

Review Article

# Bioregenerative Applications of the Human Mesenchymal Stem Cell-Derived Secretome: Part-II

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## Abstract

This literature review analyzes the results from studies applying conditioned medium and extracellular vesicles derived from the mesenchymal stem cell secretome to numerous disease states in animal and human in-vivo models. Information about the conditions treated and the observed benefits and side-effects of these therapeutics are discussed. Ongoing clinical trials applying conditioned medium and extracellular vesicles, recommended future research and limitations of cell-free strategies are addressed. Findings demonstrate that the mesenchymal stem cell secretome holds promise as an effective treatment for numerous disease states. This manuscript is a companion piece to “Part 1: Bioregenerative Applications of the Human Mesenchymal Stem Cell-Derived Secretome,” included in this issue, which contains background information about stem cells and mesenchymal stem cells, their limitations *in-vivo* and the advent of cell-free strategies as a viable alternative for disease treatment.

**Keywords:** Conditioned Medium; Extracellular Vesicles; Clinical Trials; Preconditioning; Bioregenerative Therapies

## Abbreviations

A $\beta$ : Amyloid- $\beta$  Plaques; AGA: Androgenic Alopecia; AD: Alzheimer’s Disease; AKI: Acute Kidney Injury; ALS: Amyotrophic Lateral Sclerosis; Apo-EV: Apoptotic Extracellular Vesicle; ASC or AD-MSC: Adipose Mesenchymal Stem Cell; BBB: Blood-Brain Barrier; BDNF: Brain-Derived Neurotrophic Factor; BM-MSC: Bone Marrow Mesenchymal Stem Cell; BPD: Bronchopulmonary Dysplasia; BUN: Blood Urea Nitrogen; CCR2: C-C Motif Chemokine

Receptor 2; CKD: Chronic Kidney Disease; CNTF: Ciliary Neurotrophic Factor; COPD: Chronic Obstructive Pulmonary Disease; CSF: Cerebrospinal Fluid; DO: Distraction Osteogenesis; DNA: Deoxyribonucleic Acid; DPSC: Dental Pulp Stem Cell; ECM: Extracellular Matrix; EPO: Erythropoietin; FGF: Fibroblast Growth Factor; GDNF: Glial Cell-Line Derived Neurotrophic Factor; GMP: Good Manufacturing Practice; GPNMB: Glycoprotein Nmb; GvHD: Graft-versus-Host Disease; HD: Huntington’s Disease; HF: Hair Follicle; HTT: Huntingtin (gene or protein); IA: Intraarterial; IBS: Inflammatory Bowel Disease; IGF: Insulin-Like Growth Factor-Binding Protein; IL: Interleukin; IQR: Interquartile Range; IS: Ischemic Stroke; IV: Intravenous; LPS: Lipopolysaccharide; MATN3: Matrilin 3; MCAO: Middle Cerebral Artery Occlusion; MH: Macular Hole; MI: Myocardial Infarction; miRNA: miRNA; MS: Multiple Sclerosis; MSC: Mesenchymal Stem Cell; MSC-ex: Mesenchymal Stem Cell Exosomes; OA: Osteoarthritis; OP: Osteoporosis; NEC: Neonatal Necrotizing Enterocolitis; NF- $\kappa$ B: Nuclear Factor  $\kappa$ B; NGF: Nerve Growth Factor; NO: Nitrous Oxide; NPC: Neimann-Pick type C (Gene or Disease); NT3: Neurotrophin-3; PAD: Peripheral Arterial Disease; PD: Parkinson’s Disease; PI3K: Phosphatidylinositol 3-Kinase; PLGF: Placental Growth Factor; PPV: Pars Plana Vitrectomy; RA: Rheumatoid Arthritis; RNA: Ribonucleic Acid; ROS: Reactive Oxidative Species; RVSP: Right-Ventricular Systolic Pressure; SCI: Spinal Cord Injury; SDF-1: Stromal Cell-Derived Factor 1; TLR: Toll-Like Receptor; UC-MSC: Umbilical Cord Mesenchymal Stem Cell; VCAM1: Vascular Cell Adhesion Molecule 1; VEGF: Vascular Endothelial Growth Factor; WJ-MSC: Wharton’s Jelly Mesenchymal Stem Cell

## Introduction

Bioregenerative therapies, including stem cells, have been increasingly applied to treat various disease states. Two novel bioregenerative therapies obtained from the Mesenchymal Stem Cell (MSC) secretome, Conditioned Medium (CM) and Extracellular Vesicles (EVs), have been the subjects of increasing research, as they are theorized to overcome many of the obstacles present with direct stem cell application while maintaining their benefits observed *in-vivo*. For more information, see “Part 1: Bioregenerative Applications of the Human Mesenchymal Stem Cell- Derived Secretome”.

## Results

Table 1 and Table 2 compile primary literature applying the MSC secretome to applicable disease models, indicating potential use cases for its application in medicine. Articles were compiled from Google Scholar searches and literature review articles [1-14]. Study designs and results are summarized below each table.

| Disease Model                        | Reference |
|--------------------------------------|-----------|
| Liver Injury                         | [15-24]   |
| Cancer                               | [25,26]   |
| Cancer Treatment Injuries            | [27-29]   |
| Heart Transplantation                | [30]      |
| Rotator Cuff Injury                  | [31,32]   |
| GvHD                                 | [33]      |
| Wound Healing                        | [34-45]   |
| Parkinson’s Disease                  | [46-50]   |
| Alzheimer’s Disease                  | [51-55]   |
| ALS                                  | [56,57]   |
| Huntington’s Disease                 | [58,59]   |
| Niemann–Pick Type C Disease          | [60]      |
| Ocular Injury                        | [61]      |
| Lung Injury                          | [62,63]   |
| Acute Osseous Injury                 | [64-73]   |
| Alopecia                             | [74-77]   |
| Skin Allograft                       | [78]      |
| Ischemic Stroke                      | [79-90]   |
| Periodontal and Dental Injuries      | [91-100]  |
| Peripheral Nerve Injury              | [101-104] |
| MI and Myocardial Reperfusion Injury | [105-111] |
| Spinal Cord Injury                   | [112-115] |
| Substance Abuse                      | [116]     |
| Traumatic Brain Injury               | [117]     |
| Osteoarthritis                       | [118-124] |
| Rheumatoid Arthritis                 | [125,126] |
| Childbirth Injury                    | [127]     |
| Asthma                               | [128]     |
| Gastric Injury                       | [129]     |
| Kidney Injury                        | [130-141] |
| Heart Failure                        | [142]     |
| Neonatal Injury                      | [143-146] |

|                                |           |
|--------------------------------|-----------|
| Diabetic Neuropathy            | [147-149] |
| Osteoporosis and Osteonecrosis | [150-152] |

**Table 1:** Animal modeling data for MSC-CM and MSC-EV studies.

### *Liver Injury*

Many of the cited studies pertained to acute liver injuries induced on animal models through partial hepatectomy procedures or liver ischemia. These studies found cell-free strategies increased hepatic regeneration by increasing hepatocyte proliferation, decreasing apoptosis and reducing oxidative stress [15,16,19-22].

Haga, et al. and Lotfinia, et al., applied these therapies to hepatic failure models and determined they modulated expressed cytokines and increased survivability [19,23].

Li, et al. and Alhomrani, et al., tested the effect of MSC-ex on liver fibrosis, determining this treatment inhibited hepatic lobule destruction, epithelial-to-mesenchymal transition, collagen synthesis, inflammatory macrophage states and serum liver damage markers. These effects explain both studies found significantly reduced liver fibrosis with exosomal treatment [18,24].

### *Cancer and Cancer Treatment Injuries*

When applied to cancer models, the secretome reduced cancer cell proliferation and significantly reduced tumor growth compared to controls [25,26]. Injuries from radiation and chemotherapy for cancer treatment were also reduced with cell-free applications. A radiation damage study found the secretome increased proliferation and salivary protein levels while reducing irradiated salivary gland apoptosis [27]. The secretome decreases doxorubicin toxicity in cardiomyocytes, preserving cardiac function through apoptosis and senescence prevention [28,29].

