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The versatility of mesenchymal stem cells: From regenerative medicine to COVID, what is next?

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Abstract: Mesenchymal stem cells (MSCs) play key roles in regenerative medicine by promoting tissue healing. MSCs can be isolated from different adult tissues and they are able to differentiate into several lineages. Due to their anti-inflammatory, angiogenic and immune-modulatory properties, MSCs are suitable for tissue engineering applications and, when associated with biomaterials, their benefits can be improved. Moreover, recently, MSCs have been studied for new clinical applications, such as in the treatment of patients with COVID-19. MSCs regenerative potential has been attributed to their secretome, which comprises extracellular matrix, soluble proteins and several elements, including the release of extracellular vesicles. Even though, in order to explore all their therapeutic potential, it is still necessary to advance in the investigation of their basic cell biology characteristics.

Viewpoint

Mesenchymal stem cells (MSCs) are multipotent cells found in adult tissues. They are characterized by their adherence to plastic, multilineage differentiation, and immunophenotype (Dominici *et al.*, 2006; Galgaro *et al.*, 2021; Song *et al.*, 2020). Since their discovery, MSCs have been extensively studied, mainly in the last decade. A brief timeline of the main highlights of the MSCs research can be observed in Fig. 1. MSCs were initially isolated from bone marrow, but they can actually be isolated from virtually all tissues (da Silva *et al.*, 2006). This represents a great opportunity for basic and translational studies, once it is possible to isolate MSCs from human tissues that are usually discarded after surgery procedures, such as skin, adipose tissue, umbilical cord, placenta, dental pulp, and sclerocorneal limbus (Fig. 2) (Dominici *et al.*, 2006; Galgaro *et al.*, 2021; Song *et al.*, 2020).

MSCs have anti-inflammatory, angiogenic, and immune-modulatory properties that play an important role in wound healing and regeneration (Chang *et al.*, 2021). Preclinical and clinical studies have demonstrated their promising potential to treat degenerative diseases, such as Alzheimer's disease, autoimmune diseases, bone and cartilage diseases, as well as, respiratory, cardiovascular, kidney and liver diseases (Brown *et al.*, 2019; Samadi *et al.*, 2021). The therapeutic effects of MSCs can be credited to three key mechanisms of action: 1) "homing", of the cells to the injury site; 2) cell differentiation, to promote repair of the damaged tissue; and 3) the secretion of bioactive factors (Vizoso *et al.*, 2017). MSCs can also modulate the host foreign-body immunogenic reaction toward the engineered constructs. These characteristics make them attractive for tissue engineering applications (Chen and Liu, 2016; Hanson *et al.*, 2014).

The combination of stem cells with biomaterials is a promising strategy for tissue regeneration as evidenced by several studies (Sierra-Sánchez *et al.*, 2021). Thus, our group has also contributed with the literature, evaluating the association among different scaffolds and MSCs. In our studies, we have found that Bioglass 45S5 associated with adipose-derived MSCs (ADSCs) is suitable to be tested in preclinical studies for bone regeneration. MSCs maintained their viability, natural morphology, and osteoinduction potential after exposure to Bioglass 45S5 extract. They also grew attached to the bioglass for a long time *in vitro*. Moreover, the biocompatibility of bioglass samples as scaffolds for allogeneic MSCs was confirmed after subcutaneous implantation in mice (Rodrigues *et al.*, 2019). ADSCs were also evaluated after contact with samples of 99.95% and 99.5% pure iron for cardiovascular stents. The viability, proliferation, and morphology of ADSCs were not affected *in vitro* after contact with iron (Paim *et al.*, 2020).

The use of MSCs isolated or in combination with different scaffolds is another promising strategy to treat cutaneous diseases and injuries (Sierra-Sánchez *et al.*, 2021).

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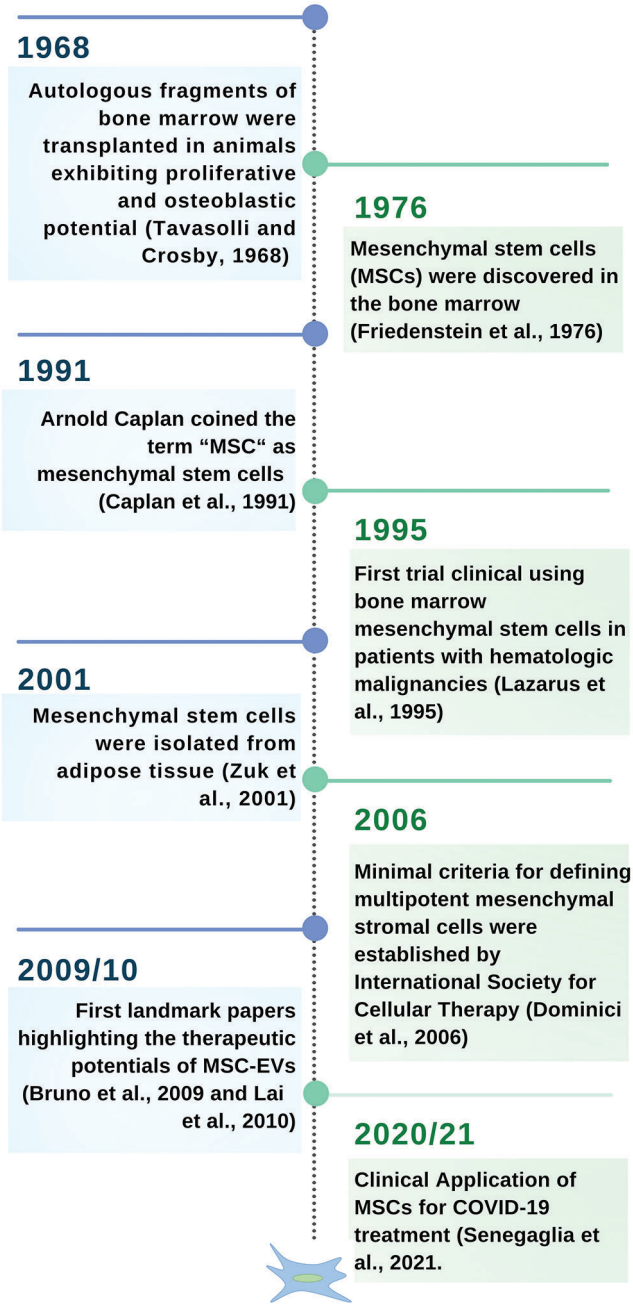

FIGURE 1. A brief timeline of MSCs.

