Delta University Scientific Journal
Journal home page: https://dusj.journals.ekb.eg

Advancing Regenerative Medicine: A Comprehensive Review of Cell-Free Therapies

Dr. Mahmoud Mohamed Aboulfotoh[1]

[1] *Lecturer of oral biology, Delta University for Science and Technology, Gamasa, Dakhlia, Egypt.*

Correspondence: Mahmoud Mohamed Aboulfotoh; Tel; 01555505577; Email: Mahmoud.abouelfotouh@deltauniv.edu.eg

ABSTRACT

This comprehensive review explores the innovative advancements in regenerative medicine, focusing on cell-free therapies and their transformative potential. Stem cells, particularly those derived from oral tissues, demonstrate exceptional capabilities for self-renewal, differentiation, and therapeutic application. Human dental stem cells (HDSCs) offer promising pathways for tissue engineering and regenerative treatments due to their stability, plasticity, and high proliferation capacities. However, limitations in cell-based therapies, including safety concerns and logistical challenges, have led to the rise of cell-free approaches. Central to these are secretomes and extracellular vesicles (EVs), which leverage bioactive compounds for tissue repair without direct cell transplantation. This review highlights the therapeutic promise of secretomes, detailing their composition, production, and advantages, such as reduced immunogenicity and tumorigenicity. Additionally, the emerging role of EVs, including exosomes and microvesicles, is examined for their precision and efficiency in clinical applications. These cell-free modalities represent a paradigm shift, offering scalable and safer alternatives for advancing regenerative medicine.

Keywords: *Stem Cells- Secretome- Extracellular Vesicles- Cell free therapy.*

Stem Cells

The unique and multipurpose stem cell type is defined by its ability to self-replicate and divide indefinitely, ultimately giving rise to specialized cell types (1). Neuronal crest cells make the craniofacial skeleton, somite sclerotome cells make the axial skeleton, and lateral plate mesoderm makes the appendicular skeleton (2). These three embryonic lineages contribute to the development of the vertebrate skeleton. Since these cells are easily accessible, can differentiate into many different types of cells, and can proliferate vigorously, they have attracted a lot of attention from scientists in recent decades (3). Embryonic pluripotent stem cells, of which oral stem cells are a subset, originate in neural crests; they undergo considerable migration and eventual engraftment into several tissues (4). Mesenchymal stem cells (MSCs) are cells in mouse bone marrow (BM) that attach to plastic and resemble fibroblasts, according to Arnold L. Caplan (5) hese cells were eventually discovered to proliferate, differentiate into a variety of tissues (including fat, bone, and reticular tissue), and even create structures resembling colonies.

Human dental stem cells (HDSC):

Stem cells found in oral tissues have the remarkable ability to self-renew and distinguish into many cell types, including odontoblasts, endothelial cells, adipocytes, and neural cells (6). The six different types of human dental stem cells that have been described in literature so far are: dental pulp stem cells (DPSCs), SHED stem cells, PDLSCs, APSCs, DFSCs, and GMSCs, which stand for gingival MSCs (7).

Oral stem cell cultures initially consist of colonies of bipolar fibroblastoid cells characterized by oval nuclei with two or three nucleoli. Research has shown that some kinds of stem cells, such as DPSCs, SHEDs, PDLSCs, and BMSCs , tend to grow and multiply more effectively than BMSCs (8). However, embryonic stem cell properties, such as proliferation and possible differentiation capacity, may be impacted by the continuous passages of adult MSCs for a longer duration (9). Additionally, MSCs sourced from oral tissues have the ability to sustain the gene expression of embryonic markers and proteins associated with proliferation in long-term passage cultures all the way up to passage 15 (10).