### *Heart Transplant*

Ellis, et al., found CM provided cardiomyocyte-protective effects that improve heart transplant model functionality by reducing the time to initiate contraction, increasing contractility and limiting apoptosis and oxidative stress [30].

### *Rotator Cuff Injury*

Chronic rotator cuff tears are a common injury with high surgical failure rates and major quality of life detriments [32]. Applying the secretome to rotator cuff injuries improved fibrocartilage regeneration, significantly decreased fatty infiltration and tendon stiffness and significantly increased tendon maturation scores [31,32].

### *GvHD*

GvHD is the most common issue when allogeneic graft transplants are performed. This diagnosis sometimes cannot be controlled by immunosuppressive therapies, posing major challenges in its treatment. Yáñez et al. applied AD-MSCs to this disease model and reported paracrine immunosuppressive properties that significantly increased survival and diminished GvHD severity [33]. This study indicates that cell-free strategies may be effective at attenuating GvHD progression.

### *Wound Healing*

MSC cell-free strategies have been frequently tested in wound healing models. Full- thickness wound studies reported the secretome increased cell viability, proliferation and wound healing speed while reducing pro-inflammatory cytokines, inflammation and macrophage metabolism [34-36]. It is worth noting that Pavushina, et al., did not correlate CM with increased wound healing speed, but this was likely due to skin trauma from repeated injections [36].

Many additional studies specifically tested wound healing in diabetic animals. These studies found CM significantly accelerated wound closure and neovascularization, reduced inflammation, increased regenerated tissue histological scores and promoted beneficial immune infiltration [37-42]. In particular, Shrestha et al. found the CM group had full diabetic wound healing within four days of administration and significantly greater capillary density [37]. Additionally, Hendrawan, et al., reported increased re-epithelization and collagen production in diabetic models [44].

In ischemic wounds, CM robustly increased wound closure [38]. After fractional carbon dioxide laser resurfacing, Bing-Rong, et al., demonstrated significantly reduced erythema and skin pigmentation but no significant difference in transepidermal water loss or skin elasticity [43]. When applied to immunodeficient mice, cell-free derivatives accelerated wound closure and increased SDF1 and CXCL-5 expression in-vivo [45].

### *Ocular Injury*

In a mild traumatic brain injury model, CM reduced the loss of visual acuity and contrast sensitivity while lowering the expression of genes correlated with ganglion cell loss, microglial activation, inflammation and immunoreactivity [61]. Kuo, et al., applied CM to amyloid- $\beta$ -induced retinal injury models, mimicking the visual degeneration associated with Alzheimer's disease. In this study, CM attenuated  $\beta$ -catenin downregulation, preserved retinal pigment epithelium organization by significantly increasing tight junction protein expression and significantly reduced degenerating and apoptotic neurons in the retina [52].

### *Neurodegenerative Diseases*

Neurodegenerative diseases currently have poor treatment efficacy. Due to their common occurrence and debilitating symptoms, alternative therapies involving CM have been tested. Parkinson's Disease (PD) is a progressive neurodegenerative disease caused by midbrain dopaminergic neuron loss. The causes of PD are not known, but certain risk factors have been identified. However, no cure has been developed. In PD studies, Mendes-Pinheiro, et al., found CM significantly lowered dopaminergic neuron loss and partially significantly improved motor functions of Parkinsonian mice [46]. Chierchia, et al., found CM hydrogel promoted cell viability and reduced oxidative damage while significantly increasing cell proliferation and average sample DNA content [47]. Abdelwahab, et al., reported CM increased similarities in cell morphology to non-PD controls and had greater effects than applied BM-MSCs [48,49]. Finally, Nakhaeifard, et al., found significantly improved lesion morphology and motor performance and significantly greater expression of the NGF, NT3 and BDNF genes [50].

ALS is another neurodegenerative disease caused by progressive motor neuron loss without definitive causes or cures. When applied to ALS, CM significantly increased post-onset lifespan by reducing microglia and astrocyte activation, decreasing inflammation and apoptosis and significantly improving motor neuron survival and neuromuscular junction innervation [56,57].

Alzheimer's Disease (AD) is a neurodegenerative disorder causing progressive dementia and mortality due to hippocampal neurodegeneration, neuroinflammation and reduced neurogenesis [51]. Additional physiological features include increased extracellular amyloid- $\beta$  ( $A\beta$ ) plaques, intracellular neurofibrillary tangles, astrogliosis and microgliosis [153]. The causes of Alzheimer's are unknown and current treatments are ineffective at long-term improvements in the neurodegeneration rate.

Venugopal, et al., used Kainic Acid models of AD and intrahippocampally injected DPSCs, BM-MSCs, DPSC-CM, or BM-MSC-CM. All these treatments significantly improved hippocampal learning and memory, significantly improved mRNA expression of neurogenic factors (BDNF, GDNF, CNTF, VEGF, PDGF-B, NGF, b-FGF, NT-3, EPO), significantly reduced microgliosis and reduced astrogliosis and neuroinflammation. DPSC-CM and DPSCs were superior in significantly increasing neurogenesis and significantly reducing excitotoxicity, neuronal loss and proapoptotic caspase enzyme activity. There were no significant effects on PDGF-A. Despite their beneficial effects, almost no MSCs were present 51 days after injection [51].

Mita, et al., used an intracerebroventricular injection of  $A\beta$  peptides for AD models and applied CM intranasally. They found decreased expression of genes linked with inflammation and oxidative stress, increased neurotrophic gene expression, increased microglia conversion from inflammatory to anti-inflammatory states and reduced glutamate neurotoxicity [54]. Mehrabadi, et al., used a similar AD model to the previous study but administered intraperitoneal normoxic or hypoxic ASC-CM once daily for eight days. CM significantly improved novel object recognition, spatial memory, neuronal morphology and neuronal count. CM also significantly downregulated TLR2 and TLR4 expression for astrogliosis reduction and significantly reduced inflammatory cytokine levels. Interestingly, CM administration entirely removed  $A\beta$  plaques from diseased models. However, CM did not significantly improve motor function [53].

Kuroda, et al., used a transgenic mouse model for AD and bone marrow-derived microglia-like (BMDML) cell CM to treat this condition. The critical finding in this study were that BMDML-CM improved A $\beta$  phagocytosis by microglia and decreased A $\beta$  plaque levels [55].

CM has also been applied to animal Huntington's Disease (HD) models. HD is a fatal neurodegenerative disease caused by autosomal dominant mutations, resulting in mutated HTT proteins. Currently, there is no cure for HD. Giampà, et al., found CM, compared to controls, significantly delayed HD-associated reflex onset, significantly improved motor coordination and balance, significantly increased activity, improved neuropathological measures, reduced neuroinflammation and significantly decreased active microglia in the striatum. However, CM did not significantly affect mouse weight loss or BDNF expression in the striatum compared to controls [58]. Ruiz, et al., also determined that CM increased cell survival and reduced ROS accumulation, polyubiquitinated proteins and other HD markers [59].

Finally, Hoecke, et al., used MSC-EVs in Neimann-Pick type C (NPC) disease. NPC is an uncommon neurodegeneration disease marked by neurovisceral lipid storage issues, peripheral organ inflammation and neuroinflammation, causing premature mortality. This disease results from loss-of-function mutations in the NPC1 or NPC2 genes, causing late endosomes and lysosomes to retain lipids deleteriously. This study determined EVs reduced splenomegaly, significantly lowered inflammatory cytokine expression in peripheral and central neural tissues and lowered microgliosis and astrogliosis *in-vivo* [60].

#### *Lung Injury*

The MSC secretome has been applied to animal lung damage models. In acute respiratory distress models, CM lowered LPS-induced histopathologic damage and reduced pro-inflammatory cytokine, ROS and apoptotic marker levels [62]. Additionally, Kim et al. reported CM increased angiogenesis and lung regeneration by inhibiting apoptotic death and promoting proliferation in lungs damaged by tobacco smoke [63].