In this setting, our research group evaluated the adhesion, morphology, and viability of ADSCs in a film composed of chitosan, gelatin, and liposomes (Vedovatto *et al.*, 2020). Our results suggested that the biomaterial was suitable for drug delivery and promoted the growth of MSCs without cell damage. In addition, the ADSCs were also studied in combination with CMC (sodium carboxymethylcellulose), a synthetic polymer used in the treatment of skin wounds. There was no significant toxicity or DNA damage on cells cultured with CMC. The association tested as a therapy in a preclinical model contributed to wound healing of skin lesions without overproduction of collagen that could cause a fibrosis (Rodrigues *et al.*, 2014). In addition, ADSCs could also proliferate *in vitro* on the human amniotic membrane (hAM), a biological scaffold generally discarded following the birth. There were no changes in their morphology, as well as, no losses on their potential of multilineage differentiation. These results indicate that hAM can be tested as a scaffold for MSCs in clinical applications (Naasani *et al.*, 2019).

Many efforts have also been put into reproducing the corneal stroma to find an alternative to corneal transplantation (Alió del Barrio *et al.*, 2021). We evaluated decellularized hAM and porcine small intestine submucosa (SIS) as scaffolds for limbal stroma–MSCs (L-MSCs), aiming to compare their performance for application in corneal regeneration. Both biological matrices maintained the cell viability, actin cytoskeleton, nuclei morphology, and mesenchymal phenotype. Nevertheless, there was a slight increase in the percentages of negative markers for L-MSCs grown on the SIS membrane for two weeks, in comparison to hAM, which was able to maintain the MSCs phenotype for a longer time. Besides, the hAM-L-MSCs construct was more transparent, which is an important characteristic to treat corneal injury (Sous Naasani *et al.*, 2018).

In our opinion, while there are many reports in the literature about the MSCs applications, it is not clear yet, whether their regenerative potential is due to the cell itself, their secretome or the combination of both. Regarding the MSCs immunomodulatory properties, researchers have shown that they can be attributed to the secretome (Bruno *et al.*, 2015; Dabrowska *et al.*, 2020). The proteins released into the microenvironment include active factors such as cytokines, chemokines, and growth factors. They can also be released encapsulated in extracellular vesicles (EVs), classified as apoptotic bodies, microvesicles (100–1000 nm), and exosomes (30–200 nm) (Harrell *et al.*, 2019). These EVs are essential to cell-cell communication, once they can carry proteins, lipids, mRNAs, and microRNAs (miRNAs) in the inner core (Groot and Lee, 2020).

Recently, several studies are revealing that extracellular vesicles secreted from MSCs have a critical therapeutic role. MSC-derived EVs have been reported in the treatment of osteoarthritis (Kim *et al.*, 2021; Li *et al.*, 2021), renal injury (Birtwistle *et al.*, 2021), traumatic brain injury (Yang *et al.*, 2017), lung injury (Huang *et al.*, 2019) and liver fibrosis (Chiabotto *et al.*, 2020). The regenerative potential of MSCs-EVs was also shown in cartilage, bone, and periodontium (Cooper *et al.*, 2019; Gholami *et al.*, 2021). The potential of MSCs and their extracellular vesicles, microvesicles, or exosomes is largely explored in clinical trials. Currently, there are over 50 phases III or IV protocols registered in clinicaltrials.gov (ongoing or completed), using MSCs from different sources, a similar proportion of allogeneic and autologous cells, and a range of variable approaches (Table S1).

One specific cargo carried by the MSC-derived EVs are the miRNAs, which are non-coding RNAs (ncRNAs) They act on post-transcriptional regulation of gene expression allowing the restoration of the injured tissue, as other effects (Saliminejad *et al.*, 2019). Several miRNAs are important regulators of gene expression during osteogenic and chondrogenic differentiation. Dysregulation of miRNA mediated mechanisms is related to the development of osteoporosis, bone fractures, and tumors (Iaquinta *et al.*, 2021). Recently, Marupanthorn found that inhibition of