However, recent studies on dental stem cells have shown promising results:

1. **Excellent stability and plasticity**: Dental stem cells remain stable (maintain their properties) and plastic (able to differentiate into various cell types) even after numerous divisions.
2. **Normal karyotype**: They retain a standard chromosome structure (karyotype), which means their genetic integrity remains intact, avoiding mutations or abnormalities that could limit their safety or effectiveness.
3. 60 population doublings: These cells don't significantly lose quality even after 60 divisions, or population doublings. Expanding in lab conditions should be restricted to around 20 to 40 population doublings, even with this stability. This rule minimizes any possible deterioration in quality or function by guaranteeing that the cells be utilized while their properties are at their best (11).

Lastly, DPSCs can have clonogenic effectiveness, high proliferation capability, multilineage differentiation ability, and self-renewal ability. They are the most promising (MSCs) for therapeutic use because of their characteristics. However, before using these cells in therapeutic therapy, a number of problems and difficulties need to be resolved(12).

Cell based therapy:

Therapy focuses on controlling the disease rather than curing it, which gives patients with terminal diseases new hope via cell-based therapies, especially stem cells. Stem cell-based treatment is an important part of regenerative medicine because it aims to improve the body's repair mechanisms by stimulating, modulating, and controlling the endogenous stem cell population and replenishing the cell pool for tissue homeostasis and regeneration (13).

Stem cells have been characterized as possible therapeutic agents after being the focus of much scientific and clinical study due to their unique self-renewal and differentiation characteristics. Regenerative medicine primarily aims to repair damaged tissues and restore lost cells by using several stem cell types, such as multipotent stem cells, progenitor cells, and human pluripotent stem cells (hPSCs) (14). One kind of stem cell used in cell-based therapy is autologous, which is the patient's own cells; another is allogeneic, which uses cells from a healthy donor (15).

Recent clinical applications have made extensive use of dental stem cells, particularly autologous dental stem cells, for periodontal tissue engineering (16). However, another case report indicated that a 61-year-old patient's periodontitis was treated with allogeneic SHEDs from a donor who was 7 years old (17). Using markers such as sarcomeric actin and cardiac troponin T, researchers were able to direct PDLSCs to differentiate into endothelial

cells and cardiomyocytes (18). It has been suggested by other researchers that PDLSCs expressing genes related to endoderm and pancreas could be used to generate islet-like cells(19).

The present standard of care for bone defect healing is autografts, which have drawbacks such as a lack of supply and potential harm to the donor site. However, a tissue engineering strategy that combines DPSCs with a biocompatible scaffold has the potential to address the increasing demand for clinically relevant bone tissue, making DPSCs an attractive option for treating a range of bone diseases (20). Also pertinent is the process of differentiating oral stem cells into various tissues.

Cell-free therapy

Some serious problems occurred as the number of authorized clinical studies on stem-cell based treatments increased, and significant restrictions have limited the use of stem-cell therapy to a narrower scope (21). Stem cells derived from donor tissues are scarce, lose their effectiveness after in vitro multiplication, and are likely contaminated. Second, there's the issue of the ineffectiveness of the injected cells; there's mounting evidence that only a small fraction, about 1-3 percent, of stem cells end in sustaining or reaching their intended destinations. Instead, most may become stuck in organs including the liver, spleen, and lungs, and a large number of cells may die soon after injection (22). The most critical issue that caused us to reevaluate the safety of stem-cell treatment was the development of tumors from some of the injected stem cells (23).

Although stem cells are vital for regenerative medicine, there has been new evidence of a way to study their therapeutic potential that does not involve cell transplantation in the past ten years, thanks to expanding understanding of how they work. A number of studies have shown significant therapeutic results across a range of experimental settings using this method, known as cell-free treatment, which relies on stem cells as a source of therapeutic molecules instead of the therapeutic drug (24).

Secretome

Low immunogenicity, emergency applicability, simplicity of storage, minimum side effects, and low tumorigenicity are only a few of the benefits of cell-free treatment over cell-based therapy (25). Extracellular matrix (ECM) proteins, membrane-shedding proteins, and vesicle-derived proteins (such as those found in exosomes and microsomal vesicles) are all part of what is known as the secretome (26).