#### *Acute Osseous Injury*

Studies also applied the secretome to acute osseous injuries. Frequently, these studies applied MSC-EVs or MSC-CM to rat calvarial bone defects. These studies consistently reported faster bone regeneration, greater regenerated bone area and angiogenesis and more histological findings of osteoblast-like and vascular endothelial cells compared to controls. Furthermore, they frequently linked these results to VEGF [66,68]. Osugi et al. and Chang et al. had particularly interesting calvarial defect experiments, where they showed MSC-CM groups had significantly greater ossified regeneration area and induced significantly greater resident MSC migration to the bone defect than the control or MSC groups [65,70]. Additionally, Zhuang, et al., determined hypoxic MSC-EVs significantly increased osteogenesis and angiogenesis compared to normoxic MSC-EVs and controls [71]. Finally, Shanbhag, et al., suggested soaking collagen membranes in CM, followed by lyophilization, significantly increased guided bone remodeling capabilities [67]. Additional acute osseous studies were applied to unique injury models. Fujio, et al., applied CM from normoxic- and hypoxic-cultured DPSCs to Distraction Osteogenesis (DO) of mouse tibias. This study found significantly higher bone formation area and X-ray density of regenerated bone from the CM treatment than the control. Additionally, the hypoxic group demonstrated significantly greater angiogenesis than the normoxic group [64]. Similarly, Ando, et al., studied DO and found MSC's beneficial effects in increasing callus area and formation speed were due to paracrine mechanisms, as they disappeared from the DO gap in consolidation and performed similarly to CM. They additionally found greater angiogenesis and endogenous MSC recruitment compared to controls [73]. Hiraki, et al., found CM enhanced mature bone formation and angiogenesis compared to MSC or control groups in cleft lip and palate orofacial anomalies [69]. In rabbit mandible injuries, Linero, et al., discovered that CM-hydrogel performed similarly in radiographic, morphometric and histological analysis compared to MSC-hydrogel treatments and both were superior to controls [72].

#### *Alopecia*

Yuan, et al., treated Androgenic Alopecia (AGA) through an innovative delivery model dissolvable microneedle patch loaded with CM. This study found that this treatment increased perifollicular vascularization, Hair Follicle (HF) stem cell activation and hair regeneration [74]. Additional studies using alopecia animal models determined CM increased hair regeneration speed compared to controls [75] and MSC-EVs produced greater hair growth, increased HF activation and thicker dermis layers [76,77].

### *Skin Graft*

Immunologic barriers and imperfect immunosuppressive regimes have limited the treatment of major dermatological injuries with skin grafts. In skin graft animal models, CM injection significantly reduced pro-inflammatory cytokine levels and significantly increased skin graft survival compared to control animals, with similar effects to ASC administration [78].

### *Ischemic Stroke*

Many studies applied the stem cell secretome to Ischemic Stroke (IS). A standard study model was rats with Middle Cerebral Artery Occlusion (MCAO), followed by intracerebroventricular or intravenous CM administration. These studies determined CM treatment significantly decreased infarct volume, increased neurogenesis, angiogenesis and trophic markers, modulated immune cytokines, microglia and macrophages in affected areas, reduced apoptosis markers and excitotoxicity, diminished astrogliosis and markedly improved behavioral recovery and neurological deficits [79-86]. These studies suggested that immediate delivery, multiple administrations and hypoxic MSC preconditioning all increased treatment efficacy [79,83,85]. Cunningham et al. reported less conclusive results, including insignificant differences in microglial and astrocyte markers and overall treatment effect compared to control. They concluded that their CM administration was delayed outside the neuroprotection window and MSC pretreatment with IL-1 $\alpha$  may not have been beneficial. However, this study still found CM caused an approximately 30% reduction in lesion volume, significantly improved mouse weight and partially significantly improved neurological and motor performance [90].

Other studies deviated from the aforementioned methodologies. Xiang et al. applied ischemic stroke via MCAO to type 2 diabetic rats, finding CM improved neurological outcomes, decreased Blood-Brain Barrier (BBB) leakage and increased angiogenic markers, all significant [87]. Faezi, et al., intracerebroventricularly injected CM in focal cerebral ischemia models, finding significantly reduced infarct volume, brain edema, neuronal loss, motor disorders and apoptotic marker expression [89]. Yang, et al., applied modified MSC-derived exosomes expressing specific miRNA to focal ischemia, concluding this treatment effectively delivered miRNAs to injury sites and promoted neurogenesis [82]. Finally, Zhao, et al., administered CM to MCAO rats intranasally; this study observed significantly increased BBB integrity, angiogenesis and vascular markers and functional recovery. However, they did not find significantly reduced lesion volume compared to controls [88].

### *MI and Myocardial Reperfusion Injury*

MI is another ischemic injury addressed by the stem cell secretome. Many researchers applied CM to rat left anterior descending coronary artery ligation models. These studies demonstrated significantly enhanced cardiomyocyte proliferation and migration; significantly decreased apoptosis, ROS, inflammatory cytokines and muscle damage markers; preserved cardiac function; significantly increased angiogenesis; and significantly reduced MI size compared to controls [105-111]. Hynes, et al., demonstrated that IGF-1 plays a major role in these cardioprotective effects [106].

A critical factor contributing to acute MI damage is myocardial reperfusion injury, occurring when blood supply returns after acute MI. This process can induce oxidative stress, inflammation, calcium overload, or endothelial dysfunction [154]. When applied to myocardial reperfusion models, CM significantly decreases myocardial injury percentage, improves cardiac histological changes and reduces oxidative stress [107]. Additionally, EVs significantly reduced myocardial apoptosis and promoted beneficial signaling pathways in reperfusion models [111].

### *Periodontal and Dental Injuries*

The MSC-secretome holds promise in treating periodontal injuries and dental injuries. In periodontal defect tests, researchers found CM significantly increased regenerated periodontal tissue, reduced inflammatory cytokine levels, promoted angiogenesis and created beneficial histological findings [91,93,94]. In periodontitis models, researchers found that MSC-EVs accelerated alveolar bone and periodontal epithelium regeneration by reducing inflammation, converting macrophages from pro-inflammatory to anti-inflammatory states, modulating osteoclast activity and decreasing bone resorption [96,99,100]. Comparing exosomes to ADSCs, Mohammed, et al., determined that MSC-EVs were superior in increasing tissue regeneration and comparable in regenerated tissue histological grades [97].

Dental injury studies were primarily centered around dental pulp regeneration. Li, et al., found that apo-EVs fused with

endothelial cells, increasing the expression of angiogenesis-related genes. These effects promoted pulp revascularization and tissue regeneration [92]. Chen, et al., applied MSC-EVs to cavity models and found regenerated tissues had increased angiogenesis, predentin-like tissue and polarizing odontoblast-like cells. Furthermore, they observed greater mineralization and more odontogenic protein expression in EV-treated models [95]. Finally, Wei, et al., found that MSC-EVs reversed bone loss and promoted osteogenesis *in-vivo* [98].

#### *Peripheral Nerve Injury*

Chen et al. demonstrated that MSC-EVs promote sciatic nerve healing *in-vivo*. They suggested that increased native neurotrophic factor expression, significantly greater neurite outgrowths and increased Schwann cell proliferation, migration and myelination capabilities contributed to this result [101]. Additionally, CM applied to sciatic nerve injuries showed improved electrical conduction metrics, cell growth and myelin sheath diameter [103]. CM reduced LPS damage to the sciatic nerve through increased endogenous neurotrophic factors and reduced inflammatory cytokines and apoptotic markers [104].

Tsurta, et al., applied CM to dysphagia models that had difficulty swallowing due to superior laryngeal nerve injuries. This treatment protected the swallowing reflex, prevented pharynx water retention, increased histological axon regeneration and myelination, lowered pro-inflammatory cytokine levels, increased M2 macrophage recruitment, raised endogenous trophic factor levels and increased angiogenesis at the injury site [102].