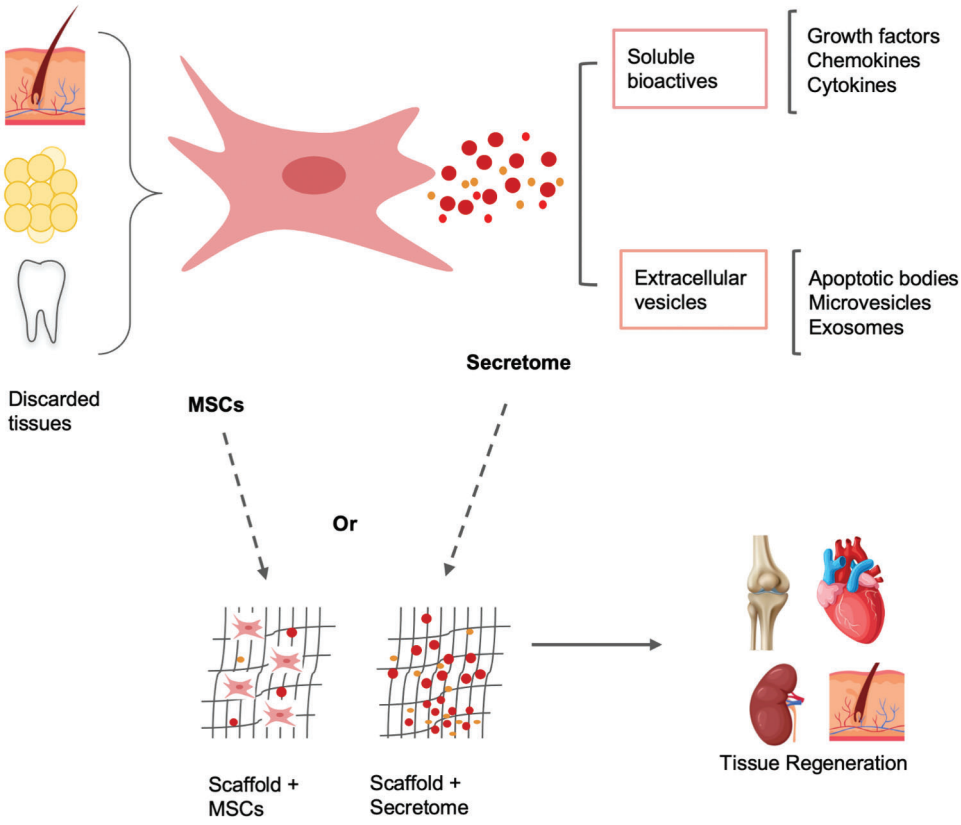

FIGURE 2. MSCs can be isolated from the discarded tissues such as: placenta, umbilical cord, teeth, adipose tissue, skin and sclerocorneal limbus. These cells secrete soluble bioactives and extracellular vesicles. MSCs or their secretome can be associated with biomaterials and promote tissue regeneration.

specific miRNAs (miR-31, miR-106a, and miR-148a) can promote osteogenic differentiation of chorion-derived mesenchymal stem cells (CH-MSCs) and placenta-derived mesenchymal stem cells (PL-MSCs) (Marupanthorn *et al.*, 2021). Likewise, Zhang showed that the silencing of miRNA-132-3p expression in the bone tissues can promote bone marrow-derived mesenchymal stromal cells (BMSC) osteogenic differentiation and osteogenesis in mice with osteopenia (Hu *et al.*, 2020). For this reason, miRNAs can be therapeutic targets to promote bone regeneration in osteogenesis-related disorders.

Currently, the MSCs-EVs have been studied to treat COVID-19 (O'Driscoll, 2020). The analysis of the miRNAs cargo carried by the MSC-EVs showed that they may reduce inflammatory responses, inhibit cell death genes and key factors of the coagulation cascade, preventing tissue damage and coagulation disturbances (Schultz *et al.*, 2021). MSCs can be administered as monotherapy or in association with other treatments. As an example, Tocilizumab, a monoclonal antibody against interleukin-6, in association with an advanced therapy product, based on umbilical cord-derived mesenchymal stromal cells (UC-MSC) was used to treat a severe COVID-19 patient. This therapeutic strategy promoted the decrease of inflammatory cytokines, the increase of regulatory cells, leading to lung repair (Senegaglia *et al.*, 2021). The presence of exosomes derived from UC-MSCs, in combination with Tocilizumab may be responsible for an additive or synergistic anti-inflammatory effect, which was translated in the improvement observed in this COVID-19 patient (Schultz *et al.*, 2021).

Another type of non-coding RNA (ncRNA), also present in MSCs, is the circular RNA (circRNA), which seem to be involved in tissue damage repair. Sun *et al.* (2018) observed an abundant expression and up-regulation of circRNAs, during the repair of human endometrial stromal cells (ESCs) by Wharton's jelly-derived MSCs (WJ-MSCs), leading to the enhance of the endometrial regeneration. Likewise, another recent study from the same group showed that circ6401 was significantly upregulated in WJ-MSCs co-cultured with damaged ESCs. The overexpression of circ6401 increased the levels of VEGFR2 and RAP1B in WJ-MSCs, promoting angiogenesis and proliferation of the damaged ESCs (Shi *et al.*, 2020).

In the recent COVID-19 scenario, the MSCs performance in clinical study protocols was a great surprise. This new open avenue begs the question, what is the next? It is notorious that MSCs have a promising use in different fields of medicine, however basic questions still remain to be answered. While the phenotype of MSCs isolated from different tissues seems very similar, they are not functionally identical (Iser *et al.*, 2014; Naasani *et al.*, 2017). This can explain, for example, the differences in therapeutic potential according to MSC origin (Eiró *et al.*, 2014; Vieira *et al.*, 2010). The secretome composition of stem cells can vary regarding different factors, such as species, tissue source, donor age, protocols for MSCs isolation, culture methods, and therapeutic protocol. Immortalization, an interesting strategy for reducing the need for primary isolation, also changes important characteristics, mainly related to levels of the extracellular adenosine, a potent immunosuppressor (Beckenkamp *et al.*, 2020). Another important aspect to be taken into consideration is the cell microenvironment. It includes the biochemical (e.g., growth factors, small bioactive molecules, and genetic regulators) and biophysical (e.g., pore size, porosity, stiffness, and topography of the

biomaterial) stimuli to which the cells are exposed (Brown *et al*., 2019; Chen and Liu, 2016; Daneshmandi *et al*., 2020). Moreover, the extracellular matrix, one of the most important components of the niche environmental signals, can impact the proliferation and differentiation of MSCs (Assis-Ribas *et al*., 2018; Novoseletskaya *et al*., 2020).

Therefore, there are important questions to be addressed before using MSCs as a cellular therapy to ensure clinical safety and efficacy: (1) Is it better to use autologous MSCs than allogeneic? (2) Are the MSCs from different donors functionally identical? (3) Are there differences between MCSs derived from young or old donors? (4) Is there relevance in the gender of the donor? (5) Can the body mass index (BMI) of donors influence the MCS performance? (6) What is the best tissue for cell isolation? (7) What is the best protocol, cultured MSCs or directly collected? (8) Is there a way of immortalizing MSCs that does not change their key functions? (9) When culturing and expanding MSCs, what is the best growth medium for clinical application? (10) What is the most suitable administration route? (11) How many cells need to be applied? (12) How many cell infusions are necessary? Answering all these variables that can impact clinical success, and other still open questions regarding the MSCs basic cell biology is the key to advance in the next therapeutic challenges.

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References

Alió Del Barrio JL, Arnalich-Montiel F, de Miguel MP, El Zarif M, Alió JL (2021). Corneal stroma regeneration: Preclinical studies. *Experimental Eye Research* **202**: 108314. DOI 10.1016/j.exer.2020.108314.

