrow MSCs	Hypoxic preconditioning; collected supernatant	↓ Infarct volume; improved neurological recovery Anti-apoptotic; pro-angiogenic; 163 differentially expressed proteins (107 ↑ in hypoxic CM)	51
Ischemic stroke	*In vivo* (MCAO rat)	Human adipose-derived stem cells	3D spheroid culture; concentrated	↓ Infarct volume; preserved motor function; ↑ microvessels; ↓ apoptosis/astrogliosis; Angiogenesis; reduced neural/microglial apoptosis	52

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Continued Table 1.

Traumatic brain injury (TBI)					
mTBI	*In vivo*: Blast mice	ASC-CCM	1 µL i.v. (~100 ng protein)	Alters glutamate proteins/AQP4 in the retina	22
TBI	*In vivo*: Weight drop mice	cMSC-EV	i.v. (5×10^8 EVs)	Reduces cystinosis/apoptosis	53
TBI	*In vivo*: CCI mice	MSC secretome	IV	Long-term neuroprotection via bioactive metabolites	54
TBI	*In vitro*: Primary cultured astrocytes and cortical neurons	2-carba-cyclic phosphatidic acid (2ccPA)	Not specified	Suppressed neuronal apoptosis; increased tenascin-C (TN-C) immunoreactivity; increased Tnc mRNA expression in astrocytes; conditioned medium from 2ccPA-treated astrocytes suppressed apoptosis of cortical neurons via TN-C	55
TBI	*In vivo*: Adult zebrafish model induced by pulsed high-intensity focused ultrasound (pHIFU)	Administration of human umbilical cord perivascular cell-conditioned medium (HUCPVC-CM)	Not specified (administered 1 hour post-injury)	Reduced reactive astrogliosis, decreased apoptosis; improved locomotor activity and reduced anxiety-like behaviors, modulation of paracrine effects for tissue recove	56
Huntington's disease (HD)					
HD	*In vivo*: R6/2 mice	LAV-BPIFB4	AAV gene therapy	Improves CXCR4-microglia crosstalk; prevents progression	57
HD	*In vitro*: HD cell model	Glia-CM	CM	Protection	58
HD	*In vivo* (R6/1 transgenic mice)	Fetal mouse glial cells	Co... striatal ...ion (15 or 30 days)	Reduced huntingtin inclusions; preserved DARPP-32+striatal and TH+ nigral neurons Enhanced autophagy (↓ p62, ↑ LC3-II, Beclin-1, LAMP-2); increased BDNF levels	59
Amyotrophic lateral sclerosis (ALS)					
ALS/FTD	*In vitro*: Motoneurons with ...CM	...RT-X agonist	0.1–10 µM	Prevents degeneration via Kv7/TSPO	60
ALS	*In vivo*: SOD1 mice; *in ...ro*: NSC-34 cells	UCMSC-CM	IV (100 µl)	Inhibits microglial activation; ameliorates neuroinflammation	61
ALS	*In vitro*: ALS-MNs (SOD1AV4 mutation); *In vivo*: SOD1G93A mice	Conditioned medium from ALS iPSCs (autologous)	*In vivo*: 200 µl CM subcutaneous	Neuroprotection, reduced apoptosis, improved survival in mice; multifaceted neuroprotection through the interactome of several key proteins	62
ALS	*In vitro* (primary motor neurons, NSC-34 cells, astrocytes, microglia from non-transgenic and SOD1G93A mice)	Mesenchymal stromal cells	Direct addition to culture medium	Concentration-dependent attenuation of staurosporine-induced apoptosis; reduced inflammation Up-regulation of GDNF and CNTF in astrocytes, VEGF in NSC-34 cells; down-regulation of TNFα, IL-6, iNOS; enhanced Fractalkine (CX3CL1)/CX3CR1 signalling	63
ALS	*In vitro* (NSC-34 cells expressing mutant TDP-43^{M337V})	Human umbilical cord mesenchymal stem cells	24 hr addition to culture medium	Increased cell viability; reduced LDH leakage, TDP-43 aggregates, oxidative damage Activation of Nrf2/HO-1 antioxidant axis (blocked by Nrf2 inhibitor ML385)	64

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Continued Table 1.

ALS	*In vitro* (NSC-34 cells expressing mutant SOD1; iPSC-derived motor neurons modeling familial and sporadic ALS)	Stem cells from human exfoliated deciduous teeth (SHED)	Direct addition to culture medium	Significantly suppressed mutant SOD1-induced intracellular aggregates and neurotoxicity in both familial and sporadic ALS models. Paracrine effects are partially mediated by heat shock proteins and activation of insulin-like growth factor-1 receptor signaling, promoting protein aggregate clearance and neuronal survival	65
Spinal cord injury (SCI)					
SCI	*In vivo*: SCI mice	hpcMSC-sEV	IV (200 µg)	Promote recovery via miR-21/JAK2/STAT3 in astrocytes	66
SCI	*In vivo*: SCI rats	NSC-CM	IT (10 µl/day)	Suppresses inflammation; promotes recovery	67
SCI	*In vitro*: Spinal cord slices	MSC-CM	CM	Increases glial reactivity, decreases neuronal survival	68
SCI	*In vitro*: Mouse spinal cord dorsal horn	ST2-CM	CM	Fosters excitability and synaptic transmission	69
SCI	*In vivo*: SCI rats; *In vitro*: hMSCs from bone marrow	NRLM-CM (conditioned medium from rapidly expanded MSCs)	Not specified (intrathecal for *in vivo*)	Superior at reducing inflammation and enhancing neurite regeneration *in vitro*; improved functional restoration in SCI rats *in vivo*	70
SCI	*In vitro*: Primary cortical neurons and astrocytes exposed to SCI-conditioned medium mimicking inflammation	Replacement with adipose tissue-derived mesenchymal stem cell-conditioned medium (ATMSC-CM)	Not specified (replacement of SCI-CM with ATMSC-CM for 72 hr)	Increased neuronal survival (MAP2-positive neurons); no significant impact on astrogliosis; stabilization via released neuroprotective and trophic factors	15
SCI	*In vitro* and *in vivo* (rat model)	Human fetal neural stem cells (hNSCs)	Not specified	Protects neurons from loss, reduces apoptotic profiles, promotes propriospinal neuron relay neural circuit reconnection, enhances axonal regeneration and functional recovery	9
SCI	*In vivo* (rat contusion model)	Human umbilical cord blood-derived CD34+ cells	Intravenous (immediate post-injury)	Attenuated motor deficits, apoptosis, inflammation, oxidative stress, astrogliosis Anti-apoptotic, anti-inflammatory, and antioxidant paracrine effects; comparable to cell therapy, synergized with 17β-estradiol	71
Multiple sclerosis (MS)/EAE					
MS	*In vivo*: EAE mice	ASC-CM	IP (300 µl)	Immunomodulatory effects	72
MS	*In vitro*: OLN-93 oligodendrocyte cell line exposed to cuprizone	Amniotic epithelial stem cell-conditioned medium (AEC-CM) combined with ponesimod	EC50 of CM: 30.78 µl; ponesimod: 5.7 nM	Synergistic increase in cell viability (up to 91%); reduced apoptosis; enhanced oligodendrocyte survival	73