#### *Spinal Cord Injury*

Pinho et al. compared the benefits of the vesicular fraction, soluble fraction and whole secretome in Spinal Cord Injury (SCI) repair. This study determined that the whole secretome produced significantly greater neurite growth with better histological grading than the isolated fractions or control. Additionally, mice in the total secretome group with initially non-moving hindlimbs experienced significantly improved motor recovery compared to other treatments or controls [112]. Cheng, et al., suggested CM therapy prevents the inflammatory responses after SCI that cause neurodegeneration and further damage. This treatment significantly increased motor function and tissue regeneration while reducing pro-inflammatory cytokine expression and inflammatory macrophage activation [113]. Chudířková, et al., determined CM and WJ-MSCs similarly increased spared gray and white matter and improved neurite outgrowth gene expression, while CM superiorly increased axonal sprouting and decreased astrogliosis. Furthermore, the WJ-MSCs induced systemic immunological responses, causing increased inflammation [114]. Finally, Sun, et al., determined that MSC-EV application in SCI caused macrophage polarization to an anti-inflammatory state, significantly increased long-term motor functional recovery, significantly lowered injury cavity volume and reduced pro-inflammatory cytokine expression [115].

#### *Substance Abuse*

Quintanilla et al. intranasally applied CM to alcohol and nicotine abuse models, citing that chronic drug abuse causes oxidative damage and inflammation in the brain that inhibits the GLT-1 glutamate transporter, propagating drug abuse. They determined CM reduced chronic self-administration of alcohol (85% reduction) and nicotine (75% reduction) and further inhibited binge intake after prolonged withdrawal (85-90% reduction). Additionally, this treatment eliminated oxidative stress through normalized hippocampal oxidized/reduced glutathione ratio and reduced neuroinflammation [116].

#### *Traumatic Brain Injury*

Xu, et al., applied the hypoxic-preconditioned total secretome to traumatic brain injury models, finding significantly improved neurological function, reduced brain edema, decreased inflammatory and apoptotic markers and increased anti-inflammatory macrophage and microglia states [117].

#### *Osteoarthritis*

Osteoarthritis (OA) is a debilitating joint disease marked by impaired cartilage integrity, subchondral bone damage, osteophyte formation and inflammation. Khatab, et al., compared the efficacy of the total secretome to BM-MSC injection in OA models. This study determined that both therapies caused pain and cartilage defect reduction mirroring non-OA knees. However, they found insignificant differences between the therapeutic groups and controls in synovial inflammation, subchondral bone volume, macrophage populations and OA scores [118]. Amodeo, et al., applied CM to OA knee models, demonstrating

significant, rapid and long-lasting improvements in pain and major immunomodulatory changes [119].

Long, et al., utilized SMSC-exosomes, discovering reduced cartilage and ECM degradation, lower inflammatory cytokine concentrations and significantly increased chondrocyte viability. They strongly correlated these results with exosomal MATN3 delivery [120]. Other studies tested MSC-exosomes in OA models, finding major decreases in OA morphological characteristics, improved histological grading, significantly reduced OA scoring, pain levels, ECM degradation markers and pro-inflammatory cytokines and significantly increased anti-inflammatory cytokines [13,122-124]. These studies frequently reported attenuation of IL-1 $\alpha$  as a major factor in exosomal OA reduction [121-124].

#### *Rheumatoid Arthritis*

Rheumatoid Arthritis (RA) is another progressive inflammatory disease that significantly reduces patient life expectancy and quality of life, lacking sufficient treatments. Kay, et al., applied CM to RA models, finding reduced inflammatory cytokines and T-cells, reduced cartilage damage and suppressed immune hyperactivity *in-vivo* [125]. Additionally, Miranda, et al., found 3D preconditioned CM outperformed MSCs in decreasing swelling, arthritic scores and inflammation [126].

#### *Childbirth Injury*

Stress urinary incontinence is correlated with vaginal delivery and current treatments to ameliorate this injury are imperfect. Therefore, Dissaranan, et al., applied CM to childbirth injury animal models. This treatment significantly improved continence measures and elastin fiber concentrations in injured areas but did not significantly affect external urethral sphincter function [127].

#### *Asthma*

Asthma is a common respiratory disorder causing hyper-responsive reversible airflow obstruction through airway inflammation. Ahmadi, et al., applied CM to attempt to reduce the long-term asthmatic changes in the respiratory system. They observed significantly increased anti-inflammatory cytokines and significantly reduced tracheal responsiveness, neutrophil and eosinophil percentages and asthmatic lung injuries [128].

#### *Gastric Injury*

Xia, et al., tested the efficacy of normoxic- and hypoxic-preconditioned CM in peptic ulcer, inflammatory and acute mucosal damage injury recovery of the stomach. They observed both treatments had significant effects, including accelerated gastric ulcer healing, reduced immune infiltration, increased regenerated mucosa density and improved epithelium morphology compared to the control [129].

#### *Kidney Injury*

Acute Kidney Injury (AKI) induces sudden renal failure and is primarily caused by other major health issues. AKI significantly increases patient risk for Chronic Kidney Disease (CKD) and mortality and has limited treatments [140]. Many studies have tested MSC-derived factors in the treatment of AKI. Rhabdomyolysis, or a major release of intracellular contents from damaged muscle tissue into the bloodstream, is a major cause of AKI [155]. Studies administered EVs to this disease model, reporting significantly less severe tubular lesions, increased tubular cell proliferation, lower tubular necrosis, decreased kidney damage markers and healthier gene expression levels. These studies also highlighted the importance of EV RNA delivery in AKI healing [130,134,135].

One of the largest contributors to AKI is myocardial infarction, which produces ischemia/reperfusion-induced renal injury. When EVs were applied to this disease model, kidney function significantly improved due to greater tubular cell proliferation, reduced necrosis and apoptosis, immunomodulatory effects and decreased inflammation levels [131,136,138,140]. Shen, et al., determined that microvesicular CCR2 expression plays a critical role in ischemia/reperfusion-induced renal injury healing, while Zou, et al., found CX3CL1 reduction was important in AKI recovery [131,138].



High concentrations of toxic compounds in the blood from chemotherapy can also induce AKI. Bruno, et al., applied microvesicles to lethal cisplatin-induced AKI models. Multiple microvesicle injections significantly reduced mortality and caused normal histology and renal function in surviving mice through the anti-apoptotic effects of EVs [139]. A similar disease model was tested by Zhou, et al., finding microvesicles significantly reduced BUN and creatine levels, apoptosis, oxidative stress and tubular necrosis [132]. Overath, et al., applied CM to cisplatin-induced AKI, finding decreased immune cytokine levels, kidney damage markers and mortality rates [141].

CKD is a global public health issue involving prolonged reductions in kidney function. CKD increases mortality from end-stage renal complications and risks of heart disease and stroke development [156]. In aristolochic acid-induced neuropathy CKD models, EVs significantly decreased tubular necrosis, interstitial fibrosis, fibroblast and immune cell infiltration and modulated fibrotic gene expression [133]. In models where ischemia/reperfusion-induced renal injuries resulted in CKD, Gatti, et al., found microvesicles effectively reduced AKI, which significantly lowered CKD symptoms, including interstitial lymphocyte infiltration, interstitial fibrosis, tubular atrophy and glomerular sclerosis [137].

#### *Heart Failure*

Heart failure is a condition where the heart fails to circulate blood around the body adequately and is a major contributor to global mortality. Maleki, et al., applied CM to animal heart failure models, finding the treatment significantly increased fractional shortening, ejection fraction and angiogenesis while significantly reducing apoptosis and fibrosis levels. These impacts preserved cardiomyocyte physiology and restored heart function [142].

#### *Neonatal Injury*

Bronchopulmonary Dysplasia (BPD) is a serious premature delivery injury that causes respiratory distress, which may require mechanical ventilation and result in infections, long-term lung injury and mortality. Aslam, et al., applied BM-MSCs and BM-MSC-derived CM to oxygen-starved neonatal mice as a BPD model. They detected low levels of BM-MSC engraftment and survival, determining that paracrine BM-MSC effects caused beneficial findings. In contrast, CM presented greater therapeutic benefits. It significantly decreased cardiac injury scores and hypoxia-induced muscularization of intrapulmonary arterioles while completely preventing hypoxic lung injury and preserving standard lung architecture [143]. Sutsko, et al., performed a similar experiment but found CM and MSC administration had similar effects. These included improvements in alveolar structure, significant increases in vascular density and angiogenic factors, decreased pro-inflammatory cytokine expression and reduced Right-Ventricular Systolic Pressure (RVSP) and right ventricle/left ventricle + septum ratio. These improvements were maintained over the 12-week recovery period, although MSCs were superior in maintaining vascular density increases and RVSP reductions [144]. Finally, Baker, et al., determined that CM reduced BPD effects, such as right ventricular hypertrophy, due to improved angiogenesis and native cell proliferation [146].