Assis-Ribas T, Forni MF, Winnischofer SMB, Sogayar MC, Trombetta-Lima M (2018). Extracellular matrix dynamics during mesenchymal stem cells differentiation. *Developmental Biology* **437**: 63–74. DOI 10.1016/j.ydbio.2018.03.002.

Beckenkamp LR, da Fontoura DMS, Korb VG, de Campos RP, Onzi GR, Iser IC, Bertoni APS, Sévigny J, Lenz G, Wink MR (2020). Immortalization of mesenchymal stromal cells by TERT affects adenosine metabolism and impairs their immunosuppressive capacity. *Stem Cell Reviews and Reports* **16**: 776–791. DOI 10.1007/s12015-020-09986-5.

Birtwistle L, Chen XM, Pollock C (2021). Mesenchymal stem cell-derived extracellular vesicles to the rescue of renal injury. *International Journal of Molecular Sciences* **22**: 6596. DOI 10.3390/ijms22126596.

Brown C, McKee C, Bakshi S, Walker K, Hakman E, Halassy S, Svinarich D, Dodds R, Govind CK, Chaudhry GR (2019). Mesenchymal stem cells: Cell therapy and regeneration potential. *Journal of Tissue Engineering and Regenerative Medicine* **13**: 1738–1755. DOI 10.1002/term.2914.

Bruno S, Deregibus MC, Camussi G (2015). The secretome of mesenchymal stromal cells: Role of extracellular vesicles in immunomodulation. *Immunology Letters* **168**: 154–158. DOI 10.1016/j.imlet.2015.06.007.

Bruno S, Grange C, Deregibus MC, Calogero RA, Saviozzi S et al. (2009). Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. *Journal of the American Society of Nephrology* **20**: 1053–1067. DOI 10.1681/ASN.2008070798.

Caplan AI (1991). Mesenchymal stem cells. *Journal of Orthopaedic Research* **9**: 641–650. DOI 10.1002/jor.1100090504.

Chang C, Yan J, Yao Z, Zhang C, Li X, Mao HQ (2021). Effects of mesenchymal stem cell-derived paracrine signals and their delivery strategies. *Advanced Healthcare Materials* **10**: e2001689. DOI 10.1002/adhm.202001689.

Chen FM, Liu X (2016). Advancing biomaterials of human origin for tissue engineering. *Progress in Polymer Science* **53**: 86–168. DOI 10.1016/j.progpolymsci.2015.02.004.

Chiabotto G, Pasquino C, Camussi G, Bruno S (2020). Molecular pathways modulated by mesenchymal stromal cells and their extracellular vesicles in experimental models of liver fibrosis. *Frontiers in Cell and Developmental Biology* **8**: 594794. DOI 10.3389/fcell.2020.594794.

Cooper LF, Ravindran S, Huang CC, Kang M (2019). A role for exosomes in craniofacial tissue engineering and regeneration. *Frontiers in Physiology* **10**: 1569. DOI 10.3389/fphys.2019.01569.

da Silva ML, Chagastelles PC, Nardi NB (2006). Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *Journal of Cell Science* **119**: 2204–2213. DOI 10.1242/jcs.02932.

Dabrowska S, Andrzejewska A, Janowski M, Lukomska B (2020). Immunomodulatory and regenerative effects of mesenchymal stem cells and extracellular vesicles: Therapeutic outlook for inflammatory and degenerative diseases. *Frontiers in Immunology* **11**: 591065. DOI 10.3389/fimmu.2020.591065.

Daneshmandi L, Shah S, Jafari T, Bhattacharjee M, Momah D, Saveh-Shemshaki N, Lo KWH, Laurencin CT (2020). Emergence of the stem cell secretome in regenerative engineering. *Trends in Biotechnology* **38**: 1373–1384. DOI 10.1016/j.tibtech.2020.04.013.

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* **8**: 315–317.

Eiró N, Sendon-Lago J, Seoane S, Bermúdez MA, Lamelas ML, Garcia-Caballero T, Schneider J, Perez-Fernandez R, Vizoso FJ (2014). Potential therapeutic effect of the secretome from human uterine cervical stem cells against both cancer and stromal cells compared with adipose tissue stem cells. *Oncotarget* **5**: 10692–10708. DOI 10.18632/oncotarget.2530.

Friedenstein AJ, Gorskaja JF, Kulagina NN (1976). Fibroblast precursors in normal and irradiated mouse hematopoietic organs. *Experimental Hematology* **4**: 267–274.

Galgaro BC, Beckenkamp LR, Nunnenkamp M, Korb VG, Naasani LIS, Roszek K, Wink MR (2021). The adenosinergic pathway in mesenchymal stem cell fate and functions. *Medicinal Research Reviews* **41**: 2316–2349. DOI 10.1002/med.21796.

Gholami L, Nooshabadi VT, Shahabi S, Jazayeri M, Tarzemany R, Afsartala Z, Khorsandi K (2021). Extracellular vesicles in bone and periodontal regeneration: Current and potential therapeutic applications. *Cell & Bioscience* **11**: 16. DOI 10.1186/s13578-020-00527-8.

Groot M, Lee H (2020). Sorting mechanisms for MicroRNAs into extracellular vesicles and their associated diseases. *Cells* **9**: 1044. DOI 10.3390/cells9041044.

Hanson S, D'Souza RN, Hematti P (2014). Biomaterial-mesenchymal stem cell constructs for immunomodulation in composite tissue engineering. *Tissue Engineering Part A* **20**: 2162–2168. DOI 10.1089/ten.tea.2013.0359.

Harrell CR, Jovicic N, Djonov V, Arsenijevic N, Volarevic V (2019). Mesenchymal stem cell-derived exosomes and other extracellular vesicles as new remedies in the therapy of inflammatory diseases. *Cells* **8**: 1605. DOI 10.3390/cells8121605.

Hu Z, Zhang L, Wang H, Wang Y, Tan Y et al. (2020). Targeted silencing of miRNA-132-3p expression rescues disuse osteopenia by promoting mesenchymal stem cell osteogenic differentiation and osteogenesis in mice. *Stem Cell Research & Therapy* **11**: 58. DOI 10.1186/s13287-020-1581-6.