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MS	*In vitro*: Primary neurons/astrocytes and oligodendrocyte precursor cells (OPCs) exposed to oxygen-glucose deprivation (OGD)	Conditioned medium from human adipose-derived mesenchymal stromal cells (adMSC-CM)	Collected after 72 hr from 1.0 × 10^4 cells/cm² (volume/concentration not specified for application)	Reduced cell death in neurons and OPCs; increased OPC differentiation into mature oligodendrocytes; correlated with growth factor levels	74
Retinal/Optic nerve injury					
Retinal oxidative stress	*In vitro*: Retinal neurons with H_2O_2	hMSC-EPO	CM	Enhance survivability against oxidative stress	75
Retinal ganglion injury	*In vitro*: RGCs with OGD	VEGF	20 ng/mL	Autocrine/paracrine neuroprotective factor	76
Retinal ganglion cell degeneration	*In vitro*: Stem cell-derived retinal ganglion cell-like cells in organoid cultures	Co-culture with adult Müller cells or addition of Müller cell-conditioned medium (CM)	Not specified (co-culture or CM addition to organoids)	Enhanced RGC-like cell survival; increased neurite length; up-regulation of Atoh7 (RGC development factor); promotion of neuronal maturation and neurogenesis	77
Oxidative stress-induced retinal degeneration (H_2O_2 on ARPE-19 cells; sodium iodate-induced retinal damage *in vivo*)	*In vitro* and *in vivo* (rat model)	Human amniotic mesenchymal stem cells (hAMSC)	Not specified	Ameliorates mitochondrial dysfunction, suppresses intrinsic apoptosis, reduces RPE and retinal damage, inhibits H_2O_2-induced decrease in cell viability	78
Retinal disorders	*In vitro/in vivo* (light-induced mouse)	Human adipose-derived stem cells	Standard collection	Preserved retinal structure/function; ↓ photoreceptor death Anti-apoptotic (Akt pathway); TIMP-1, SPARC	79

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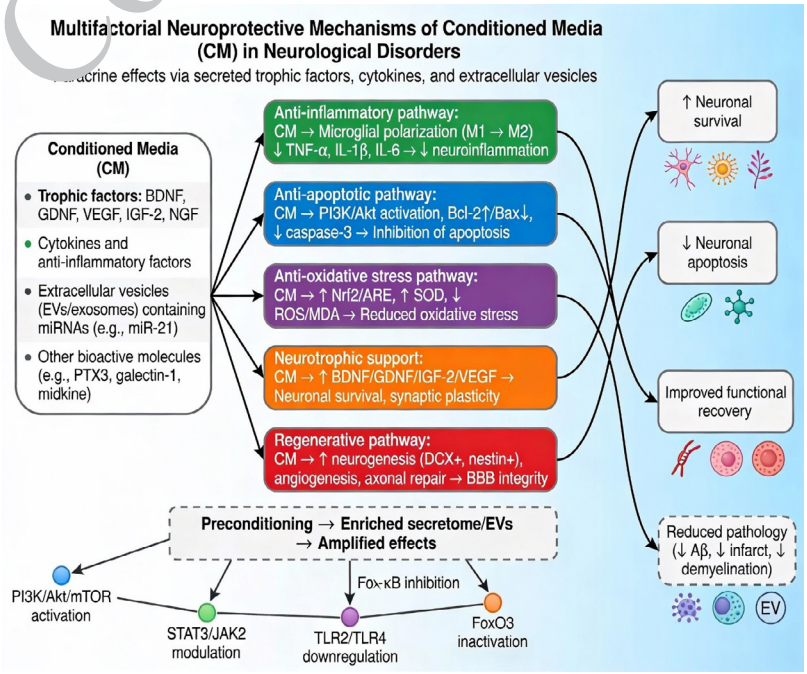


Figure 2. Multifactorial neuroprotective mechanisms of CM in neurological disorders

Table 2. Risk of Bias Summary of included studies between 2010 and July 2026

Disease	Model type	Randomization	Allocation concealment	Blinding of personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias	Overall risk	Ref.
AD	*In vitro*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	27
AD	*In vivo*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	28
AD	*In vivo*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	29
AD	*In vitro*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	7
AD	*In vivo*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	30
PD	*In vitro*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	31
PD	*In vitro*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	32
PD	*In vivo*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	33
PD	*In vitro*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	34
PD	*In vivo*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	35
PD	*In vitro*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	36
PD	*In vitro/In vivo*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	37
PD	*In vivo*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	38
PD	*In vitro*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	39
PD	*In vivo*	Low	Unclear	Low	Low	Low	Low	Low	Unclear	40
HIN	*In vitro*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	24
Stroke	*In vitro*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	41
Stroke	*In vivo*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	42
Stroke	*In vivo*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	43
Stroke	*In vivo*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	44
Stroke	*In vivo*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	45
Stroke	*In vitro*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	46
Stroke	*In vitro*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	47
Stroke	*In vivo*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	48
Stroke	*In vivo*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	49
Stroke	*In vitro*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	50
Stroke	*In vivo*	Low	Unclear	Unclear	Low	Low	Low	Low	Unclear	51
Stroke	*In vivo*	Low	Unclear	Unclear	Low	Low	Low	Low	Unclear	52
TBI	*In vivo*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	22
TBI	*In vivo*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	53
TBI	*In vivo*	Unclear	Unclear	Unclear	Unclear	Low	Low	Low	Unclear	54

- Randomization: Low if the study mentioned random group assignment (common in animal studies); Unclear otherwise.
- Allocation Concealment: Unclear in most, as not explicitly described.
- Blinding of Personnel: Unclear, rarely specified for animal handling.
- Blinding of Outcome Assessment: Low for histological/behavioral assessments if blinded (often mentioned); Unclear for others.
- Incomplete Outcome Data: Low, as most studies reported all outcomes without significant attrition.
- Selective Reporting: Low, as outcomes matched methods.
- Other Bias: Low, no apparent biases like funding conflicts.
- Overall Risk: Unclear for most due to incomplete reporting typical in preclinical studies.

Risk of bias assessment

SYRCLE tool for *in vivo* studies; ToxRTool for *in vitro*. Assessed selection, performance, detection, and reporting biases, as shown in Table 2. Common issues: unclear blinding, incomplete CM details.

Results

Study selection

Fifty-eight studies included, covering AD (n=5), PD (n=10), ischemic stroke (n=12), TBI (n=5), HD (n=3), ALS (n=6), SCI (n=8), MS (n=3), retinal disorders (n=5), and hypoxic neural injury (n=1).

Quantitative summary

All 58 included studies (100%) reported positive neuroprotective effects of CM, including improvements in neuronal survival, functional recovery, or related outcomes. No studies reported absence of effect or negative outcomes.

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Study characteristics

Risk of Bias Assessment

Below is the risk-of-bias assessment for all 58 studies, using SYRCLE for *in vivo* studies and ToxRTool for *in vitro* studies.

Alzheimer's disease (AD)

In one study authors demonstrated that TGF-β1 released from astrocytes halted the loss of synapses caused by amyloid-β oligomers (AβOs) in an AD model. They demonstrated how astrocytes were important in synaptic loss due to the release of TGF-β1. Osteocalcin prevented AβOs from binding to neurons, significantly reduced dendritic spine loss in the hippocampus, and prevented AβOs from entering astrocytes. The release of TGF-β1 from astrocytes was important, as AβO clearance was shown to prevent synaptic loss and perhaps reduce memory deficits by regulating astrocytic neuroprotective signaling cascades (27).

CM from stem cell conditioned medium (SC-CM) derived from human exfoliated deciduous teeth (SHED-CM) enhanced cognitive function in a mouse model of β-amyloid plaques when administered intranasally. Mechanistically, the SC-CM reduced pro-inflammatory sources, induced M2-like microglia, and ultimately increased neuroregeneration through pathways to support axonal elongation and neurotransmitting properties. This occurred as the SC-CM created an environment conducive to an anti-inflammatory state (28).

Mehrabadi *et al.* (2020) examined the therapeutic potential of hypoxic-conditioned medium from adipose tissue mesenchymal stem cells (ATSC-CM) in a rat model of AD caused by intra-hippocampal injection of Aβ1-40. ATSC-CM was delivered through intraperitoneal injection (200 µl) for 8 consecutive days. They reported positive alterations in spatial and recognition memory by behavioral assays, a drastic reduction in beta-amyloid plaques, and increased neuronal survival in the hippocampus. They speculated that the observed neuroprotective effects were largely due to the anti-inflammatory action of the CM, which, through down-regulation of TLR2 and TLR4, also reduced pro-inflammatory cytokine levels of IL-1β and TNF-α. They further argued that the neuroprotective effects identified through hypoxic preconditioning allow CM to function therapeutically without the need to transplant cells to provide a milieu that alters toll-like receptor pathways that protect against neuroinflammation and increase neuronal survival without using live cell-based therapeutic strategies, thus providing a cell-free therapy to reduce important aspects of AD (29).