The rapid manufacturing and transportation of secretomes' medium makes them a good alternative to stem cells in times of crisis, when large amounts may be produced in advance to save money and ensure that patients have access to therapy when they need it(25). It also has a reduced likelihood of recipient rejection response following therapy since it is cell-free (27).

The secretome is a collection of substances released into the extracellular space by cells that are essential for biological control and cell-to-cell communication. These substances include soluble proteins, growth factors, cytokines, free nucleic acids, lipids, and extracellular vesicles. Conditions media (CM) are the recipients of secretomes released into the environment by stem cells (25). The whole milieu of cell-sourced secretomic and vesicular materials is represented in a conditioned medium, which is a kind of culture medium that includes biologically active components secreted into the media substances from cells or tissues that have been cultivated before (28).

Secretomes contain a variety of soluble factors that include growth factors and cytokines, including: (1) PDGF, VEGF, HGF, and M-CSF that are important for hair growth; (2) PDGF, IL-6, IL-11, and TGF that are important for wound healing; (3) laminins and TGFβ1 that contribute to epithelization; (4) FGF-4, FGF-6, and FGF-7/KGF that are known to induce fibroblast and keratinocyte proliferation; (5) EGF, TGFα, PDGF-AA, PDGF-AB, M-CSF, and PLGF that are known to promote epithelial cell migration and proliferation; (6) and-1, VEGF, PLGF, IGF-1, and HGF that are important for angiogenesis; (7) IGFBP that are known to induce cell chemotaxis and proliferation; and (8) TIMP-1 and TIMP-2 that are known to inhibit ECM degradation (29). Due to its essential bioactive ingredients, secretomes may become a potential cell-free therapy for body regeneration.

Bone marrow, adipose-derived, dermal, epidermal, and extra-embryonic perinatal stem cells (placenta, fetal membrane) are among the many possible sources of secretomes (30). Injured cells may respond directly to soluble molecules and vesicles released into CM by stem cells, or they might respond indirectly by stimulating the release of functionally active products from neighboring tissues (25). The micropores in the dermis allow the bioactive chemicals contained in these soluble factors to penetrate, where they activate fibroblasts, boost collagen synthesis and remodeling, and encourage skin regeneration(27).

Secretome preparation

The third passage of stem cells was used to prepare the secretome. The stem cells were rinsed three times with PBS once they reached 80 to 90% confluence, and serum-free DMEM was added to the medium. To release their secretome into the growth medium, cells were cultured in serum-free media for 48 hours. Centrifugation of the collected supernatant yielded CM. The next step was to collect the media, filter them through a 0.2 μm filter to exclude any cellular debris, and then store them at -80°C until they were needed (31). To characterize bone marrow-derived (BMSCs), Assi et al. (32). employed an Enzyme-Linked Immunosorbent Assay (ELISA) to quantify the amounts of two proteins, PDGF and IL-10, in conditioned medium (CM). Yet, enzyme-linked immunosorbent assays (ELISAs) play a vital role in CM characterisation, particularly when it comes to identifying therapeutically important proteins. Although it can't fully replace comprehensive proteomic approaches, it is an essential tool for CM analysis because to its specificity, sensitivity, and user-friendliness.

Extracellular vesicles (EVs)

The vesicles that make up the secretome are called extracellular vesicles (EVs). These tiny particles, which range in size from 30 to 5000 nm, are encased in phospholipids, making them lipid bilayer-bound vesicles. Inside, they hold a diverse and intricate mixture of substances, including proteins, lipids, and nucleic acids. Methods based on polymer precipitation, size exclusion chromatography, ultra-centrifugation, filtering, and ion exchange chromatography may be used to separate extracellular vesicles from soluble components in CM (33). In contrast to soluble components and free nucleic acids in CM, which are susceptible to rapid destruction, EVs' membranes prevent this from happening. Since EVs need less dose, take up less space, and may be stored for longer than conditioned media, they are more suited for transit and storage in clinical applications (34). Because CM were so simple to isolate, studies on them began in the 1950s. Nevertheless, EVs were first thought of as products of cellular metabolism until their biological functions were acknowledged (35).