Huang R, Qin C, Wang J, Hu Y, Zheng G, Qiu G, Ge M, Tao H, Shu Q, Xu J (2019). Differential effects of extracellular vesicles from aging and young mesenchymal stem cells in acute lung injury. *Sedentary Life and Nutrition* **11**: 7996–8014. DOI 10.18632/aging.102314.

Iaquinta MR, Lanzillotti C, Mazziotta C, Bononi I, Frontini F et al. (2021). The role of microRNAs in the osteogenic and chondrogenic differentiation of mesenchymal stem cells and bone pathologies. *Theranostics* **11**: 6573–6591. DOI 10.7150/thno.55664.

Iser IC, Bracco PA, Gonçalves CEI, Zanin RF, Nardi NB, Lenz G, Battastini AMO, Wink MR (2014). Mesenchymal stem cells from different murine tissues have differential capacity to metabolize extracellular nucleotides. *Journal of Cellular Biochemistry* **115**: 1673–1682. DOI 10.1002/jcb.24830.

Kim GB, Shon OJ, Seo MS, Choi Y, Park WT, Lee GW (2021). Mesenchymal stem cell-derived exosomes and their therapeutic potential for osteoarthritis. *Biology* **10**: 285. DOI 10.3390/biology10040285.

Lai RC, Arslan F, Lee MM, Sze NSK, Choo A et al. (2010). Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. *Stem Cell Research* **4**: 214–222. DOI 10.1016/j.scr.2009.12.003.

Lazarus HM, Haynesworth SE, Gerson SL, Rosenthal NS, Caplan AI (1995). *Ex vivo* expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells): Implications for therapeutic use. *Bone Marrow Transplantation* **16**: 557–564.

Li S, Liu J, Liu S, Jiao W, Wang X (2021). Mesenchymal stem cell-derived extracellular vesicles prevent the development of osteoarthritis via the circHIPK3/miR-124-3p/MYH9 axis. *Journal of Nanobiotechnology* **19**: 1–20.

Marupanthorn K, Tantrawatpan C, Kheolamai P, Tantikanlayaporn D, Manochantr S (2021). MicroRNA treatment modulates osteogenic differentiation potential of mesenchymal stem cells derived from human chorion and placenta. *Scientific Reports* **11**: 7670. DOI 10.1038/s41598-021-87298-5.

Naasani LIS, Rodrigues C, de Campos RP, Beckenkamp LR, Iser IC, Bertoni APS, Wink MR (2017). Extracellular nucleotide hydrolysis in dermal and limbal mesenchymal stem cells: A source of adenosine production. *Journal of Cellular Biochemistry* **118**: 2430–2442. DOI 10.1002/jcb.25909.

Naasani LIS, Souza AFD, Rodrigues C, Vedovatto S, Azevedo JG, Bertoni APS, da Cruz Fernandes M, Buchner S, Wink MR (2019). Decellularized human amniotic membrane associated with adipose derived mesenchymal stromal cells as a bioscaffold: Physical, histological and molecular analysis. *Biochemical Engineering Journal* **152**: 107366. DOI 10.1016/j.bej.2019.107366.

Novoseletskaya E, Grigorieva O, Nimiritsky P, Basalova N, Eremichev R et al. (2020). Mesenchymal stromal cell-produced components of extracellular matrix potentiate multipotent stem cell response to differentiation stimuli. *Frontiers in Cell and Developmental Biology* **8**: 555378. DOI 10.3389/fcell.2020.555378.

O'Driscoll L (2020). Extracellular vesicles from mesenchymal stem cells as a COVID-19 treatment. *Drug Discovery Today* **25**: 1124–1125. DOI 10.1016/j.drudis.2020.04.022.

Paim TC, Wermuth DP, Bertaco I, Zanatelli C, Naasani LIS, Slaviero M, Driemeier D, Schaeffer L, Wink MR (2020). Evaluation of *in vitro* and *in vivo* biocompatibility of iron produced by powder metallurgy. *Materials Science & Engineering: C* **115**. DOI 10.1016/j.msec.2020.111129.

Rodrigues C, de Assis AM, Moura DJ, Halmenschlager G, Saffi J, Xavier LL, da Cruz Fernandes M, Wink MR (2014). New therapy of skin repair combining adipose-derived mesenchymal stem cells with sodium carboxymethylcellulose scaffold in a pre-clinical rat model. *PLoS One* **9**: e96241. DOI 10.1371/journal.pone.0096241.

Rodrigues C, Naasani LIS, Zanatelli C, Paim TC, Azevedo JG, de Lima JC, da Cruz Fernandes M, Buchner S, Wink MR (2019). Bioglass 45S5: Structural characterization of short range order and analysis of biocompatibility with adipose-derived mesenchymal stromal cells *in vitro* and *in vivo*. *Materials Science & Engineering C, Materials for Biological Applications* **103**: 109781. DOI 10.1016/j.msec.2019.109781.

Saliminejad K, Khorram Khorshid HR, Soleymani Fard S, Ghaffari SH (2019). An overview of microRNAs: Biology, functions, therapeutics, and analysis methods. *Journal of Cellular Physiology* **234**: 5451–5465. DOI 10.1002/jcp.27486.

Samadi P, Saki S, Manoochehri H, Sheykhhasan M (2021). Therapeutic applications of mesenchymal stem cells: A comprehensive review. *Current Stem Cell Research & Therapy* **16**: 323–353. DOI 10.2174/1574888X15666200914142709.

Schultz IC, Bertoni APS, Wink MR (2021). Mesenchymal stem cell-derived extracellular vesicles carrying miRNA as a potential multi target therapy to COVID-19: An *in silico* analysis. *Stem Cell Reviews and Reports* **17**: 341–356. DOI 10.1007/s12015-021-10122-0.

Senegaglia AC, Rebelatto CLK, Franck CL, Lima JS, Boldrini-Leite LM et al. (2021). Combined use of tocilizumab and mesenchymal stromal cells in the treatment of severe COVID-19: Case report. *Cell Transplantation* **30**: 9636897211021008.