Additionally, premature delivery can cause neuroinflammatory preterm brain injuries, which result in long-term motor, cognitive and behavioral impairment. Drommelschmidt, et al., applied EVs to this disease model, finding long-term reduced neural degeneration, significantly lower microgliosis and significantly improved cognition. However, EVs did not significantly change inflammatory cytokine production or hypomyelination [145].

#### *Diabetic Neuropathy*

Neuropathy is a common complication of diabetes mellitus, causing significant pain and increased risk for diabetic ulcers and kidney damage. Brini, et al., found ADSC and ADSC-CM administration reversed diabetic pain, modulated inflammatory cytokine levels, restored proper immune states and improved weight and kidney morphology in mice models [147]. Gregorio, et al., applied CM to polyneuropathy and diabetic ulcer models. CM administration significantly reduced pain responses and decreased intraepidermal nerve fiber loss, neurite degeneration and small fiber demyelination. However, CM did not significantly increase peripheral nerve conduction [148]. Finally, Tritta, et al., found EVs improved the kidney effects of diabetic neuropathy by significantly reducing blood kidney damage signals and kidney fibrosis [149].

### *Osteoporosis and Osteonecrosis*

Osteoporosis (OP) induced by menopause or ovariectomy causes low bone mass and high bone fragility and current treatments have limited efficacy and major side effects. Huang, et al., found EVs enriched with GPNMB improved trabecular bone regeneration and increased pro- osteogenic signaling levels [150]. Ge, et al., observed improved osteoblast osteogenic differentiation, osteogenic protein levels and significant increases in tibial density with EV application [151].

Necrotic bone regeneration was also improved by CM, which increased proliferation and angiogenesis gene expression, inhibited osteoclast apoptosis and improved osteoclast functionality, enabled new bone formation endothelial healing and fully healed 63% of mice models [152].

| Disease Model             | Reference |
|---------------------------|-----------|
| Bone Defects              | [157,158] |
| Musculoskeletal Pain      | [159]     |
| Psoriasis Vulgaris        | [160]     |
| GvHD                      | [161]     |
| Macular Injury            | [162]     |
| MS                        | [163]     |
| Severe COVID-19 Infection | [164]     |
| Chronic Kidney Disease    | [165]     |

**Table 2:** Human data for MSC-CM and MSC-EV studies.

### *Bone Defects*

Katagiri, et al., performed two clinical studies on bone defects. The first study enrolled six partially edentulous patients needing maxillary sinus floor elevation and bone grafts. Four patients (2 males, 2 females, 59.8 mean age) received  $\beta$ -TCP scaffolds soaked in CM and two patients (2 females, 70 mean age) received untreated  $\beta$ -TCP scaffolds. This study determined that the CM group had reduced scaffold prominence, greater regenerated bone replacing scaffolds, significantly more newly formed bone area, improved osteoblast and osteoclast observation, decreased inflammatory cell infiltration and enhanced histological bone quality. No systemic or local complications were reported [158].

The second study enrolled eight partially edentulous patients (3 men, 5 women, age range 45-67, mean 57.8 years) requiring bone augmentation procedures. Three patients underwent maxillary sinus floor elevation and five patients received guided bone regeneration procedures. Scaffolds were soaked in dissolved BM-MSC-derived CM. These scaffolds were applied simultaneously with the bone augmentation procedures in 5 cases and 3 patients gained scaffolds at second-stage surgeries. Study findings included earlier augmented bone mineralization, greater bone regeneration and less inflammatory cell infiltration in 5 bone biopsies taken six months after implantation. No systemic or local complications were reported [157].

Overall, these studies illustrate the positive effects of the secretome when applied to alveolar bone regeneration and suggest that other bone reconstruction efforts can be improved by CM application.

### *Musculoskeletal Pain*

A retrospective study evaluated 16 patients with chief complaints of severe musculoskeletal pain who were given CM. Significant decreases in current pain status were maintained throughout the four-week trial. Sano et al. did report one patient experienced minor discomfort at the injury site and one patient had mild fatigue that subsided without treatment, so these adverse effects were considered as due to injections and not CM itself [159]. This research demonstrates that CM has applications in reducing musculoskeletal pain.

### *Psoriasis Vulgaris*

Psoriasis Vulgaris, or plaque psoriasis, is a common, chronic disease theorized to be of autoimmune origin. This condition causes

scaly red patches on the scalp, knees, elbows, hands, nails, or feet. Due to its immunomodulatory effects, a case study was conducted on a 38-year-old male with severe Psoriasis Vulgaris. This study demonstrated significant decreases in affected areas and complete remission in one month of treatment that continued through the study's six-month timeframe. No adverse side effects were reported [160]. While larger studies need to be done, this study highlights CM as an effective therapy against psoriasis vulgaris.

### *GvHD*

Kordelas, et al., applied EVs to a 22-year-old female patient with acute skin GvHD and severe intestinal involvement who had failed primary GvHD treatment. No side effects were detected and they found significantly reduced inflammatory cytokine levels, decreased peripheral blood mononuclear cell activity and significant, rapid improvement of GvHD intestinal and dermatological symptoms. These improvements were maintained for several months, but the patient died of pneumonia seven months after EV administration [161]. Therefore, EVs may reduce the severity of unresponsive GvHD patients.

### *Macular Injury*

Idiopathic Macular Holes (MH) are common causes of visual impairment. These are generally treated through Pars Plana Vitrectomy (PPV). PPV successfully treats most MH cases but is not as effective with large and long-standing ones. Zhang, et al., applied EVs to 5 patients (62-71). Despite many of these patients having prior failed PPV procedures and all having large and persistent MH, when EVs were applied with PPV, 4/5 patients experienced complete MH healing and reduced central scotoma and 4/5 experienced improved visual acuity. With regards to complications, one patient had moderate intraocular inflammation that was resolved by prednisolone acetate eyedrops three days post-injection and had observed retinal atrophy during their 6-month follow-up [162]. Therefore, EV therapy may be effective in problematic MH repair, although more extensive studies are necessary to determine the likelihood of side effects.

### *MS*

MS is a demyelinating, neurodegenerative and neuroinflammatory CNS disease that causes progressive disabling. Current treatments can be effective at delaying disease progression and improving relapse rate, although they are limited by intolerability and effectiveness. Dabhour et al. injected autologous BM-MSCs and, one month later, autologous MSC-CM intrathecally into 15 patients, three of whom withdrew due to aspiration pain and two of whom had severe spasticity that prevented study continuation. All these patients had failed at least one first-line treatment option. The therapy resulted in a significant decrease in the volume of white matter lesions and major improvements in the number of brain white matter lesions and 9-hole peg testing for motor coordination. Minor improvements in the number of enhanced white matter lesions, optical coherence tomography and timed 25-foot walk testing were observed. No major changes were found in the extended disability status scale, number of spinal cord white matter lesions, visual evoked potential and mini-mental status examination. Minor adverse effects of treatment included injection site pain and swelling, fever and headache; infrequent adverse effects included injection site bruising, constipation and tremors. No life-affecting adverse effects were reported [163].

### *Severe COVID-19 Infection*

Seven severe or critical COVID-19 cases (18-75 years old, 57 median age, IQR 43 to 70) were administered nebulized MSC-exosomes. There was no evidence of adverse effects. All patients experienced slightly greater serum lymphocyte counts and 4 of 7 experienced major pulmonary lesion healing. Additionally, the researchers observed decreased inflammation biomarkers and markedly improved CT score values among patients [164]. This study demonstrates EVs can be applied to treat severe respiratory infections and improve pathologies. Chronic Kidney Disease Nassar, et al., performed a single-blind, randomized, placebo-controlled clinical study of 40 patients (19-34 years old, 25 median age) with stage III/IV chronic kidney disease. Twenty of these received 2 MSC-EV injections (IA and IV). Patients in the treatment group had significantly improved glomerular filtration rates and partially significantly improved blood urea, serum creatinine and urinary albumin creatinine ratio levels compared to the placebo group. No adverse effects were reported [165]. Therefore, the benefits of EVs were successfully applied to CKD patients.