Several distinct kinds of EVs, produced by either endosomal or non-endosomal processes, have been identified via investigations into their biogenesis. While EVs may be released by both prokaryotic and eukaryotic cells, the latter have been the subjects of most extensive research. So far, every kind of mammalian cell—neuronal, endothelium, MSC, and epithelial—has been discovered to secrete EVs. Biological fluids including blood, synovial fluid, urine, and saliva also contain EVs (36).

To date, extracellular vesicles (EVs) have been classified into three subtypes based on their biogenesis and diameters: exosomes, microvesicles, and apoptotic bodies. These subtypes can be retained as a dynamic extracellular vesicular compartment, which is advantageous for their paracrine or autocrine biological effects on tissue metabolism (37). Without a working nucleus, they are unable to reproduce (38). Extracellular vesicles (EVs) may be secreted by any cell, including MSCs. Almost all bodily fluids include EVs; this includes skin, amniotic fluid, breast milk, plasma, serum, saliva, and urine. As we become older, it is believed that EVs help maintain skin homeostasis (39). Conditioned media may also be used to isolate and characterize EVs. Further, EVs were able to function as cell-free therapeutics by way of bioactive compounds (38). Because of their capacity to convey proteins, lipids, nucleic acids (such as mRNAs and miRNAs), and other molecules, EVs are crucial in the process of cell-to-cell communication. (40).

Exosomes

Small extracellular vesicles (EVs) may contain a diverse population of exosomes, ranging from "classical" to "non-classical," and have a diameter of 50-150 nanometers. The multivesicular body (MVB) is a classical exosome that forms when the late endosomal membrane is invaginated inward. A group of proteins called the endosomal sorting complex required for transport (ESCRT) is responsible for recruiting cargo and creating the inward invagination of the late endosomal membrane. ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III are all involved in this process (41).

It is the job of ESCRT-0, ESCRT-I, and ESCRT-II to identify ubiquitinated cargo and load it into the endosome lumen. Intraluminal vesicles (ILVs) in MVBs are orchestrated by ESCRT-III, which is assembled by ESCRT-II proteins. ESCRT-III recruits auxiliary proteins such VPS4 and ALG-2-interacting protein X (ALIX). Sphingomyelinase hydrolysis and neutral sphingomyelinase 2 ceramide production are two additional mechanisms that mediate the inward budding of the MVB that are not reliant on ESCRT (42).

Microvesicles

Microvesicles, also known as big extracellular vesicles (EVs), may have any size between 150 nm and more than 1,000 nm in diameter, with 250–400 nm being the typical (43). One way in which microvesicles are made is via the process of the cell membrane detaching and blossoming outward. Although cytosolic biomolecules undergo random specific exocytosis, membrane proteins and receptors are directed to the plasma membrane prior to microvesicle budding. Various processes, including phospholipid redistribution, Rho-kinase-mediated myosin light chain phosphorylation, and contractile machinery, contribute to the budding of the plasma membrane at specific sites, enabling vesicle pinching and separation (36). The biological origin of microvesicles may be inferred from their lipid content, receptors on the plasma membrane, and other substances. A wide variety of biomolecules make

up the "cargo" of microvesicles. These macromolecules, which usually comprise ribosomal RNA (rRNA) and messenger RNA (mRNA) fragments, are transported into the plasma membrane (44).

Apoptotic bodies

The apoptotic bodies, which range in size from 1 to 5 μm, make up the biggest category of environmental variables (EVs). The blebbing and protrusion of the apoptotic cell membrane during cell death is the process that produces them. Blebbing of the membrane, leading to the formation of apoptotic protrusions such as microtubule spikes, apoptopodia, and beaded apoptopodia, happens during programmed cell death, which may be produced by either normal physiological signaling or pathologic events. Disassembling into apoptotic structures, these projections encase whole cellular organelles, nuclear genomic DNA fragments, nucleic acids, and unexpected cargo. Removing apoptotic bodies typically involves macrophage phagocytosis, which is triggered by the identification of signals on the plasma membrane (36).