Tolstova *et al.* (2024) investigated the enhanced neuroprotective benefits of CM from preconditioned mesenchymal stem cells (MSCs) across various *in vitro* AD models, including oxidative stress exposure (H2O2) in PC12 neuron-like cells and LPS-stimulated THP-1 microglia, to create an inflammatory-like environment. The CM from the MSCs was either preconditioned with a TLR3 ligand (pCM) or CoCl2 to induce hypoxia (hCM), and was ultimately undosed, meaning each sample was not specifically dosed for each cell type evaluated. In this study, pCM and hCM had dose-dependent immunosuppressive effects mainly on the M1 macrophages via inhibiting pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) while promoting anti-inflammatory cytokines (IL-4, IL-10, TGF-β). hCM promoted PC12 electrophysiological viability that was subjected to oxidative stress and showed relative specialization of growth factors such as VEGF and promoted the Nrf2/ARE pathway while inhibiting the levels of ROS and apoptosis. The research suggests the therapeutic implications of preconditioned CM for improved bioactivity and neuroprotective efficacy, as it furthered improved immunomodulatory and antioxidant activity most likely by enriching the secretome to battle neuroinflammation and oxidative damage more effectively than non-preconditioned CM. (7). Wihadmadyatami *et al.* famously found that CM from bovine umbilical MSC-CM (BUMSC-CM) exaggerated BDNF and NGF levels while reducing IL-1β and TNF-α in a trimethyltin rat model. Increased density of differentiated hippocampal neurons by the BUMSC-CM was also accompanied by proliferation. In addition, *in silico* analysis revealed that CM amino acids bind to amyloid-beta, potentially reducing aggregation (30).

Parkinson's disease (PD)

The secretome of hydrogel-embedded adipose mesenchymal stem cells demonstrated neuroprotective properties against the 6-hydroxydopamine (6-OHDA) neurotoxin associated with PD. In particular, conditioned medium (CM) attenuated measurable neurotoxicity and oxidative stress by promoting microglial M2-type activation and SIRT3 up-regulation, inhibiting pro-inflammatory pathways, and thereby decreasing dopaminergic neuron loss in both *in vitro* and *in vivo* models (31). By providing functional mitochondria, Human astrocytes derived from iPSCs were able to rescue rotenone-induced mitochondrial dysfunction and dopaminergic neurodegeneration. The mechanism of mitochondrial rescue is intercellular mitochondrial transfer via tunneling nanotubes, which restores mitochondrial membrane potential, decreases ROS, and inhibits apoptosis mediated via PI3K/Akt signaling in models of rotenone-induced toxicity (32).

Neural-induced human adipose tissue-derived stem cell-derived secretome has neuroprotective effects in rotenone-induced PD. The neuroprotective mechanism involves a secretome-mediated microglial M2 shift via brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF), thereby reducing oxidative stress and apoptosis (33). CM from endothelial progenitor cells also protects dopaminergic neuron survival. The mechanism of action involves CM from endothelial progenitor cells mediating up-regulation of GDNF and BDNF secretion, thereby reducing oxidative stress and promoting M2 microglial polarization (34).

Evidence indicated that conditioned medium from adipose-derived stem cells (ASCs) significantly protects dopaminergic neurons in parkinsonian rat models from degeneration through the action of neurotrophic factors (e.g., BDNF and GDNF). Their study also revealed that the ASC preconditioned medium appeared to modulate the type of microglial activation from M1 phagocytic activity to M2 repair activity (35). In one study, conditioned medium from human menstrual blood-derived endometrial stem cells protected nigral dopaminergic neurons against MPP+ in a PD toxicity model. The conditioned medium protected against destruction by reducing oxidative stress through up-regulation of manganese superoxide dismutase and against oxidative stress-induced apoptosis through a Bcl-2/Bax modulation pathway (36).

CM derived from skin-derived precursor Schwann cells (SKP-SCs) considerably increased SH-SY5Y cell activity and viability against 6-OHDA-induced toxicity, indicating that

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IGF-2 is a significant mechanism of action, as neutralizing antibodies against IGF-2 significantly inhibited this effect. Elevated IGF-2 was found in the peripheral blood of patients with PD and in the mouse model. Pre-treatment of IGF-2 (in concentrations of 5-100 ng/ml *in vitro*) or through intranasal delivery *in vivo* elicited activation of the PI3K/AKT pathway, restored tyrosine hydroxylase (a marker of dopamine neurons), reduced α-synuclein promiscuous aggregation, inhibited IGF-2 receptor down-regulation, reduced oxidative stress, and shifted microglia toward the anti-inflammatory M2 subtype. These data suggest that IGF-2 can be identified in cell-derived secretomes and CM as a paracrine factor that reverses PD pathology and promotes dopaminergic neuron survival (37).

In a study authors assessed the role of STAT4 in the context of PD using MPP+-treated SH-SY5Y neuroblastoma cell lines (*in vitro*) and an MPTP-induced mouse model (*in vivo*). STAT4 up-regulation inhibited KISS1 expression, leading to reduced oxidative stress (lower ROS and MDA; higher SOD), reduced inflammation (lower IL-1β, IL-6, TNF-α), and decreased neuronal apoptosis (lower caspase-3 and Bax; higher Bcl-2) in PD *in vitro* and *in vivo* models. Recovery of MAPK inactivation induced by STAT4 over-expression only occurred when KISS1 was over-expressed, while specific inhibitors of MAPK signaling reduced effects induced by the MPP+. The mechanism appears to specifically target and negatively regulate KISS1 transcription through STAT4, leading to inactivation of the MAPK signaling pathway linked to detrimental PD pathology associated with the loss of dopaminergic neurons and associated motor deficits. Overall findings suggest that conservation of STAT4 signaling may be a therapeutic target for neuroprotection in the context of PD (38).

The researchers, used an experimental cellular model of PD by applying neural-induced adipose-derived stem cell conditioned medium (CSC) to treat SH-SY5Y cells exposed to Rotenone, a pesticide known to induce PD-like symptoms in animals and humans. The results demonstrated that the application of CSC was able to reverse the dysregulation of autophagy caused by Rotenone, and to restore the normal ratios of p-mTORC1/mTORC2 and ULK1 in the cells, as well as the levels of the ATG13 and Beclin-1 proteins, and to decrease the levels of p-AMPK and DEP-TOR proteins, and to enhance the amounts of p-Akt/p-ERK1/2 proteins, while decreasing GSK3β activity, returning to the normal morphological and functional form the LC3B, p62, ATG3/5/7/12, and lysosomal markers LAMP1/LAMP2/TFEB, ultimately restoring protection against alpha-synuclein aggregation and associated toxicity by a comprehensive restoration of the mTOR-dependent autophagic flux and lysosomal function (39).

The authors investigated the therapeutic potential of SHED-CM (CM from human exfoliated deciduous teeth) in a rat model of PD induced by rotenone via IHBM (IVSHQM) administration. The results show that administration of SHED-CM (100μg/mL) significantly improved motor function as measured by the Rotarod test and demonstrated increased levels of tyrosine hydroxylase in the striatum and SNc, decreased levels of α-synuclein in both regions, and decreased levels of the microglial marker Iba-1 and T-cell marker CD4, indicating decreased levels of neuroinflammation. Proteomic analysis identified several key factors secreted in SHED-CM, including IGFBP-6, TIMP-1, TIMP-2, and TGF-β1. Transcriptomic and pathway analyses demonstrated that the effects of SHED-CM on motor function, neuroinflammation, and regeneration were mediated by the modulation of cholinergic synapses, calcium signaling pathways, serotonergic synapses, and axon guidance. Thus, the findings of this study suggest that SHED-CM possesses neuroregenerative and anti-inflammatory properties (40).