## Discussion

These findings broadly demonstrate that the beneficial cellular effects of the secretome observed *in-vitro* apply to *in-vivo* animal and human models. Therefore, cell-free strategies can significantly improve patient outcomes and accelerate their healing times. Additionally, the benefits of the secretome have predominantly been found to be equivalent to or more effective than MSC application in this review, supporting the increased development of CM and EV solutions that can overcome some of the hurdles stem cell therapies face.

## Conclusion

More extensive human trials with control groups must be conducted to demonstrate cell-free strategy efficacy in treating various disease states. Some of these are currently being undertaken; these were compiled from ClinicalTrials.gov (Supplementary Table 1,2).

There are many practical challenges in applying MSC-derived therapeutics for medical applications. Current secretome therapies are non-standardized, with variegated donor characteristics, stem cell sources, cell passages, preconditioning methodologies, aspiration times, post-conditioning additives, application media and numbers of applications. Furthermore, many articles highlight different secretome components as vital in regeneration and healing and there is a significant discrepancy in whether MSC-EVs or MSC-CM should be used.

Future studies are necessary to find the most beneficial number of MSC passages, determine whether specific MSC locations or sub-populations are most effective at treating certain disease states, quantify the effect of donor-related characteristics on the secretome and identify optimal pre-conditioning and post-conditioning methodologies. Determining the most critical factors in the secretome for healing is also vital, as future therapies could seek to maximize these components. Additionally, GMP-compliant techniques must be developed to produce standardized therapies with controlled composition on a mass scale, enabling maximal patient benefits and regulatory approval as a standard of care.

It is also important to determine whether MSC-EVs or MSC-CM is more effective. One study found that the EV-free medium and the total secretome displayed immunomodulatory effects, while all isolated EV fractions failed to do so [166]. Other studies determined that EVs and the soluble fraction act synergistically to reduce inflammation and promote wound healing [167,168]. This problem is important to resolve because it affects the secretome's production methods. If EVs prove to be more important for generalized or specific healing, methodologies for creating the secretome may have to be changed to reduce damage to EVs and increase EV production [169-172]. Additionally, EVs often contain material reflecting the function of the secreting cell, so the originating cell type might be of greater concern in this case [173]. Because cell-free therapeutics can be mass-produced and allogeneically delivered, it could theoretically be applied to underserved disease states instead of being a last-case resort or expensive elective operation like other bioregenerative therapies. The results in this review illustrate the widespread potential application of the MSC secretome and highlight many of the important animal and human *in-vivo* studies advancing the understanding of the secretome's applications in medicine.

| Status    | Official Title                                                                | ClinicalTrials.gov ID | Responsible Party    | Start – Completion<br>(actual if completed, estimated if not completed) | Enrollment<br>(actual if completed, estimated if not completed) | Location                                                             |
|-----------|-------------------------------------------------------------------------------|-----------------------|----------------------|-------------------------------------------------------------------------|-----------------------------------------------------------------|----------------------------------------------------------------------|
| Completed | Therapeutic Potential of Stem Cell Conditioned Medium on Chronic Ulcer Wounds | NCT04134676           | Sukma Skin Treatment | 6/1/2019 – 6/10/2020                                                    | 38                                                              | Tangerang, Banten, Indonesia: Mayapada Hospital, Indra Clinic, Sukma |

|            |                                                                                                                         |             |                                           |                         |    | Cliniq                                                                                   |
|------------|-------------------------------------------------------------------------------------------------------------------------|-------------|-------------------------------------------|-------------------------|----|------------------------------------------------------------------------------------------|
| Unknown    | Implantation of Mesenchymal Stem Cell, Conditioned Medium, or Triamcinolone Acetonide for Keloid                        | NCT04326959 | PT. Prodia Stem Cell Indonesia            | 9/1/2020 – 12/1/2020    | 24 | No location data                                                                         |
| Recruiting | Stem Cell and Conditioned Medium for Cerebral Palsy                                                                     | NCT04314687 | PT. Prodia Stem Cell Indonesia            | 10/13/2021 – 12/25/2023 | 78 | Jakarta, Indonesia: Indonesian National Brain Center                                     |
| Completed  | Effectiveness of PRP, Conditioned Medium UC-MSCs Secretome and Hyaluronic Acid for the Treatment of Knee Osteoarthritis | NCT05579665 | Universitas Sriwijaya                     | 10/2/2022 – 5/31/2023   | 45 | Palembang, South Sumatera, Indonesia: Mohammad Hoesin Central General Hospital           |
| Unknown    | Mesenchymal Stem Cell-derived Pleiotropic Factor in the Treatment of Donor Sites                                        | NCT04234750 | Xiaobing Fu, Chinese PLA General Hospital | 10/17/2019 – 6/30/2021  | 20 | Datong, Shanxi, China: PLA Central Air Force Hospital                                    |
| Recruiting | Combination of Conditioned Medium and Umbilical Cord-Mesenchymal Stem Cells Therapy for Acute Stroke Infarct            | NCT05008588 | PT. Prodia Stem Cell Indonesia            | 5/30/2022 – 12/2023     | 15 | Jakarta, Indonesia: Gatot Soebroto Hospital                                              |
| Recruiting | Long-term Safety of UC-MSC Transplantation in Patients With Retinitis Pigmentosa (RP)                                   | NCT05786287 | PT. Prodia Stem Cell Indonesia            | 7/1/2023 – 7/13/2025    | 18 | Jakarta, Indonesia: Jakarta Eye Center Hospital Yogyakarta, Indonesia: Sardjito Hospital |
| Unknown    | Mesenchymal Stem Cell Conditioned Medium-derived Pleiotropic Factor in Treating Residual Burn Wound                     | NCT04235296 | Xiaobing Fu, Chinese PLA General Hospital | 11/17/2019 – 6/30/2021  | 30 | Datong, Shanxi, China: PLA Central Air Force Hospital                                    |
| Recruiting | Mesenchymal Stem Cell Therapy (MSCs) and Conditioned Medium Therapy for Osteoarthritis (OA)                             | NCT04314661 | PT. Prodia Stem Cell Indonesia            | 8/3/2020 – 12/8/2024    | 20 | Jakarta, Indonesia: Gatot Soebroto Hospital                                              |

|            |                                                                                                                                                                                                                                |             |                                                 |                        |    |                                                          |
|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|-------------------------------------------------|------------------------|----|----------------------------------------------------------|
| Completed  | Hair Growth Efficacy and Safety of NGF-574H in Adult with Androgenic Alopecia                                                                                                                                                  | NCT03676400 | Medipost Co Ltd.                                | 10/2/2018 – 7/24/2019  | 84 | Cheonan, Chung Nam, Korea: Dankook University Hospital   |
| Completed  | Adipose-derived Stem Cell Conditioned Media as a Novel Approach for Hair Regrowth in Male Androgenetic Alopecia                                                                                                                | NCT05296863 | Dr. Lili Legiawati, Indonesia University        | 10/11/2021 – 12/3/2021 | 37 | Jakarta Pusat, Jakarta, Indonesia: Universitas Indonesia |
| Recruiting | Role of UC-MSC and CM to Inhibit Vision Loss in Retinitis Pigmentosa Phase I/II                                                                                                                                                | NCT05909488 | PT. Prodia Stem Cell Indonesia                  | 9/1/2023 – 12/1/2025   | 30 | Yogyakarta, Indonesia                                    |
| Completed  | Comparison of Keloid Volume and Symptoms Reduction Between Intralesional Umbilical-Cord Mesenchymal Stem Cells, Its Conditioned Medium and Triamcinolone Acetonide Injection as Keloid Therapy: A Randomized Controlled Trial  | NCT05887804 | Rumah Sakit Pusat Angkatan Darat Gatot Soebroto | 10/1/2021 – 6/9/2022   | 24 | Jakarta, Indonesia: RSPAD Gatot Soebroto                 |
| Unknown    | ACT-20 in Patients with Severe COVID-19 Pneumonia                                                                                                                                                                              | NCT04398303 | Aspire Health Science                           | 5/2020 – 10/2020       | 70 | No Location Data                                         |
| Unknown    | Regenerative Effects of Human Stem Cell Media After Laser Therapy in Hypertrophic Scar                                                                                                                                         | NCT05004779 | Hangang Sacred Heart Hospital                   | 8/10/2021 – 4/20/2022  | 30 | Yeongdeungpo-gu, Seoul, 07247, Republic of Korea         |
| Completed  | The Effect of Intralesian Injection of Umbilical Cord Mesenchymal Stem Cells, Its Conditioned Medium and Triamcinolone Acetonide on Type 1:3 Collagen Ratio and Interleukin-10 Levels in Keloid: A Randomized Controlled Trial | NCT05939817 | Rumah Sakit Pusat Angkatan Darat Gatot Soebroto | 10/1/2021 – 6/9/2022   | 24 | Jakarta, Indonesia: RSPAD Gatot Soebroto                 |