Finally, Longwei et al. (2019) [58] describe the roles of EVs in various types of tissue regeneration based on where EVs were first developed for regenerative medicine. Nonetheless, it primarily falls into four categories: (a) related cells in the regenerative niche, such as osteoblasts and bone marrow-derived stem cells (BMMSCs); (b) mesenchymal stem cells (MSCs), such as BMMSCs, adipose-derived stem cells (ASCs), induced pluripotent stem cell (iPSC)–derived mesenchymalstem cells (iPSC-MSCs, and umbilical cord MSCs (ucMSCs); (c) immune cells, such as dendritic cells, monocytes, and macrophages; and (d) bodily fluids, such as platelet-rich plasma (PRP) and human umbilical cord blood (UCB). Though identical cells produce different EVs in response to environmental changes, secretomes are cell-specific. For highly effective precision medicine, it is necessary to understand how EVs regulate complicated tissue regeneration in order to create customized cells and EVs.

Conclusion

In conclusion, advancements in cell-free therapies signify a paradigm shift in regenerative medicine, emphasizing safety, scalability, and therapeutic efficacy. By leveraging the secretome and extracellular vesicles, researchers can bypass the challenges associated with traditional stem-cell-based therapies, such as immunogenicity and tumorigenicity. These innovative approaches harness the intrinsic bioactive potential of stem cells to promote tissue repair and regeneration without direct cell transplantation. Moving forward, integrating cell-free therapies with precision medicine holds immense promise for addressing a broad spectrum of clinical conditions, paving the way for groundbreaking treatments that prioritize patient safety and outcome optimization.