Shi Q, Sun B, Wang D, Zhu Y, Zhao X, Yang X, Zhang Y (2020). Circ6401, a novel circular RNA, is implicated in repair of the damaged endometrium by Wharton's jelly-derived

mesenchymal stem cells through regulation of the miR-29b-1-5p/RAP1B axis. *Stem Cell Research & Therapy* **11**: 1–16.

Sierra-Sánchez Á., Montero-Vilchez T, Quiñones-Vico MI, Sanchez-Diaz M, Arias-Santiago S (2021). Current advanced therapies based on human mesenchymal stem cells for skin diseases. *Frontiers in Cell and Developmental Biology* **9**: 643125. DOI 10.3389/fcell.2021.643125.

Song N, Scholtemeijer M, Shah K (2020). Mesenchymal stem cell immunomodulation: Mechanisms and therapeutic potential. *Trends in Pharmacological Sciences* **41**: 653–664. DOI 10.1016/j.tips.2020.06.009.

Sous Naasani LI, Rodrigues C, Azevedo JG, Damo Souza AF, Buchner S, Wink MR (2018). Comparison of human denuded amniotic membrane and porcine small intestine submucosa as scaffolds for limbal mesenchymal stem cells. *Stem Cell Reviews and Reports* **14**: 744–754. DOI 10.1007/s12015-018-9819-8.

Sun B, Shi L, Shi Q, Jiang Y, Su Z, Yang X, Zhang Y (2018). Circular RNAs are abundantly expressed and upregulated during repair of the damaged endometrium by Wharton's jelly-derived mesenchymal stem cells. *Stem Cell Research & Therapy* **9**: 314. DOI 10.1186/s13287-018-1046-3.

Tavassoli M, Crosby WH (1968). Transplantation of marrow to extramedullary sites. *Science* **161**: 54–56. DOI 10.1126/science.161.3836.54.

Vedovatto S, Facchini JC, Batista RK, Paim TC, Lionzo MIZ, Wink MR (2020). Development of chitosan, gelatin and liposome film and analysis of its biocompatibility *in vitro*. *International Journal of Biological Macromolecules* **160**: 750–757. DOI 10.1016/j.ijbiomac.2020.05.229.

Vieira NM, Zucconi E, Bueno CR Jr, Secco M, Suzuki MF, Bartolini P, Vainzof M, Zatz M (2010). Human multipotent mesenchymal stromal cells from distinct sources show different *in vivo* potential to differentiate into muscle cells when injected in dystrophic mice. *Stem Cell Reviews and Reports* **6**: 560–566. DOI 10.1007/s12015-010-9187-5.

Vizoso FJ, Eiro N, Cid S, Schneider J, Perez-Fernandez R (2017). Mesenchymal stem cell secretome: Toward cell-free therapeutic strategies in regenerative medicine. *International Journal of Molecular Sciences* **18**: 9. DOI 10.3390/ijms18091852.

Yang Y, Ye Y, Su X, He J, Bai W, He X (2017). MSCs-derived exosomes and neuroinflammation, neurogenesis and therapy of traumatic brain injury. *Frontiers in Cellular Neuroscience* **11**: 55. DOI 10.3389/fncel.2017.00055.

Zuk PA, Zhu M, Mizuno H, Huang J, William Futrell J, Katz AJ, Benhaim P, Peter Lorenz H, Hedrick MH (2001). Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Engineering* **7**: 211–228. DOI 10.1089/107632701300062859.

TABLE S1

Clinical trials with mesenchymal stem cells.

Cell type/ source	Study brief title	Condition	Interventions	Phase status	Reference
Autologous Bone Marrow-derived MSCs	Combination of autologous MSC and HSC infusion in patients with decompensated cirrhosis	Cirrhosis	- CD34 and MSC infusion - Standard of care for cirrhosis management	Phase 4 Completed	NCT0424368
	Bone regeneration with mesenchymal stem cells	Mandibular Fractures	- Application of MSCs	Phase 3 Completed	NCT02755922
	Safety and efficacy of autologous mesenchymal stem cells in chronic spinal cord injury	Spinal Cord Injury	- Posterior cervical laminectomy and MSCs transplantation	Phase 2/3 Completed	NCT01676441
	Safety and efficacy of intracoronary adult human mesenchymal stem cells after acute myocardial infarction	Acute Myocardial Infarction	- Intracoronary injection of MSCs - Control (aspirin and clopidogrel)	Phase 2/3 Completed	NCT01392105
	A comparative study of 2 doses of BM autologous H-MSC + biomaterial *vs.* iliac crest autograft for bone healing in non-union	Non-Union Fracture	- MSCs low dose + biphasic calcium phosphate (BCP) - MSCs high dose + BCP - Control (Autologous iliac crest graft)	Phase 3 Recruiting	NCT03325504
	To evaluate the efficacy and safety of HEARTICELGRAM®-AMI in patients with acute myocardial infarction	Acute Myocardial Infarction	- MSCs and contemporary drug treatment - Control (contemporary drug treatment)	Phase 3 Recruiting	NCT01652209
	Clinical trial to evaluate the efficacy and safety of Cellgram-LC administration in patients with alcoholic cirrhosis	Alcoholic Cirrhosis	- Injection of MSCs in hepatic artery - Best supportive care	Phase 3 Not yet recruiting	NCT04689152
	Bone marrow mesenchymal stem cells transfer in patients with ST-segment elevation myocardial infarction	Myocardial Infarction	- Bone marrow MSC transfer - Best medical treatment - Percutaneous coronary intervention	Phase 2/3 Completed	NCT04421274

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Table S1 (continued).