Hypoxic neural injury

On SH-SY5Y cells exposed to CoCl2 for hypoxia-induced-like neural damage: G-CSF, CM from dental pulp stem cells (DPSCs-CM), and CM from DPSCs that were transfected with a plasmid encoding G-CSF (DPSC-CMT). DPSCs-CM and G-CSF were effective in significantly protecting SH-SY5Y against neural damage compared with controls, as indicated by resazurin and LDH assessments. The combination of DPSCs-CM and G-CSF reduced the percentage of apoptotic cells. The protective capacities of DPSC-CMT significantly improved as a result of the transfection of DPSCs with the G-CSF plasmid. Western blotting indicated significant decreases in the levels of β-catenin, cleaved PARP, and caspase-3 in hypoxic SH-SY5Y cells treated with DPSCs-CM plus G-CSF, while surviving expression significantly increased. The low levels of IL-6/IL-10 that were exposed to CoCl2 and that were treated with DPSCs-CM indicated that IL-6/IL-10 inflammatory mediators have been suppressed from their elevated base level. The proposed mechanism for therapies was the suppression of inflammatory mediators and the modulation of apoptotic pathways as part of the protective effect, with added effectiveness from a combination of G-CSF and DPSCs-CM, yielding a greater overall neuroprotective effect (29).

Ischemic stroke

The findings of this *in vitro* study using cell culture models of ischemic stroke (e.g., oxygen-glucose deprivation (OGD), hypoxia, and hydrogen peroxide exposure) showed that CM from bone marrow mononuclear cells (BM-MNCs) can serve as an important therapeutic agent by directly protecting primary cortical neurons from injury. CM from BM-MNCs decreased neuronal death by as much as 52% in OGD models. CM from BM-MNCs also modulated microglial activation, shifting it from neurotoxic, pro-inflammatory to anti-inflammatory. Furthermore, the study used CM from activated macrophages/microglia to induce toxicity in the neural culture model, a toxicity that was reversed by BM-MNC conditioned media, indicating paracrine neuroprotective effects mediated by secreted factors such as cytokines and growth factors. These findings suggest that BM-MNC conditioned media may provide a cell-free therapeutic option to protect against ischemia-reperfusion injury through neuronal survival and inflammatory inhibition (41).

This trial utilized both *in vivo* (middle cerebral artery occlusion (MCAO) in rats) and *in vitro* models. Results showed that astrocyte-conditioned media (ACM) containing pentraxin 3 (PTX3) has potential as a therapeutic agent by promoting blood-brain barrier (BBB) integrity during the acute phase of ischemic stroke. Results found that ACM from wild-type astrocytes prevented the permeability of brain endothelial cell cultures treated with oxygen-glucose deprivation (OGD), whereas ACM from PTX3-knockout astrocytes did not prevent permeability. It

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was determined that PTX3 is a key bioactive component of ACM. PTX3 in ACM regulated vascular endothelial growth factor (VEGF) signaling, enhancing tight junctions and decreasing BBB permeability and integrity. Furthermore, the *in vivo* experiment showed that PTX3 deficiency resulted in increased edema and larger slice infarction size, while exogenous PTX3 was potentially able to rescue BBB function, at least through CM delivery of PTX3. Importantly, ACM is a paracrine therapeutic approach to prevent BBB permeability dysfunction caused by injury to the brain and limit damage due to stroke via anti-permeability (42).

Using a rat permanent middle cerebral artery occlusion model, the authors examined ultracentrifuged conditioned medium from human pluripotent stem cell-derived neural precursor cells. Continuous intravenous (IV) dosing (200 µg) at either one (24 hr) or multiple doses (24 hr, 3, 6, 11 days post-occlusion) generated a 29% reduction in infarct volume (single dose) and a 24% decrease (multiple doses), which was less than a 56% decrease in the controls. It was associated with improved recovery (grasping, balance & neurological scores) for up to 15 days. Moreover, the NPC-CM treatments reduced microglial/astrocyte activation, increased M2 microglia, and increased neurogenesis (DCX+/BrdU+ neuroblasts, nestin+cells). The proteome and transcriptome data suggested multiple potential secreted factors (such as galectin-1, midkine, VEGF-beta, etc.) during treatment that may have supported cellular development and angiogenesis (and thus recovery, as measured by functional activity), along with up-regulation of BDNF and Neurod1 and down-regulation of inflammation. Overall, multiple doses of NPC-CM were superior at the time points examined compared with a single dose, highlighting the potential of NPC-CM as a clinically relevant cell-free therapy but warranting long-term studies to reproduce and confirm these findings (43).

Conditioned medium of interleukin-1α-primed mesenchymal stem cells, or their secretome medium, on recovery from stroke in mice produced a 30% decrease in lesion volume when administered early in lesion formation and improved neurological outcomes even when administered days after occlusion. This secretome medium, rich in a plethora of growth factors and vesicles, has since been shown to promote anti-inflammatory and regenerative pathways that support recovery from functional loss (44).

Shooshtari *et al.* (2025) evaluated the neuroprotective ability of conditioned medium from adipose and liver MSCs in a rat model of global cerebral ischemia-reperfusion injury. Suppose we consider both the rAD-MSC-CM, which was retrieved from the adipose tissue surrounding the epididymis of male Wistar rats, and the cLD-MSC-CM, which was derived from the liver of 10-day-old chicken embryos. The *in vivo* study involved male Wistar rats in which a basal global cerebral ischemia was induced using the four-vessel occlusion (4VO) model, and the CM was administered intravenously at a dose of 0.5 mg/kg 30 min after reperfusion. The study reports that the administration of rAD-MSC-CM and cLD-MSC-CM significantly improved memory function through behavioral testing; reduced anxiety- and depression-like behaviors, exploratory behavior, and BBB permeability; decreased cerebral edema (brain water content); enhanced antioxidant properties (reduced oxidative stress); and improved histopathological aspects of hippocampal tissue. Some of the neuroprotective properties are likely related to the antioxidant properties of the MSC-CM (45).

Scheibe *et al.* (2012) examined the neuroprotective properties of mesenchymal stromal cell conditioned medium on cortical neurons in an *in vitro* model of cerebral ischemia using oxygen-glucose deprivation (OGD). Co-culture of primary cortical neurons and MSCs in a transwell system for 48 hr prior to OGD reduced neuronal cell death by 30-35%. Equivalent protection against apoptosis was demonstrated with MSC-CM added 48 hr prior to OGD, 30 min prior to OGD, or even after OGD. Western blotting found increased phosphorylation of both STAT3 and Akt in neuronal cultures following treatment with MSC-conditioned media. Inhibition of the PI3K/Akt pathway eliminated the neuroprotective properties of MSC-conditioned media, suggesting that MSCs enhance neuronal survival through an Akt-dependent anti-apoptotic signaling cascade. Using mass spectrometry, plasminogen activator inhibitor-1 was also identified as an active compound in MSC-conditioned media. Thus, paracrine factors released by MSCs exert a protective effect against neuronal apoptosis in the OGD model of cerebral ischemia (46).

In an *in vitro* model of cerebral ischemia/reperfusion following Oxygen glucose deprivation (OGD) of astrocytes, the hair follicle stem cell (HFSC) cretome significantly improved astrocyte viability and function during OGD-induced injury, with reduced cell death and cellular loss observed with its presence. In addition to the *in vitro* studies, *in vivo* studies were performed using the middle cerebral artery occlusion (MCAO) stroke model in rats. HFSC or HFSC secretome was delivered intravenously and was found to improve neurological scores, decrease infarct volume, and improve recovery. The mechanistic sections of the studies outlined are an important aspect of the work. It was shown that the HFSC secretome, containing multiple growth factors, including hepatocyte growth factor (HGF), diminished the inflammatory response in the MCAO model, attenuated BBB permeability, enhanced angiogenesis in the penumbra, diminished oxidative stress, and attenuated apoptosis in the ischemic condition. The study concluded that the HFSC secretome could act via a paracrine-mediated neuroprotective mechanism in ischemic neural injuries (47).

Faezi *et al.* (2018) showed, through their research on a rat model that underwent a 60-minute occlusion of the middle cerebral artery (MCA), that the infusion of amniotic mesenchymal stem cell-derived conditioned medium (CM) i.c.v. 30 min post-reperfusion significantly reduced the size of the infarcted area, brain swelling, serum extravasation index, neuronal loss within the motor cortex, and the increased production of apoptosis initiators, specifically caspase-3 and Bax, compared with control animals. In contrast, the infusion of CM increased Bcl-2 levels, a known anti-apoptotic agent, indicating that CM acted both systemically to provide neuroprotection and locally via direct anti-apoptotic action during acute injury (48).