References

1. Ahmadian S, Mahdipour M, Pazhang M, Sheshpari S, Mobarak H, Bedate AM, et al. Effectiveness of Stem Cell Therapy in the Treatment of Ovarian Disorders and Female Infertility: A Systematic Review. Curr Stem Cell Res Ther [Internet]. 2020 Mar 26;15(2):173–86. Available from: http://www.eurekaselect.com/176825/article
2. Florencio-Silva R, Sasso GR da S, Sasso-Cerri E, Simões MJ, Cerri PS. Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells. Biomed Res Int [Internet]. 2015;2015:1–17. Available from: http://www.hindawi.com/journals/bmri/2015/421746/
3. Spagnuolo G, Codispoti B, Marrelli M, Rengo C, Rengo S, Tatullo M. Commitment of Oral-Derived Stem Cells in Dental and Maxillofacial Applications. Dent J [Internet]. 2018 Dec 13;6(4):72. Available from: https://www.mdpi.com/2304-6767/6/4/72
4. Ibarretxe G, Crende O, Aurrekoetxea M, García-Murga V, Etxaniz J, Unda F. Neural Crest Stem Cells from Dental Tissues: A New Hope for Dental and Neural Regeneration. Stem Cells Int [Internet]. 2012;2012:1–12. Available from: http://www.hindawi.com/journals/sci/2012/103503/
5. Caplan AI. Mesenchymal Stem Cells: Time to Change the Name! Stem Cells Transl Med [Internet]. 2017 Jun 1;6(6):1445–51. Available from: https://academic.oup.com/stcltm/article/6/6/1445/6448531
6. Diomede F, Zini N, Pizzicannella J, Merciaro I, Pizzicannella G, D'Orazio M, et al. 5-Aza Exposure Improves Reprogramming Process Through Embryoid Body Formation in Human Gingival Stem Cells. Front Genet [Internet]. 2018 Oct 8;9. Available from: https://www.frontiersin.org/article/10.3389/fgene.2018.00419/full
7. Park Y-J, Cha S, Park Y-S. Regenerative Applications Using Tooth Derived Stem Cells in Other Than Tooth Regeneration: A Literature Review. Keating A, editor. Stem Cells Int [Internet]. 2016 Jan 20;2016(1). Available from: https://onlinelibrary.wiley.com/doi/10.1155/2016/9305986
8. Ivanovski S, Gronthos S, Shi S, Bartold P. Stem cells in the periodontal ligament. Oral Dis [Internet]. 2006 Jul 21;12(4):358–63. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1601-0825.2006.01253.x
9. Yu J, He H, Tang C, Zhang G, Li Y, Wang R, et al. Differentiation potential of STRO-1+ dental pulp stem cells changes during cell passaging. BMC Cell Biol [Internet]. 2010 Dec 8;11(1):32. Available from: https://bmcmolcellbiol.biomedcentral.com/articles/10.1186/1471-2121-11-32
10. Diomede F, Rajan TS, Gatta V, D'Aurora M, Merciaro I, Marchisio M, et al. Stemness Maintenance Properties in Human Oral Stem Cells after Long-Term Passage. Stem Cells Int [Internet]. 2017;2017:1–14. Available from: https://www.hindawi.com/journals/sci/2017/5651287/
11. Avinash K, Malaippan S, Dooraiswamy JN. Methods of Isolation and Characterization of Stem Cells from Different Regions of Oral Cavity Using Markers: A Systematic Review. Int J Stem Cells [Internet]. 2017 May 30;10(1):12–20. Available from: http://www.ijstemcell.com/journal/view.html?doi=10.15283/ijsc17010
12. Borrelli MR, Hu MS, Longaker MT, Lorenz HP. Tissue Engineering and Regenerative Medicine in Craniofacial Reconstruction and Facial Aesthetics. J Craniofac Surg [Internet]. 2020 Jan;31(1):15–27. Available from: https://journals.lww.com/10.1097/SCS.0000000000005840
13. O'Brien T, Barry FP. Stem cell therapy and regenerative medicine. Mayo Clin Proc. 2009;84(10):859–61.
14. Mousaei Ghasroldasht M, Seok J, Park H-S, Liakath Ali FB, Al-Hendy A. Stem Cell Therapy: From Idea to Clinical Practice. Int J Mol Sci [Internet]. 2022 Mar 5;23(5):2850. Available from: https://www.mdpi.com/1422-0067/23/5/2850
15. Srijaya TC, Ramasamy TS, Kasim NHA. Advancing stem cell therapy from bench to bedside: lessons from drug therapies. J Transl Med [Internet]. 2014 Dec 4;12(1):243. Available from: https://translational-medicine.biomedcentral.com/articles/10.1186/s12967-014-0243-9

16. Apatzidou DA, Bakopoulou AA, Kouzi-Koliakou K, Karagiannis V, Konstantinidis A. A tissue-engineered biocomplex for periodontal reconstruction. A proof-of-principle randomized clinical study. J Clin Periodontol [Internet]. 2021 Aug 14;48(8):1111–25. Available from: https://onlinelibrary.wiley.com/doi/10.1111/jcpe.13474

17. Hernández-Monjaraz B, Santiago-Osorio E, Ledesma-Martínez E, Alcauter-Zavala A, Mendoza-Núñez VM. Retrieval of a periodontally compromised tooth by allogeneic grafting of mesenchymal stem cells from dental pulp: A case report. J Int Med Res [Internet]. 2018 Jul 18;46(7):2983–93. Available from: https://journals.sagepub.com/doi/10.1177/0300060518773244

18. Pizzicannella J, Diomede F, Merciaro I, Caputi S, Tartaro A, Guarnieri S, et al. Endothelial committed oral stem cells as modelling in the relationship between periodontal and cardiovascular disease. J Cell Physiol [Internet]. 2018 Oct 30;233(10):6734–47. Available from: https://onlinelibrary.wiley.com/doi/10.1002/jcp.26515

19. Lee JS, An SY, Kwon IK, Heo JS. Transdifferentiation of human periodontal ligament stem cells into pancreatic cell lineage. Cell Biochem Funct [Internet]. 2014 Oct 4;32(7):605–11. Available from: https://onlinelibrary.wiley.com/doi/10.1002/cbf.3057