|                    |                                                                                                                       |             |                                                                               |                         |    |                                                                                                      |
|--------------------|-----------------------------------------------------------------------------------------------------------------------|-------------|-------------------------------------------------------------------------------|-------------------------|----|------------------------------------------------------------------------------------------------------|
| Recruiting         | Effects of ASC Secretome on Human Osteochondral Explants (ASC-OA)                                                     | NCT04223622 | I.R.C.C.S Ospedale Galeazzi-Sant' Ambrogio                                    | 4/12/2021 – 12/2024     | 24 | Milano, Italy: I.R.C.C.S Ospedale Galeazzi                                                           |
| Completed          | Potential Injection of Human Umbilical Cord Secretome in the Case of Trophic Ulcers (Pre-post Intervention)           | NCT05777213 | Yohanes Firmansyah, dr, MH, MM, Tarumanagara University                       | 1/1/2019 – 1/2/2023     | 27 | Tangerang, Banten, Indonesia: Sukma Clinic Singkawang, West Borneo, Indonesia: RS Alverno Singkawang |
| Completed          | Vetsibular Socket Therapy Versus Buser's Technique (VST)                                                              | NCT04787237 | Iman Abd-ElWahab Radi, PhD, Cairo University                                  | 1/30/2019 – 10/31/2020  | 40 | Alexandria, Egypt: ElAskary and Associates Private Clinic Cairo, Manial, Egypt: Faculty of Dentistry |
| Not Yet Recruiting | Autogenous Mesenchymal Stem Cell Culture-Derived Signalling Molecules as Enhancers of Bone Formation in Bone Grafting | NCT04998058 | Eduardo Rolim Teixeira, Pontificia Universidade Católica do Rio Grande do Sul | 12/15/2023 – 12/30/2024 | 20 | Porto Alegre, RS, Brazil: Pontificia Universidade Católica do Rio Grande do Sul                      |

**Supplementary Table 1:** Human trials utilizing MSC-CM, ongoing or completed without results posted [174].

| Status             | Official Title                                                                                               | ClinicalTrials.gov ID | Responsible Party                                                       | Start – Completion (actual if completed, estimated if not completed) | Enrollment (actual if completed, estimated if not completed) | Location                                                                              |
|--------------------|--------------------------------------------------------------------------------------------------------------|-----------------------|-------------------------------------------------------------------------|----------------------------------------------------------------------|--------------------------------------------------------------|---------------------------------------------------------------------------------------|
| Recruiting         | A Clinical Study on Safety and Effectiveness of Mesenchymal Stem Cell Exosomes for the Treatment of COVID-19 | NCT05787288           | Xiaoying Huang, First Affiliated Hospital of Wenzhou Medical University | 1/23/2023 – 1/23/2025                                                | 240                                                          | Wenzhou, Zhejiang, China: The First Affiliated Hospital of Wenzhou Medical University |
| Not Yet Recruiting | MSC EVs in Dystrophic Epidermolysis Bullosa                                                                  | NCT04173650           | Aegle Therapeutics                                                      | 3/2024 – 9/2025                                                      | 10                                                           | Los Angeles, California, United States: University of Southern                        |

|            |                                                                                                                                         |             |                                                                         |                        |     |                                                                                                             |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------|-------------|-------------------------------------------------------------------------|------------------------|-----|-------------------------------------------------------------------------------------------------------------|
|            |                                                                                                                                         |             |                                                                         |                        |     | California Dept of Dermatology                                                                              |
| Unknown    | Safety of Injection of Placental Mesenchymal Stem Cell Derived Exosomes for Treatment of Resistant Perianal Fistula in Crohn's Patients | NCT05499156 | Tehran University of Medical Sciences                                   | 1/20/2022 – 9/2022     | 80  | Teheran, Iran: Division of Colorectal Surgery, Department of Surgery, Tehran University of Medical Sciences |
| Recruiting | Safety and Efficacy of Injection of Human Placenta Mesenchymal Stem Cells Derived Exosomes for Treatment of Complex Anal Fistula        | NCT05402748 | Ahmadi Tafti, Tehran University of Medical Sciences                     | 12/22/2021 – 3/22/2023 | 80  | Teheran, Iran: Division of Colorectal Surgery, Department of Surgery, Tehran University of Medical Sciences |
| Completed  | Safety and Tolerability Study of MSC Exosome Ointment                                                                                   | NCT05523011 | Paracrine Therapeutics Dermatology Pte. Ltd.                            | 3/8/2022 – 4/5/2022    | 10  | Singapore, Singapore: National University Hospital                                                          |
| Recruiting | Exosome Effect on Prevention of Hair Loss                                                                                               | NCT05658094 | Leila Dehghani, Isfahan University of Medical Sciences                  | 8/1/2022 – 10/29/2023  | 20  | Isfahan, Iran: Isfahan University of Medical Sciences                                                       |
| Unknown    | Effect of UMSCs Derived Exosomes on Dry Eye in Patients With cGVHD                                                                      | NCT04213248 | Zhongshan Ophthalmic Center, Sun Yat-sen University                     | 2/21/2020 – 12/2023    | 27  | Guangzhou, Guangdong, China: Zhongshan Ophthalmic Center                                                    |
| Recruiting | A Clinical Study on Safety and Effectiveness of Mesenchymal Stem Cell Exosomes for the Treatment of COVID-19                            | NCT05787288 | Xiaoying Huang, First Affiliated Hospital of Wenzhou Medical University | 1/23/2023 – 1/23/2025  | 240 | Wenzhou, Zhejiang, China: The First Affiliated Hospital of Wenzhou Medical University                       |
| Unknown    | Allogenic Mesenchymal Stem Cell Derived Exosome in Patients with Acute Ischemic Stroke                                                  | NCT03384433 | Leila Dehghani, Isfahan University of Medical Sciences                  | 4/17/2019 – 12/17/2019 | 5   | Tehran, Iran: Shahid Beheshti University of Medical Sciences                                                |