Injected concentrated amniotic fluid-derived stem cells into the brain 30 min after inducing injury from poor blood flow in a rat's brain. Reduced neuronal and DNA damage, as measured by Nissl and TUNEL staining, and activated molecular processes that led to the generation of ERK1/ERK2, BDNF, VEGF, and NGF, thereby promoting the formation of new neurons and blood vessels and decreasing neuronal death (49).

Exposed astrocytes to low oxygen and glucose levels for 6, 24, or 48 hr. Followed by 24 hr of returning to normal

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conditions to test whether CM produced from astrocytes treated with 6 hr of low oxygen and glucose treatment would be able to protect neurons from dying during the first 6 hr of low oxygen and glucose treatment. Results indicated that CM produced from astrocytes treated with 6 hr of low oxygen and glucose was able to increase the number of living neurons significantly, maintain the healthy structure of the neurons, support normal mitochondrial activity, decrease the number of dead cells, and stimulate the process that breaks down and recycles damaged cellular components (autophagy), resulting in the up-regulation of a protein called LC3b-II and down-regulation of a protein called p62. However, the result was not replicated using CM from astrocytes treated for more than 6 hr under low-oxygen, low-glucose conditions (50).

Hypoxic preconditioned medium (hypoxic BMSC-CM) from bone marrow mesenchymal stromal cells using a model of ischemic stroke (middle cerebral artery occlusion in the rat). Given intravenously, reduced stroke size to levels comparable to those of normoxic CM or DMEM (Dulbecco's Modified Eagle's Medium) alone and also provided additional benefits, including reducing apoptotic cells and increasing blood vessel formation, compared with the other two treatment groups. Cytokine array and label-free proteomics resulted in the identification of 163 proteins found to be differentially expressed between hypoxic BMSC-CM and DMEM, 107 of which were still expressed in greater amounts (i.e., increased concentration of secreted proteins) when created from BMSCs subjected to hypoxic conditions. This study demonstrated that hypoxic preconditioning increased the potency of the secretome resulting from BMSCs (51).

In a separate study, authors tested the use of adipose-derived stem cells grown in a three-dimensional spheroid system to generate conditioned medium (adipose SC-CM). When tested in the rat middle cerebral artery occlusion model, the continuous infusion of this medium (started at day 8 post-stroke) showed reductions in the volume of the infarct, maintenance of motor function as measured by the Rotarod test, as well as an increase in the number of blood vessels, as indicated by the number of CD31 positive micro vessels and decreased numbers of TUNEL positive apoptotic cells located within the penumbral area. Most notably, there were marked decreases in microglial apoptosis and astrogliosis within the penumbral area (52).

Traumatic brain injury (TBI)

Amini *et al*., in their publication in 2019, outlined the influence of CM from adipose-derived mesenchymal stem cell concentrate on glutamate-regulating proteins and synaptic transmission in TBI, and provided a mechanism by which they efficiently modulate the M2 polarization of microglial cells, down-regulating TNF-α and stimulating BDNF release to block excitotoxicity as well as enhancing neuronal repair (22). Furthermore, other research has shown the extracellular vesicles from clonal mesenchymal stem cells derived have also shown to improve injury in a mouse model of TBI through decreasing injury through a reduction of cystosis and apoptosis, the mechanism of action was through uptake of miR-21 by the extracellular vesicles taken them up and inhibition of the TLR4/NF-κB pathway to suppress inflammation, as well they activated the PI3K/Akt pathway to provide neuroprotection (53).

Systemic treatment with conditioned medium from interleukin-1-primed mesenchymal stem cells (MSCs) enhances recovery from TBI. The proposed mechanisms include direct promotion of BDNF and GDNF activity, reduced microglial activation, and induction of neurogenesis (54). This *in vitro* study investigated the neuroprotective mechanisms of 2-carba-cyclic phosphatidic acid (2ccPA), a lipid mediator, in a TBI model, with a key focus on the therapeutic role of CM derived from 2ccPA-treated primary cultured astrocytes. Astrocytes treated with 2ccPA showed up-regulated expression of tenascin-C (TN-C) at both mRNA (dose- and time-dependent via RT-PCR) and protein levels (via immunoblotting and immunocytochemistry), indicating enhanced astrocytic reactivity. In a scratch injury model using primary cortical neurons, CM collected from these 2ccPA-treated astrocytes significantly suppressed neuronal apoptosis, as evidenced by reduced levels of cell death markers. This paracrine neuroprotective effect was mediated specifically by TN-C, as TN-C knockdown in astrocytes abolished the CM's anti-apoptotic benefits. The findings suggest that 2ccPA-induced CM acts as a cell-free therapeutic agent in TBI by delivering astrocyte-secreted TN-C to promote neuronal survival, highlighting its potential to modulate glial responses and mitigate secondary injury without direct cell administration (55).

Liu *et al*. (2020) examined the influence of human umbilical cord perivascular cell-conditioned media (HUCPVC-CM) in an adult zebrafish model of TBI induced by pulsed high-intensity focused ultrasound (pHIFU). The authors evaluated the impact of HUCPVC-CM administered at 1 hour postinjury. The authors concluded that HUCPVC-CM administration improved outcomes following pHIFU-induced TBI. The western blotting (WB) and immunohistochemistry (IHC) results were consistent with the authors' conclusions, as HUCPVC-CM significantly reduced reactive astrogliosis at 24 hr post-injury ($P<0.05$). Most notably, at 24 hr postinjury, the HUCPVC-CM treatment showed fewer apoptotic cells in HUCPVC-CM-treated zebrafish, as indicated by IHC analysis. The authors' behavioral analysis showed improvements in locomotion ($P<0.05$) and anxiety ($P<0.05$) at 6 hr and 24 hr postinjury following HUCPVC-CM treatment. In conclusion, the authors evaluated HUCPVC-CM for the treatment of acute TBI-associated outcomes in a pHIFU zebrafish model. The data demonstrate that a cell-free therapeutic approach can be used to treat severe TBI, likely through paracrine mechanisms to reduce astrogliosis and apoptosis (56).

Huntington's disease (HD)

Investigation on the longevity-associated variant of BPIFB4 (LAV-BPIFB4) for its ability to enhance CXCR4-mediated striatal-microglial crosstalk in HD using R6/2 mice. AAV gene therapy with LAV-BPIFB4 improved motor dysfunction, theorized to be through preventing body weight loss associated with mutant huntingtin aggregation. Cell lines derived from striatum with expanded Htt (STHdh Q111/111) showed LAV-BPIFB4 overexpression through sBPIFB4 secretion and proliferative/survival response. CM from LAV-BPIFB4-overexpressing cells directed microglia toward a pro-resolving, anti-inflammatory CD163+ IL-10-high phenotype with help from CXCR4 and stromal cell-derived factor-1 (SDF-1). In an *in vivo* model, LAV-BPIFB4 conferred CXCR4-dependent neuroprotection, delaying disease progression (57).

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In the study, the researchers utilized a striatal neuronal cell line expressing either wild-type (HTT) or mutant (mHTT) huntingtin to model HD. The study showed that GCM isolated from glia increased neuronal survival in the HD cell model under stress conditions such as serum deprivation. The study discovered a mechanism by which GCM exerts neuroprotective effects: namely, by decreasing oxidative stress and improving mitochondrial function. The GCM did this by decreasing reactive oxygen species (ROS) in the HD-model neuronal cells and stabilizing mitochondrial membrane potential. This is especially important because mHtt is known to cause mitochondrial damage, thereby increasing oxidative stress and neuronal death. The researchers suggest that glial-derived factors are likely antioxidants or growth factors that prevent pathological processes by enhancing cellular resilience. Although the specific molecular factors in GCM responsible for this protective effect are not elucidated in this study, this work suggests that interactions between glial cells and neurons protect cells from neurotoxic activity associated with HD (58).