Cell type/ source	Study brief title	Condition	Interventions	Phase status	Reference
	Parkinson's disease therapy using cell technology	Transplantation: Mesenchymal Stem Cell	- MSCs transplantation - Placebo	Phase 2/3 Recruiting	NCT04146519
	Bone marrow derived stem cell transplantation in T2DM	Type 2 Diabetes Mellitus	- MSCs transplantation - MNC's transplantation - Control	Phase 3 Recruiting	NCT01759823
	Efficacy in alveolar bone regeneration with autologous mscs and biomaterial in comparison to autologous bone grafting	Alveolar Bone Atrophy	- MSC combined with BCP - Autologous bone graft	Phase 3 Recruiting	NCT04297813
	Evaluatiton the efficacy and safety of mutiple lenzumestrocel (neuronata-r® inj.) Treatment in patients with ALS	Amyotrophic Lateral Sclerosis	- Lenzumestrocel - Riluzole - Placebo	Phase 3 Recruiting	NCT04745299
	Cardiovascular clinical project to evaluate the regenerative capacity of cardiocell in patients with acute myocardial infarction (AMI)	Myocardial Infarction	- MSCs administration - Placebo	Phases 2 and 3 Completed	NCT03404063
	Safety and efficacy of repeated administrations of NurOwn® in ALS patients	Amyotrophic Lateral Sclerosis (ALS)	- NurOwn® (MSC-NTF cells) - Placebo	Phase 3 Completed	NCT03280056
	Stem cell therapy for treatment of female stress urinary incontinence	Urinary Incontinence, Stress	- MSCs injection - Surgery (TVT)	Phase 3 Completed	NCT02334878
	Transplantation of bone marrow derived mesenchymal stem cells in affected knee osteoarthritis by rheumatoid arthritis	Rheumatoid Arthritis	- MSCs transplantation - Placebo	Phase 2/3 Completed	NCT01873625
	Bone marrow *vs.* adipose autologous mesenchymal stem cells for the treatment of knee osteoarthritis*	Knee Osteoarthritis	- Bone marrow derived MSC - Adipose derived MSC - Bone marrow & adipose derived MSC injection	Phase 3 Not yet recruiting	NCT04351932
	Multicenter trial of stem cell therapy for osteoarthritis (miles)*	Osteoarthritis	- Autologous Bone Marrow Concentrate - Adipose-derived Stromal Vascular Fraction - Umbilical Cord Tissue - Corticosteroid injection	Phase 3 Active, not recruiting	NCT03818737
Allogeneic Bone Marrow-derived MSCs	Mesenchymal stem cell infusion in haploidentical hematopoietic stem cell transplantation in patients with hematological malignancies	Hematopoietic Stem Cell Transplantation	- MSCs infusion - Cyclophosphamide administration	Phase 3 Completed	NCT03106662
	A study of allogeneic low oxygen mesenchymal bone marrow cells in subjects with myocardial infarction	Myocardial Infarction	- MSCs administration - Placebo	Phase 3 Completed	NCT02672267
	Left ventricular assist device combined with allogeneic mesenchymal stem cells implantation in patients with end-stage heart failure.	Heart Failure Ischemic Cardiomyopathy	- MSCs implantation	Phase 2/3 Active, not recruiting	NCT01759212
	MSCS in COVID-19 ARDS	Acute Respiratory Distress Syndrom, COVID	- MSCs infusion - Placebo	Phase 3 Active, not recruiting	NCT04371393
	Evaluation of PROCHYMAl® adult human stem cells for treatment-resistant moderate-to-severe crohn's disease	Crohn's Disease	- *Ex Vivo* Cultured Adult Human Mesenchymal Stem Cells-Prochymal® - Placebo	Phase 3 Completed	NCT00482092

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Table S1 (continued).

Cell type/ source	Study brief title	Condition	Interventions	Phase status	Reference
	Extended evaluation of PROCHYMAL® adult human stem cells for treatment-resistant moderate-to-severe crohn's disease	Crohn's Disease	- Placebo - *Ex Vivo* Cultured Adult Human Mesenchymal Stem Cells- Prochymal®	Phase 3 Completed	NCT00543374
	Evaluation of PROCHYMAL® for treatment-refractory moderate-to-severe crohn's disease	Crohn's Disease	- *Ex Vivo* Cultured Adult Human Mesenchymal Stem Cells- Prochymal®	Phase 3 Completed	NCT01233960
	Efficacy and safety of PROCHYMAL™ infusion in combination with corticosteroids for the treatment of newly diagnosed acute graft *versus* host disease (GVHD)	Graft *vs.* Host Disease	- *Ex Vivo* Cultured Adult Human Mesenchymal Stem Cells- Prochymal® - Placebo	Phase 3 Completed	NCT00562497
Autologous Adipose-derived MSCs	Clinical study to evaluate efficacy and safety of ASC and fibringlue or fibringlue in patients with crohn's fistula	Crohn's Fistula	- MSCs injection - Fibrin Glue	Phase 3 Recruiting	NCT04612465
	A phase 2b/3a study to evaluate the efficacy and safety of JOINTSTEM in patients diagnosed as knee osteoarthritis	Osteoarthritis, Knee	- JOINTSTEM - Placebo Control	Phases 2 and 3 Recruiting	NCT04368806
	A phase 3 study to evaluate the efficacy and safety of JOINTSTEM in treatment of osteoarthritis	Degenerative Arthritis Knee Osteoarthritis	- JOINTSTEM - Saline	Phase 3 Completed	NCT03990805
	Clinical trial to evaluate the efficacy and safety of stem cells	Anal Fistula	- MSCs + fibrin glue - fibrin glue	Phase 3 Completed	NCT01803347
	Effects of α mat *versus* steroid injection in knee osteoarthritis (stα mat-knee study)	Knee Osteoarthritis	-Microfragmented Adipose Tissue Transplant - Corticosteroid injection	Phase 3 Recruiting	NCT04230902
	Follow-up study for participants of JOINTSTEM	Knee Osteoarthritis	- JOINTSTEM - Saline	Phase 3 Enrolling by invitation	NCT04427930
	Bone marrow *versus* adipose autologous mesenchymal stem cells for the treatment of knee osteoarthritis*	Knee Osteoarthritis	- Bone marrow derived-MSC - Adipose derived MSC - Bone marrow & adipose derived MSC injection	Phase 3 Not yet recruiting	NCT04351932
	Multicenter trial of stem cell therapy for osteoarthritis (miles)*	Osteoarthritis	- Autologous Bone Marrow Concentrate - Adipose-derived Stromal Vascular Fraction - Umbilical Cord Tissue - Corticosteroid injection	Phase 3 Active, not recruiting	NCT03818737
Allogeneic Adipose-derived MSC	Clinical study to evaluate efficacy and safety of ALLO-ASC-DFU in patients with diabetic foot ulcers.	Diabetic Foot Ulcer	- ALLO-ASC-DFU - Vehicle sheet	Phase 3 Active, not recruiting	NCT03370874
	Adipose derived mesenchymal stem cells for induction of remission in perianal fistulizing crohn's disease	Crohn's Disease	- MSCs injection - Saline solution	Phase 3 Completed	NCT01541579
	Clinical study to evaluate efficacy and safety of ALLO-ASC-DFU in patients with diabetic wagner grade 2 foot ulcers	Diabetic Foot Ulcer	- Hydrogel sheet containing MSC - Vehicle Sheet without MSCs	Phase 3 Recruiting	NCT04569409