20. Namjoynik A, Islam MA, Islam M. Evaluating the efficacy of human dental pulp stem cells and scaffold combination for bone regeneration in animal models: a systematic review and meta-analysis. Stem Cell Res Ther [Internet]. 2023 May 15;14(1):132. Available from: https://stemcellres.biomedcentral.com/articles/10.1186/s13287-023-03357-w

21. Soteriou D, Fuchs Y. A matter of life and death: stem cell survival in tissue regeneration and tumour formation. Nat Rev Cancer [Internet]. 2018 Mar 19;18(3):187–201. Available from: https://www.nature.com/articles/nrc.2017.122

22. Toma C, Wagner WR, Bowry S, Schwartz A, Villanueva F. Fate Of Culture-Expanded Mesenchymal Stem Cells in The Microvasculature. Circ Res [Internet]. 2009 Feb 13;104(3):398–402. Available from: https://www.ahajournals.org/doi/10.1161/CIRCRESAHA.108.187724

23. Volarevic V, Markovic BS, Gazdic M, Volarevic A, Jovicic N, Arsenijevic N, et al. Ethical and Safety Issues of Stem Cell-Based Therapy. Int J Med Sci [Internet]. 2018;15(1):36–45. Available from: http://www.medsci.org/v15p0036.htm

24. Evangelista A, Soares MP, Villarreal C. Cell-free therapy: a neuroregenerative approach to sensory neuropathy? Neural Regen Res [Internet]. 2019;14(8):1383. Available from: https://journals.lww.com/10.4103/1673-5374.253522

25. Xia J, Minamino S, Kuwabara K, Arai S. Stem cell secretome as a new booster for regenerative medicine. Biosci Trends [Internet]. 2019 Aug 31;13(4):299–307. Available from: https://www.jstage.jst.go.jp/article/bst/13/4/13_2019.01226/_article

26. Makridakis M, Vlahou A. Secretome proteomics for discovery of cancer biomarkers. J Proteomics [Internet]. 2010 Nov;73(12):2291–305. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1874391910002058

27. Cai Y, Li J, Jia C, He Y, Deng C. Therapeutic applications of adipose cell-free derivatives: a review. Stem Cell Res Ther [Internet]. 2020 Dec 22;11(1):312. Available from: https://stemcellres.biomedcentral.com/articles/10.1186/s13287-020-01831-3

28. Yu D, Li Y, Wang M, Gu J, Xu W, Cai H, et al. Exosomes as a new frontier of cancer liquid biopsy. Mol Cancer [Internet]. 2022 Dec 18;21(1):56. Available from: https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-022-01509-9

29. Balasubramanian S, Thej C, Walvekar A, Swamynathan P, Gupta PK, Seetharam RN, et al. Evaluation of the Secretome Profile and Functional Characteristics of Human Bone Marrow Mesenchymal Stromal Cells-Derived Conditioned Medium Suggest Potential for Skin Rejuvenation. J Cosmet Dermatological Sci Appl [Internet]. 2017;07(01):99–117. Available from: http://www.scirp.org/journal/doi.aspx?DOI=10.4236/jcdsa.2017.71010

30. Foo JB, Looi QH, Chong PP, Hassan NH, Yeo GEC, Ng CY, et al. Comparing the Therapeutic Potential of Stem Cells and their Secretory Products in Regenerative Medicine. Yong KW, editor. Stem Cells Int

[Internet]. 2021 Aug 19;2021:1–30. Available from: https://www.hindawi.com/journals/sci/2021/2616807/

31. Yang C, Lei D, Ouyang W, Ren J, Li H, Hu J, et al. Conditioned Media from Human Adipose Tissue-Derived Mesenchymal Stem Cells and Umbilical Cord-Derived Mesenchymal Stem Cells Efficiently Induced the Apoptosis and Differentiation in Human Glioma Cell Lines In Vitro. Biomed Res Int [Internet]. 2014;2014:1–13. Available from: http://www.hindawi.com/journals/bmri/2014/109389/