Infusion of Glial-Cell Conditioned Medium Derived From Fetal Mice Into R6/1 Transgenic Mice Enhances Autophagy Activity And Supports Trophic Factors, The continuous infusion of fetal mouse glial-conditioned medium over 15 or 30 days into the striatum of R6/1 transgenic mice resulted in significant decreases in the levels of huntingtin aggregates in the ipsilateral cortex and both striata; preservation of medium-sized spiny DARPP-32+ neurons within the striatum; preservation of dopaminergic TH+ neurons within the substantia nigra; decreases in the p62 autophagy substrate; increases in autophagic indicators including LC3-II, Beclin-1 and LAMP-2; and increases in levels of BDNF, which suggests that Glial-Cell Conditioned Medium Derived From Fetal Mice Provides Neuroprotective Effects Through Enhancements In Autophagy Activity And Support Of Trophic Factors (59).

Amyotrophic lateral sclerosis (ALS)

The use of spontaneous primary rat ventral spinal cord neuronal cultures, as well as organotypic chunks of spinal cord cultures exposed to ACM from primary mouse ALS astrocytes carrying mutant SOD1G93A, as well as iPSC-derived human astrocytes carrying SOD1D90A and TDP43A90V mutations, revealed that these ALS/FTD-ACMs dramatically reduced MN viability and exaggerated measures of oxidative stress concurrently - replicating the toxic paracrine effects of diseased astrocytes - and that GRT-X treatment consistently protected MNs from degeneration using ACM, and did not allow oxidative stress to increase in MNs. In sum, ACM could be used to mimic astrocytic-mediated toxicity in ALS/FTD and suggest that bi-activation of Kv7.2/3 and TSPO may oppose the deleterious effects (60).

Tang *et al.* (2023) studied the various anti-inflammatory and neuroprotective effects of umbilical cord mesenchymal stem cell conditioned medium (UCMSC-CM) using the SOD1-G93A transgenic mouse model and under *in vitro* conditions of BV-2 microglia overexpressing SOD1-G93A. In this study, systemic intravenous UCMSC-CM (100 µl) increased the lifespan of SOD1-G93A mice, inhibited microglial activation and astrogliosis, reduced proinflammatory cytokine and iNOS expression (TNF-α, IL-1β) in the spinal cord, and normalized the inflammatory environment. In LPS-stimulated BV-2 cells under *in vitro* conditions, UCMSC-CM reduced the levels of iNOS and proinflammatory cytokines; when using transwell co-culture conditions with BV-2 cells and astrocytes, and with microglia CM, UCMSC-CM regulated the microglial secretome and prevented the conversion of astrocytes to neurotoxic A1 astrocytes. Collectively, these results support the view that UCMSC-CM's paracrine mechanisms inhibit glial-mediated neuroinflammation and provide evidence for the use of UCMSC-CM as a cell-free therapy for ALS (61).

In vitro, CM from ALS iPSCs, CM from human embryonic stem cells (hESCs), as well as CM from wild-type iPSCs, were tested on ALS-MNs, with significant protection against both apoptosis and cytotoxicity, enhanced formation and maintenance of neurites, restored mitochondrial function (i.e., membrane potential and reduced ROS), and decreased inflammatory genes such as TNF-α, IFN-β1, and IP-10. Notably, CM rescues were markedly distinct from cyclosporine A (CsA) administration, which stabilized mitochondrial membrane potential and reduced the inflammatory response but failed to protect against MN death. CM from ALS iPSCs rescued denervation and neuron death totally unlike CsA. *In vivo*, the effect of ALS iPSC-CM was confirmed in SOD1 G93A transgenic mice, with 200 µl of ALS iPSC-CM (from CS07 and CS053) given subcutaneously. ALS iPSC-CM enhanced neuromuscular junction integrity, muscle innervation, muscle mass, posture, strength, and neurological scores; delayed the onset of paralysis; and increased lifespan when compared with vehicle or fibroblast-CM controls. The CM was subsequently characterized biochemically, and the data suggested that CM-spurred activity reflects pluripotency (pluripotency is lost when differentiating iPSCs into fibroblasts) and that the CM activity occurs via an EZH2-dependent epigenetic process (62).

Sun *et al.* (2013) assessed the protective effect of the conditioned medium of MSC (mesenchymal stromal cells) on primary motor neurons, NSC34 (neuroblastoma) cells, and astrocytes derived from both wild-type and SOD1(G93A) mutant mice (a model of ALS) in an *in vitro* experiment. MSC-CM reduced staurosporine-induced apoptosis in a dose-dependent fashion and also stimulated astrocytes' up-regulation of GDNF (glial-derived neurotrophic factor) and CNTF (ciliary neurotrophic factor), as well as stimulated NSC34 cells' up-regulation of VEGF (vascular endothelial growth factor). In addition, treatment with MSC-CM reduced the expression of the pro-inflammatory cytokines TNF-α and IL-6. It decreased iNOS (inducible nitric oxide synthase) production in astrocytes while simultaneously increasing neuroprotective fractalkine (CX3CL1) levels in mutant astrocytes and up-regulating CX3CR1 in mutant microglia. These findings suggest paracrine modulation of apoptosis, inflammation, and neurotrophic support (63).

Lan *et al.* (2022) showed that 24 hr of treatment with conditioned medium from human umbilical cord-derived mesenchymal stem cells (HUC-MSCs) significantly increased cell viability, decreased LDH (lactate dehydrogenase) release, and decreased aggregation of TDP-43 (M337V) protein in motoneuron-like NSC34 cells. In addition, treatment with conditioned medium from HUC-MSCs decreased oxidative stress markers (MDA, 4HNE, protein carbonyl, and 8-OHdG) and down-regulated nuclear Nrf2 and HO-1 (heme oxygenase-1) protein levels. Finally,

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ML385, which inhibits the translocation of the Nrf2 protein to the nucleus, attenuated the neuroprotection conferred by treatment with HUC-MSCs conditioned medium. Therefore, neuroprotection conferred by HUC-MSCs conditioned medium appears to occur primarily through the activation of the Nrf2/HO-1 antioxidant pathway (64).

The human exfoliated deciduous tooth conditioned medium significantly decreased the intracellular aggregates and neurotoxicity induced by the mutant SOD1 transfected NSC-34 cells and the model of familial and sporadic ALS derived via inducing pluripotent stem cells differentiating into motor neurons. The decrease observed with the use of the CM is partially attributable to the presence of heat shock proteins and the activation of the Insulin-Like Growth Factor-1 Receptor Signaling Pathway, which supports the notion of paracrine-mediated inhibition of protein aggregation and enhancement of neuronal survival in both familial and sporadic ALS (65).

Spinal cord injury (SCI)

The article by Yang *et al.* (2024) reported that hypoxic preconditioned mesenchymal stem cell (MSC)-derived small extracellular vesicles (sEVs) promoted recovery from SCI by delivering miR-21 from H-sEVs, thereby converting astrocytes to an A2 phenotype via STAT3, limiting inflammation, and promoting neurogenesis (66). Cheng *et al.* (2017) studied neural stem cell-conditioned medium for SCI treatment *in vitro* and in mice, which improved motor function and lesion healing mainly by reducing pro-inflammatory cytokines and reprogramming macrophages to an M2 phenotype. Secreted neurotrophic factors suppress inflammation and promote tissue repair, thereby supporting adequate neuroprotection (67).

In one publication, mesenchymal stem cell-conditioned medium was associated with increased glial reactivity but decreased neuronal survival after SCI. The attribution of conditioned medium to this mechanism could be related to, but not limited to, CM up-regulating GFAP and promoting astrocyte activation. Again, this model may increase inflammation, but it still assesses some degree of neuroprotection through BDNF up-regulation (68). ST2 conditioned medium was associated with dorsal horn cell excitability and transmission in SCI. Yet again, the attribution of conditioned medium to this process depends on the mechanisms already discussed but conditioned medium may contribute to relative increases in microglial activation and neuropathic pain (69). Conversely, the utility of conditioned medium derived from rapidly expanded mesenchymal stem cells may provide an even better alternative for neural repair in SCI. This utility likely depends on CM abundant in GDNF and VEGF to stimulate remyelination and minimize inflammatory events (70).