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Table S1 (continued).

Cell type/ source	Study brief title	Condition	Interventions	Phase status	Reference
Allogeneic Umbilical Cord-derived MSCs	Different efficacy between rehabilitation therapy and stem cells transplantation in patients with sci in China	Spinal Cord Injury	- Cell therapy - Cehabilitation	Phase 3 Completed	NCT01873547
	The effectiveness of adding allogenic stem cells after traditional treatment of osteochondral lesions of the talus	Osteochondral Fracture of Talus	- Platelet-poor plasma scaffold embedded in MSCs added to the traditional treatment for osteochondral lesions - Traditional treatment (debridement and microfracture)	Phase 3 Recruiting	NCT03905824
	Efficacy of stem cell transplantation compared to rehabilitation treatment of patients with cerebral paralysis	Cerebral Palsy	- Rehabilitation - MSCs injection	Phase 3 Completed	NCT01929434
	Efficacy and safety of UC-MSCS for the treatment of steroid-resistant agvhd following allo-hsct	Graft *vs.* Host Disease	- MSCs and Anti-CD25 mAb - Anti-CD25 mAb	Phase 3 Not yet Recruiting	NCT04738981
Allogeneic Wharton's Jelly derived MSCs	Management of retinitis pigmentosa by mesenchymal stem cells by Wharton's jelly derived mesenchymal stem cells	Retinitis Pigmentosa Inherited Retinal Dystrophy	- MSCs administration	Phase 3 Completed	NCT04224207
	Randomized clinical trial to evaluate the regenerative capacity of cardiocell in patients with chronic ischaemic heart failure (CIHF)	Heart Failure	- CardioCell - Placebos	Phases 2 and 3 Completed	NCT03418233
	Therapy of toxic optic neuropathy via combination of stem cells with electromagnetic stimulation	Methanol Poisoning Toxic Optic Neuropathy Stem Cell Tyrosine Kinase 1 Y842X Magnetic Field Exposure	- MSCs and repetitive electromagnetic stimulation - MSCs injection - Repetitive electromagnetic stimulation	Phase 3 Completed	NCT04877067
	Cardiovascular clinical project to evaluate the regenerative capacity of cardiocell in patients with no-option critical limb ischemia (n-o cli)	Critical Limb Ischemia	- CardioCell - Placebos	Phases 2 and 3 Active, not recruiting	NCT03423732
Allogeneic Human Umbilical Cord Blood-derived MSCs	Efficacy and safety of allogenic stem cell product (CARTISTEM®) for osteochondral lesion of talus	Chondral or Osteochondral Lesion of Talus	- CARTISTEM® (product based MSCs) - Microfracture	Phase 3 Active, not recruiting	NCT04310215
	Follow-up study of CARTISTEM® *versus* microfracture for the treatment of knee articular cartilage injury or defect	Degenerative Osteoarthritis Defect of Articular Cartilage	- CARTISTEM - Microfracture	Phase 3 Completed	NCT01626677
	Multicenter trial of stem cell therapy for osteoarthritis (MILES)	Osteoarthritis	- Autologous Bone Marrow Concentrate - Adipose-derived Stromal Vascular Fraction - Umbilical Cord Tissue - Corticosteroid injection	Phase 3 Active, not recruiting	NCT03818737
	Study to compare efficacy and safety of CARTISTEM and microfracture in patients with knee articular cartilage injury	Cartilage Injury Osteoarthritis	- Cartistem - Microfracture treatment	Phase 3 Completed	NCT01041001

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Table S1 (continued).

Cell type/ source	Study brief title	Condition	Interventions	Phase status	Reference
Autologous Deciduous Dental Pulp-derived MSCs	Bone tissue engineering with dental pulp stem cells for alveolar cleft repair	Cleft Lip and Palate	- MSCs associated with hydroxyapatita/collagen - Iliac crest autogenous bone graft	Phase 3 Completed	NCT03766217
Non-Informed	Comparative study of strategies for management of Duchenne Myopathy (DM)	Myopathy	- Sildenafil (Phosphodiesterase inhibitors - Prednisolone (Steroids) - MSC transplantation	Phase 4 Not yet recruiting	NCT03633565
	Mesenchymal stem cell therapy for SARS-CoV-2-related Acute Respiratory Distress Syndrome	COVID-19	- MSC therapy protocol 1 -MSC therapy protocol 2	Phase 2/3 Recruiting	NCT04366063
	MSC for Severe aGVHD	Steroid-resistant Severe aGVHD	- MSCs	Phase 2/3 Recruiting	NCT03631589
	Clinical extension study for safety and efficacy evaluation of Cellavita-HD Administration in Huntington's patients	Huntington Disease	- Injection of Cellavita-HD	Phase 2/3 Active, not Recruiting	NCT04219241

Notes: Free text terms searched in ClinicalTrials.gov on August 2021: "Mesenchymal stem cells" or "Mesenchymal stromal cells" and "extracellular vesicles" or Exosomes or microvesicles, with no language or time restrictions. Studies with status Recruiting, not yet recruiting, active, not recruiting, completed, enrolling by invitation and terminated studies were included. *Studies involving 2 or more MSCs types.