32. Assi MM, Grawish ME, Elsabaa HM, Helal ME, Ezzat SK. Therapeutic potential of hyaluronic acid hydrogel combined with bone marrow stem cells-conditioned medium on arthritic rats' TMJs. Sci Rep [Internet]. 2024 Nov 5;14(1):26828. Available from: https://www.nature.com/articles/s41598-024-77325-6

33. Tenchov R, Sasso JM, Wang X, Liaw W-S, Chen C-A, Zhou QA. Exosomes─Nature's Lipid Nanoparticles, a Rising Star in Drug Delivery and Diagnostics. ACS Nano [Internet]. 2022 Nov 22;16(11):17802–46. Available from: https://pubs.acs.org/doi/10.1021/acsnano.2c08774

34. Lai RC, Arslan F, Lee MM, Sze NSK, Choo A, Chen TS, et al. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem Cell Res [Internet]. 2010 May;4(3):214–22. Available from: https://linkinghub.elsevier.com/retrieve/pii/S187350610900141X

35. Lv L, Sheng C, Zhou Y. Extracellular vesicles as a novel therapeutic tool for cell-free regenerative medicine in oral rehabilitation. J Oral Rehabil [Internet]. 2020 Nov 27;47(S1):29–54. Available from: https://onlinelibrary.wiley.com/doi/10.1111/joor.12885

36. Cheng L, Hill AF. Therapeutically harnessing extracellular vesicles. Nat Rev Drug Discov [Internet]. 2022 May 2;21(5):379–99. Available from: https://www.nature.com/articles/s41573-022-00410-w

37. György B, Szabó TG, Pásztói M, Pál Z, Misják P, Aradi B, et al. Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. Cell Mol Life Sci [Internet]. 2011 Aug 11;68(16):2667–88. Available from: http://link.springer.com/10.1007/s00018-011-0689-3

38. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles [Internet]. 2018 Dec 23;7(1). Available from: https://onlinelibrary.wiley.com/doi/10.1080/20013078.2018.1535750

39. Terlecki-Zaniewicz L, Pils V, Bobbili MR, Lämmermann I, Perrotta I, Grillenberger T, et al. Extracellular Vesicles in Human Skin: Cross-Talk from Senescent Fibroblasts to Keratinocytes by miRNAs. J Invest Dermatol [Internet]. 2019 Dec;139(12):2425-2436.e5. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022202X19317543

40. Jarrige M, Frank E, Herardot E, Martineau S, Darle A, Benabides M, et al. The Future of Regenerative Medicine: Cell Therapy Using Pluripotent Stem Cells and Acellular Therapies Based on Extracellular Vesicles. Cells [Internet]. 2021 Jan 27;10(2):240. Available from: https://www.mdpi.com/2073-4409/10/2/240

41. Wollert T, Hurley JH. Molecular mechanism of multivesicular body biogenesis by ESCRT complexes. Nature [Internet]. 2010 Apr 8;464(7290):864–9. Available from: https://www.nature.com/articles/nature08849

42. Verderio C, Gabrielli M, Giussani P. Role of sphingolipids in the biogenesis and biological activity of extracellular vesicles. J Lipid Res [Internet]. 2018 Aug;59(8):1325–40. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022227520330467

43. van Niel G, Carter DRF, Clayton A, Lambert DW, Raposo G, Vader P. Challenges and directions in studying cell–cell communication by extracellular vesicles. Nat Rev Mol Cell Biol [Internet]. 2022 May 8;23(5):369–82. Available from: https://www.nature.com/articles/s41580-022-00460-3

44. Lunavat TR, Cheng L, Kim D-K, Bhadury J, Jang SC, Lässer C, et al. Small RNA deep sequencing discriminates subsets of extracellular vesicles released by melanoma cells – Evidence of unique microRNA cargos. RNA Biol [Internet]. 2015 Aug 3;12(8):810–23. Available from: http://www.tandfonline.com/doi/full/10.1080/15476286.2015.1056975