Szekiova *et al.* (2018) examined the neuroprotective properties of conditioned medium from rat adipose tissue-derived mesenchymal stem cells (ATMSC-CM) in an *in vitro* model of SCI inflammation using cortical neurons. Primary cultures from postnatal day (P6) Wistar rat brain cortex were challenged with SCI-CM from a central lesion and from rostral and caudal segments of the injured spinal cord. After 48 hr of incubation with SCI-CM, the culture medium was replaced with ATMSC-CM for a further 72 hr. Immunocytochemical assessment indicated a significant reduction in the number of MAP2-positive neurons exposed to SCI-CM compared with controls. ATMSC-CM is protective relative to primary cultures exposed only to DMEM, clearly supporting the secretion of neuronal survival factors by ATMSCs. The effect on astrocytes was more variable, with no apparent effect. The study shows that inflammation-like SCI-CM can negatively affect the survival of cortical neurons, and that exposure to ATMSC-CM can rescue that neuronal population, likely via neuroprotective and trophic factors released. That astrogliosis was unaffected by ATMSC-CM (15).

Liang *et al.* (2014) researched the neuroprotective and regenerative effects of neural stem cell conditioned medium (NSCM) from human fetal neural stem cells (hNSCs) in a rat model of SCI. In their contusion injury model, NSCM administered continuously after injury reduced apoptotic profiles, as evidenced by reduced caspase-3 activation at 7 days, and protected against neuronal loss at 6 weeks post-injury. There was also extensive growth of the corticospinal tract (CST) in the cervical region, along with greater than tripling of synaptic contacts made by CST collaterals and propriospinal interneurons projecting from cervical to lumbar levels; this synaptic activity would help re-establish a relay circuit, and nerve axons were repaired and regenerated, which is important. This mechanism is thought to be mediated by the paracrine secretion of neurotrophic factors that promote neuronal survival, inhibit apoptosis, and support axonal elongation and synaptic remodeling. The authors conclude that NSCM has the potential to serve as a cell-free therapy for recovery after SCI (9).

In rats with spinal contusion injuries by injecting umbilical cord blood $CD34^+$ conditioned medium intravenously immediately following the injury conditioned medium decreased the severity of motor deficits, decreased the number of cells undergoing apoptosis, reduced inflammatory phenomena and the oxidative stress associated with the contusion, and diminished astrogliosis within the spinal cord of the injured rats, demonstrating that umbilical cord blood $CD34^+$ conditioned medium produces similar neuroprotective effects as do $CD34^+$ cell transplants, and that co-administration of 17β-estradiol further enhances the effects of umbilical cord blood $CD34^+$ conditioned medium. Data confirmed that umbilical cord blood $CD34^+$ conditioned medium provides neuroprotection via a paracrinally mediated mechanism, owing to its anti-apoptotic, anti-inflammatory, and anti-oxidant properties (71).

Multiple sclerosis (MS)

Yousefi *et al.* (2016) investigated the *in vivo* immunomodulatory effects of adipose-derived mesenchymal stem cell (AMSC) conditioned medium (CM) as a therapeutic approach to experimental autoimmune encephalomyelitis. They reported that AMSC-CM modulated autoimmune responses via mechanisms that decrease the pro-inflammatory cytokines IFN-γ and IL-17, induce Treg cells, and reduce demyelination via IL-10 (72).

Safaeinejad *et al.* (2021) explored the synergistic effect of amniotic epithelial stem cell-conditioned medium (AEC-CM) and ponesimod in an *in vitro* model of MS using cuprizone-exposed OLN-93 oligodendrocytes. The combination therapy not only increased cell viability and decreased apoptosis in the large majority of NEW/OLN-93 cells, but also appeared to mediate these effects by down-

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regulating apoptotic markers (caspase-3, caspase-8, Bax, Annexin V) and up-regulating the BCL-2/Bax apoptotic ratio, suggesting modulation of mitochondrial events of apoptosis to induce neuroprotection (73). adMSC-conditioned medium in an *in vitro* model of MS-relevant neurological damage, using OGD in primary rat C6 glial cells and OPCs. Reduced cell death in this model and shifted cells toward a more immature state, with the effect size positively correlated with levels of VEGF-A, BDNF, and PDGF-AA, suggesting growth factor-mediated, neuroprotective, and promyelinating effects (74).

Retinal disorders

In one study, human mesenchymal stem cells expressing erythropoietin improved retinal neuron survival in an oxygen-induced retinopathy model by reducing oxidative stress through Nrf2 activation, decreasing apoptosis, and promoting M2 macrophage polarization (75). In this *in vitro* study using pure adult retinal ganglion cells (RGCs) subjected to either oxygen-glucose deprivation (OGD) or excitotoxic injury, CM from mixed retinal cells or mesenchymal stem cells (MSCs) provided paracrine neuroprotection via vascular endothelial growth factor (VEGF). CM containing VEGF increased RGC survival by 2-3-fold (along with reduced apoptosis and increased neurite outgrowth) through VEGFR2 activation of PI3K/Akt signaling downstream. The findings also demonstrated autocrine release of VEGF from RGCs; however, the paracrine effect of CM conferred a greater protective effect, primarily from MSCs. The study neutralized VEGF in the CM and, importantly, diminished protection, demonstrating the significance of VEGF as the predominant bioactive factor in paracrine neuroprotection of RGCs. The clear implication is that CM is a practical therapeutic delivery vehicle for VEGF in retinal disorders, such as glaucoma, thereby promoting non-invasive, paracrine-based neuroprotection of injured RGCs (76).

Pereiro *et al.* (2020) evaluated the influence of adult Müller cells and Müller cell-conditioned media on the survival of stem cell-derived retinal ganglion cells. During co-culture with stem cell-derived RGC-like cells (i.e., cultures that were submitted to Müller cell-conditioned media), RGC-like viability was significantly improved across both culture conditions, and neurite length increased significantly. Notably, in stem cell-derived organoids exposed to Müller cell-conditioned media, Atoh7, a transcription factor required for RGC development, was up-regulated, suggesting that Müller cells can improve the survival of retinal progenitors and/or postmitotic precursor cells. The mechanism suggests that Müller cells secrete factors that promote neuronal maturation and neurogenesis (77).

Peng *et al.* (2024) evaluated the regenerative capacity of human amniotic mesenchymal stem cell-derived conditioned medium (hAMSC-CM) and exosomes (hAMSC-exo) in oxidative stress-induced retinal degeneration using H2O2-treated ARPE-19 retinal pigment epithelial (RPE) cells *in vitro* and sodium iodate-induced models in rats *in vivo*. *In vitro* data indicated that hAMSC-CM and hAMSC-exo resulted in greater cell viability, inhibited intrinsic apoptosis, and improved mitochondrial dysfunction. At the same time, the use of an exosome inhibitor, GW4869, confirmed that exosomes played a central functional role in these effects. *In vivo*, hAMSC-CM (hAMSC-molecule therapy) or hAMSC-exo administered intravitreally (IVT) up-regulated RPE degeneration and improved retinal structure. The proposed mechanism of action of hAMSC-derived factors involves the PI3K/Akt/FoxO3 signaling pathway to suppress apoptosis, reduce oxidative damage, and promote cellular survival, collectively positioning hAMSC-derived factors as a new cell-free therapeutic for managing retinal degenerative disease (78).

In 2013, researchers wrote about the use of conditioned medium from Human adipose-derived stem cells (hASC-CM) as a possible treatment for photoreceptor degeneration using both) *in vitro*)tunicamycin/H2O2/ light exposed photoreceptors). These researchers tested hASC-CM through both in though hASC-CM provided photoreceptor degeneration protection via reduced cell death and preservation of outer nuclear layer thickness on an electroretinography basis and) *in vivo* (light-inflicted retinal damage in mouse models). Through the use of antibody microarrays, hASC-CM provided for the identification of two critical factors in the mechanism of action; tissue inhibitor of metalloproteinases 1 (TIMP-1)and secreted protein, acidic, and rich in cysteine (SPARC), both of which appeared to provide protective benefits from hASC-CM in both *in vitro* and *in vivo* through distinct mechanisms (79).

Results

This systematic review included 58 preclinical studies evaluating the neuroprotective effects of CM across models of AD (AD; n=5), PD (PD; n=10), ischemic stroke (n=12), TBI (TBI; n=5), HD (HD; n=3), ALS (ALS; n=6), SCI (SCI; n=6), MS (MS; n=3), retinal disorders (n=5), and hypoxic neural injury (n=1).