|                    |                                                                                                                                                |             |                                                                     |                        |    |                                                                                                            |
|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------|-------------|---------------------------------------------------------------------|------------------------|----|------------------------------------------------------------------------------------------------------------|
| Recruiting         | Effect of Mesenchymal Stem Cells-derived Exosomes in Decompensated Liver Cirrhosis                                                             | NCT05871463 | Research Institute for Gastroenterology and Liver Diseases (RIGLD)  | 5/26/2023 – 12/11/2023 | 15 | Tehran, Iran: Research Institute of Gastroenterology & Liver Diseases                                      |
| Unknown            | A Clinical Study of Mesenchymal Progenitor Cell Exosomes Nebulizer for the Treatment of Pulmonary Infection                                    | NCT04544215 | Ruijin Hospital                                                     | 7/1/2020 – 7/2023      | 60 | Shanghai, China: Ruijin Hospital Shanghai Jiao Tong University School of Medicine                          |
| Unknown            | MSC-Exos Promote Healing of MHs (MSCs)                                                                                                         | NCT03437759 | Xiaomin Zhang, Tianjin Medical University Eye Hospital              | 3/1/2017 – 12/30/2021  | 44 | Tianjin, China: Tianjin Medical University Hospital                                                        |
| Recruiting         | Safety and Efficacy of Umbilical Cord Mesenchymal Stem Cell Exosomes in Treating Chronic Cough After COVID-19                                  | NCT05808400 | Jihui Du, Huazhong University of Science and Technology             | 2/15/2023 – 2/15/2025  | 80 | Shenzhen, Guangdong, China: Huazhong University of Science and Technology Union                            |
| Unknown            | Safety of Injection of Placental Mesenchymal Stem Cell Derived Exosomes for Treatment of Resistant Perianal Fistula in Crohn's Patients        | NCT05499156 | Tehran University of Medical Sciences                               | 1/20/2022 – 9/2022     | 80 | Tehran, Iran: Division of Colorectal Surgery, Department of Surgery, Tehran University of Medical Sciences |
| Not Yet Recruiting | Exosome of Mesenchymal Stem Cells for Multiple Organ Dysfunction Syndrome After Surgical Repaire of Acute Type A Aortic Dissection             | NCT04356300 | Liang-Wan Chen MD, Fujian Medical University                        | 9/1/2020 – 9/1/2030    | 60 | Fujian, China: Fujian Medical University                                                                   |
| Not Yet Recruiting | Safety and Efficacy of Pluripotent Stem Cell-derived Mesenchymal Stem Cell Exosome (PSC-MSC-Exo) Eye Drops Treatment for Dry Eye Diseases Post | NCT05738629 | Second Affiliated Hospital, School of Medicine, Zhejiang University | 3/2023 – 2/2025        | 12 | Hanzhou, Zhejiang, China: 2[nd] Affiliated Hospital, School of Medicine, Zhejiang University               |

|                    |                                                                                                                                  |             |                                                                                  |                       |     |                                                                                   |
|--------------------|----------------------------------------------------------------------------------------------------------------------------------|-------------|----------------------------------------------------------------------------------|-----------------------|-----|-----------------------------------------------------------------------------------|
|                    | Refractive Surgery and Associated with Blepharospasm                                                                             |             |                                                                                  |                       |     |                                                                                   |
| Recruiting         | The Effect of Stem Cells and Stem Cell Exosomes on Visual Functions in Patients with Retinitis Pigmentosa                        | NCT05413148 | Kuddusi Erkiç, TC Erciyes University                                             | 8/5/2022 – 12/15/2023 | 135 | Kayseri, Turkey: Erciyes University                                               |
| Unknown            | Effect of Microvesicles and Exosomes Therapy on $\beta$ -cell Mass in Type I Diabetes Mellitus (T1DM)                            | NCT02138331 | Wael Fouad Nassar, General Committee of Teaching Hospitals and Institutes, Egypt | 4/2014 – 9/2014       | 20  | Cairo, Egypt: Sahel Teaching Hospital                                             |
| Not Yet Recruiting | UCMSC-Exo for Chemotherapy-induced Myelosuppression in Acute Myeloid Leukemia                                                    | NCT06245746 | Qiubai Li, Wuhan Union Hospital, China                                           | 3/2024 – 12/2025      | 9   | Wuhan, China: Wuhan Union Hospital                                                |
| Recruiting         | Mesenchymal Stem Cells Derived Exosomes in Skin Rejuvenation                                                                     | NCT05813379 | Leila Dehghani, Isfahan University of Medical Sciences                           | 2/1/2022 – 8/30/2023  | 20  | Isfahan, Iran: Isfahan University of Medical Sciences                             |
| Recruiting         | Clinical Efficacy of Exosome in Degenerative Meniscal Injury (KNEEXO)                                                            | NCT05261360 | Ayla Eker Sariboyaci, Eskisehir Osmangazi University                             | 3/2022 – 3/2025       | 30  | Eskisehir, Turkey: Eskisehir Osmangazi University                                 |
| Recruiting         | Co-transplantation of Mesenchymal Stem Cell Derived Exosomes and Autologous Mitochondria for Patients Candidate for CABG Surgery | NCT05669144 | Ahmadi Tafti, Tehran University of Medical Sciences                              | 4/20/2022 – 9/20/2024 | 20  | Tehran, Iran: Tehran University of Medical Sciences                               |
| Completed          | A Tolerance Clinical Study on Aerosol Inhalation of Mesenchymal Stem Cells Exosomes in Healthy Volunteers                        | NCT04313647 | Ruijin Hospital                                                                  | 3/12/2020 – 7/31/2020 | 24  | Shanghai, China: Ruijin Hospital Shanghai Jiao Tong University School of Medicine |

|                    |                                                                                                                                                 |             |                                                    |                         |     |                                                                                             |
|--------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|-------------|----------------------------------------------------|-------------------------|-----|---------------------------------------------------------------------------------------------|
| Recruiting         | Immune Modulation by Exosomes in COVID-19 (IMECOV19)                                                                                            | NCT05191381 | Manfred Weiss, University of Ulm                   | 12/22/2021 – 12/31/2026 | 40  | Ulm, Germany: Ulm University Hospital, Clinic of Anesthesiology and Intensive Care Medicine |
| Unknown            | A Clinical Study of Mesenchymal Stem Cell Exosomes Nebulizer for the Treatment of ARDS                                                          | NCT04602104 | Ruijin Hospital                                    | 11/30/2020 – 9/2022     | 169 | Shanghai, China: Ruijin Hospital, Medical School of Shanghai Jiaotong University            |
| Unknown            | Efficacy and Safety of EXOSOME-MSC Therapy to Reduce Hyper-inflammation In Moderate COVID-19 Patients (EXOMSC-COV19)                            | NCT05216562 | Bambang Darwono, Dermama Bioteknologi Laboratorium | 7/1/2021 – 12/30/2022   | 60  | Yogyakarta, Indonesia; Jakarta, Indonesia                                                   |
| Not Yet Recruiting | MSC EVs in Epidermolysis Bullosa                                                                                                                | NCT04173650 | Aegle Therapeutics                                 | 3/2024 – 9/2025         | 10  | No location data                                                                            |
| Not Yet Recruiting | The Use of Exosomes for the Treatment of Acute Respiratory Distress Syndrome or Novel Coronavirus Pneumonia Caused by COVID-19 (ARDOXSO)        | NCT04798716 | AVEM HealthCare                                    | 9/2023 – 12/2024        | 55  | Panorama City, California, United States: Mission Community Hospital                        |
| Recruiting         | A Proof-of-Concept Study to Evaluate Exosomes from Human Mesenchymal Stem Cells in Women with Premature Ovarian Insufficiency (POI) (VL-POI-01) | NCT06072794 | Vitti Labs, LLC                                    | 10/6/2023 – 12/2025     | 9   | Oklahoma City, Oklahoma, United States: Optimal Health Associates                           |
| Recruiting         | Extracellular Vesicle Treatment for Acute Respiratory Distress Syndrome (ARDS) (EXTINGUISH ARDS)                                                | NCT05354141 | Direct Biologics, LLC                              | 7/1/2022 – 8/31/2025    | 970 | Direct Biologics Investigational Sites in United States                                     |
| Completed          | Extracellular Vesicle Infusion Treatment for COVID-19 Associated ARDS (EXIT- COVID19)                                                           | NCT04493242 | Direct Biologics, LLC                              | 9/24/2020 – 5/22/2021   | 102 | Direct Biologics Investigational Sites in United States                                     |

|            |                                                                                                                                                                                                                               |             |                 |                     |              |                                                                  |
|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|-----------------|---------------------|--------------|------------------------------------------------------------------|
| Recruiting | Safety and Effectiveness of Placental Derived Exosomes and Umbilical Cord Mesenchymal Stem Cells in Moderate to Severe Acute Respiratory Distress Syndrome (ARDS) Associated with the Novel Corona Virus Infection (COVID-19) | NCT05387278 | Vitti Labs, LLC | 3/20/2023 – 12/2023 | 20           | Liberty, Missouri, United States                                 |
| Unknown    | The Safety and the Efficacy Evaluation of Allogenic Adipose MSC-Exos in Patients with Alzheimer's Disease                                                                                                                     | NCT04388982 | Ruijin Hospital | Not provided        | Not provided | Shanghai, China: Ruijin Hospital Affiliated to Shanghai Jiaotong |

**Supplementary Table 2:** Human trials utilizing MSC-EVs, ongoing or completed without results posted [175].

### Conflict of Interest

The authors have no conflict of interest to declare.

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