CM, which originates from MSCs, astrocytes, and glial cells, has been shown to exert protective effects on the nervous system. CM reduces neuronal apoptosis, neuroinflammation, and oxidative stress; promotes neuronal survival, neurogenesis, and angiogenesis; stimulates axon repair; and enhances functional recovery.

In AD models, CM reduces beta-amyloid pathology, synaptic loss, pro-inflammatory cytokines, and plaque burden, while also promoting neuronal survival, cognitive function, and the expression of neurotrophic factors such as BDNF and NGF.

In models of PD, CM prevents dopaminergic neuronal damage caused by toxins (such as 6-OHDA, rotenone, and MPP+), reduces oxidative stress and apoptosis, shifts the microglial population towards an anti-inflammatory phenotype, and promotes the expression of neuroprotective factors such as BDNF, GDNF, and IGF-2.

In ischemic stroke models, CM reduced infarct volume, edema, and BBB permeability; modulated microglial activation; promoted angiogenesis and neurogenesis; and improved neurological scores and behavioral outcomes.

In TBI models, CM attenuated glutamate excitotoxicity, oxidative stress, apoptosis, and neuroinflammation, thereby supporting long-term recovery.

In models of HD and ALS, CM protected motor neurons from apoptosis, oxidative stress, and glial-mediated neuroinflammation, while preserving motor neuron function and extending the lifespan of transgenic models.

The administration of CM was also shown to reduce inflammatory cytokine levels and apoptosis in SCI models. The administration of CM promoted axonal regeneration, remyelination, and reconnection of propriospinal relay pathways, thereby improving motor function in SCI models.

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In MS models, CM reduced pro-inflammatory cytokine levels, reduced demyelination and apoptosis, and induced regulatory T cells with pro-myelinating effects.

In models of different types of retinal degenerative diseases, CM has been shown to enhance survival rates of retinal neurons, reduce oxidative stress and apoptosis, promote neurite outgrowth, and improve retinal structure and function by using various growth factors, such as vascular endothelial growth factor (VEGF) and erythropoietin.

In many cases, optimizing results requires both preconditioning the source cells (e.g., hypoxia and/or chemical treatment agents) and using components such as extracellular vesicles, which have been shown to enhance CM effects significantly. Due to inadequate reporting of blinding and CM characterization, most studies have unclear risk of bias.

Cross-disease mechanistic comparison

Across multiple neurological and neurodegenerative disorders the reduction of neuroinflammation (via M2 polarization of microglia in PD stroke, TBI, ALS and SCI), anti-apoptotic activity (observed in all disorders), decreased oxidative stress (a major factor in AD, PD, TBI, HD and retinal disorders) and increased neurotrophic support (e.g., up-regulation of BDNF/GDNF in AD, PD, stroke and SCI), were the most common biological mechanisms. In contrast, in acute injury models (stroke, TBI, and SCI), angiogenesis and neurogenesis were often enhanced. While synaptic preservation predominated in degenerative disease models (e.g., AD, PD). Preconditioning enhanced multiple effects in AD, PD, stroke, and SCI, primarily by enriching trophic factors and extracellular vesicles (EVS). EVs also emerged as a critical mediator in TBI, SCI, and retinal disorders.

Discussion

The 58 studies that have been successfully reviewed have been able to demonstrate a substantial amount of preclinical evidence demonstrating how CM from mesenchymal stem cells (MSCs) and glial cells, as well as the neuroprotective characteristics of CM, have demonstrated effects in the area of neuroprotection through the multiple spectra of neuroprotection, including neuroprotection in the treatment of neurological disorders in humans. The inclusion of CM in the treatment of AD, PD, TBI, HD, ALS, SCI, MS, and Retinal Disorders has led to the significant reduction of many of the main pathologies associated with each of these disorders, including neuronal apoptosis, neuroinflammation, oxidative stress, and neurotransmitter imbalances, as well as the promotion of neurogenesis, neuronal survival, and functional recovery. The complete agreement of the findings with 100% of the studies is, without question, an indication of the reliability of CM in reversing the effects of pathological processes resulting from neurological disorders.

Upon analysis of the overall effects of MSC-derived CM across a variety of neurological disorders and injuries, we see that MSC-CM exerts paracrine-based mechanisms, including immunomodulation, anti-apoptotic effects, and enhanced synaptic plasticity. For example, CM from MSCs in animal models of AD preserved synaptic integrity and decreased amyloid-beta pathology through factors secreted by either astrocytes or stem cells. In PD models, CM protected dopaminergic neurons by modulating microglial polarization, rescuing mitochondria, and providing neurotrophic support. Models of acute injury, such as stroke and TBI, demonstrate that CM increases BBB integrity, reduces infarct size, and promotes the return to more functional behaviors, potentially through the roles of VEGF, IGF-2, and other growth factors. Similar functions to those noted in the above models were observed in models of SCI and retinal injury.

In addition, comparisons among the various diseases demonstrate potential commonalities, such as changes in microglial phenotype to promote an anti-inflammatory response and enhanced anti-apoptotic mechanisms across conditions, suggesting that CM may apply to a wide range of conditions. However, for acute injuries (i.e., stroke, TBI, and SCI), the literature appears to focus more on regenerative processes (i.e., angiogenesis and axonal repair). In contrast, in chronic neurodegenerative conditions (i.e., Alzheimer's, Parkinson's, Huntington's, and ALS), the focus has been more on providing trophic support and reducing protein aggregation and/or mitochondrial dysfunction.

These results highlight CM's potential as a cell-free therapy, offering advantages over direct cell transplantation, including reduced risks of immune rejection and tumorigenesis. However, translational challenges persist. Heterogeneity in CM preparation (e.g., cell source, conditioning duration, concentration methods) hinders comparability and reproducibility. Many studies showed unclear risk of bias, particularly regarding blinding and detailed CM characterization. Furthermore, the evidence is predominantly preclinical, with limited direct replication of human disease complexity and few clinical data available.

Limitations

There are some limitations associated with this review. Firstly, the protocol was not prospectively registered (e.g., on PROSPERO) and therefore lacks transparency. Secondly, due to heterogeneity of outcomes and models amongst the studies, no quantitative meta-analysis was conducted; instead, the results have been reported narratively. Cross-disease comparisons have also been added; however, they remain limited relative to within-disease analyses. Mechanistic insights were previously synthesized; however, they would benefit from more in-depth and comparative analyses across studies.

To facilitate the progression of CM into clinical applications, there is a need for standardized, scalable protocols, comprehensive omics characterization of bioactive compounds, and studies to identify key mechanisms of action. Given the focus on humans as the source material for CM, robustly designed preclinical and controlled clinical trials should be conducted to determine the efficacy, optimal dosing, and safety of CM. CM may represent a novel standalone or adjunct combination therapy for treating neurological disorders because it is a novel approach with multiple mechanisms of action and a favorable safety profile.

Conclusion

The present systematic review provides strong evidence that CM, particularly from mesenchymal stem cells and glial cells, holds considerable therapeutic potential across multiple neurological disorders. CM-induced reductions in neuronal apoptosis, inflammation, and other disease-associated pathological features, while also promoting or enhancing neurorestoration across a variety of preclinical

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models of AD, PD, stroke, and TBI. All included studies (100%) reported positive effects. Nevertheless, variation in preparation methods, study design limitations, lack of protocol registration, and absence of a meta-analysis highlight the need for standardized protocols and further research. Future research using omic-based characterization of CM and well-designed clinical trials will be important to fully exploit the therapeutic potential of CM for brain injury versus degenerative conditions. Because of its multifactorial neuroprotective mechanisms and low risk of adverse effects, CM has the potential to serve as a cell-free therapy, used alone or in conjunction with other therapies, to improve outcomes in individuals with neurological disorders.

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Authors' Contributions

A A and Z T conceived and designed the study; A A conducted the literature search, data collection, and screening; A A and Z T analyzed and interpreted the results; A A drafted the manuscript and visualizations; Z T critically revised and edited the article; Z T supervised and secured funding; A A and Z T approved the final version for publication.

Conflicts of Interest

The authors reported no potential conflicts of interest.

Declaration

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Corrected Proof