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EXOSOMES – THE REGAL SCEPTER IN THE EMPIRE OF REGENERATION

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ABSTRACT

Periodontitis is a chronic inflammatory disease characterised by the irreversible destruction and mutilation of the of the teeth supporting complex, christened the periodontium. While treating the malady deems arresting the state of pathogenecity, it also encompasses the aspect of restitutio ad integrum of the lost periodontal structures. While myriad modalities have been tested as means to justify this end, mesenchymal stem cells derived exosomes are the exalted avant garde contrivance in the treatment arsenal. Exosomes/extracellular vesicles are small particles (<100 nm) released from cells possessing a lipid bilayer structure and housing various cellular components, proteins, mRNAs, DNA, and miRNAs. Mesenchymal stem cell derived exosomes contain molecules that modulate wound healing and exert a plethora of actions including angiogenesis, anti-inflammation, osteoblast proliferation, intercellular communication , anti-apoptosis, and immune regulation and all these adeptly work in harmony to succour periodontal regeneration. While overcoming the operational and logistical challenges of stem cells, they have revolutionized the field of periodontal regeneration and this paper is a succinctly comprehensive dossier reviewing this modish yet august stem cell based cell free treatment weaving the magic of regeneration.

KEYWORDS: Exosome, periodontal regeneration, stem cell, miRNA.

INTRODUCTION

The lexicon categorizes periodontitis as one of the most common chronic inflammatory diseases attributed to the colonization and subsequent invasion by the periodontopathic bacteria. What ensues this perplexing quandary of a pathogenic bacteria rich niche is the genesis of a pernicious host response culminating in the progressive destruction of connective tissue and tooth-supporting structures including the gingiva, periodontal ligament, and alveolar bone (Tonetti et al. 2018).[1] Current treatment modalities for periodontitis are efficacious in arresting the pathogenic condition but fail to orchestrate predictable regeneration of lost periodontal structures in sundry clinical scenarios. This lacuna warrants the inception of avant garde therapeutics to confer verisimilitude upon this regenerative reverie .Although recent times have seen the evolution of mesenchymal stem cells (MSC) as potential candidates engineering regeneration, it is marred with several shortcomings. Given that it is a cell based therapy, it incurs copious operational and logistical challenges in the manufacture, delivery and storage of cells for transplantation. The use of MSCs for tissue repair was originally attributed to their singular unmatched potential of differentiation to generate multitudinous cell types that could indemnify the lost cells in injured or dead tissues. However, it is increasingly evident that MSCs secrete copious factors that have the capacities to alleviate tissue injury and promote repair and regeneration. At the nascent stages, the therapeutic efficiency of MSCs was attributed to their ability to migrate and engraft in target tissues; however, studies unveiled that systemically administered MSCs seldom harbour to the target in significant numbers (Gao et al., 2001), evincing that the biological effects observed due to stem cell administration are the upshot of their secreted factors.[3] Enshrouded amidst these intriguing secreted factors are exosomes which are nano-sized membrane vesicles of about 50–200 nm shown to possess prodigious replicative potential justifying their pragmatic far-reaching applications in in animal models for myocardial ischemia reperfusion injury, cutaneous wound, graft-versus-host disease (GVHD) , drug-induced hepatic injury, and more recently bone and cartilage injuries.[2]

EXOSOMES – AN EXPOSITORY EXORDIUM

Extracellular vesicles are designated as particles that are naturally released from the cell and delimited by a lipid bilayer, carrying intravesicular components from the cytosol but not from the nucleus of the secreting cells.

Initially, EVs were presumed to be secreted by the outward budding of the plasma membrane of cells and ostensibly donned the role of "waste bins" of the cells facilitating the disposal of cellular detritus. In 1983, the endosomal pathway was touted to be an alternative way for EV secretion; following which was the emergence of a novel terminology - "exosome" which was proposed to earmark the EVs that originated from the endosome. Originally identified by **Pan and Johnston(1983**), the cell type–dependent exosomal contents may implicate the therapeutic application of exosomes in myriad fields encompassing tissue regeneration, and the discovery of exosomes as biomarkers for diseases. Owing to their variegated fons et origo, they are categorize into three broad groups based on their biogenesis:apoptotic bodies, microvesicles/ectosomes and exosomes. Apoptotic bodies are EVs with the most heterogeneous size (200 nm–5 μm) and are secretory products of dying cell, while microvesicles/ectosomes (100 nm –800 nm) and exosomes (30 nm–150 nm) are secreted by viable cells either through outward budding of the plasma membrane or alternatively stem from intracellular endosomal pathways. Exosomes were initially considered as serendipitous carriers of nugatory flotsams and jetsam of cellular metabolism. Pioneering work by **Raposo et al** led to the levitation of exosomes from the boulevard of frivolity as they were eminently implicated in cellular signalling with the demonstration of lymphocyte exosomes carrying MHC class II antigens from cells to the extracellular fluid and inducing a MHC class II T cell response.[5]

Although the mechanism of exosome biogenesis continue to elude the human mind, esoteric research into the same has implicated the endosomal sorting complex required for transport (ESCRT)–dependent and ESCRT-independent machinery. Endocytosis brings about the advent of intracellular formation of early endosomes, which further mature and form multi-vesicular bodies (MVBs) by inward budding of the late endosomal membrane culminating in the assemblage of intraluminal vesicles (ILVs).

The human ESCRT consists of four complexes, ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III, which are characterised by the assemblage of 33 proteins and are numbered in accordance with the order they act in the pathway and play distinct **roles (Hanson and Cashikar).** In brevity, ESCRT-0 garnering the assistance of clathrin recognize and sequester ubiquitinated transmembrane proteins in the endosomal membrane. Following the sequential recruitment of ESCRT-I and ESCRT-II to ESCRT-0, the complexes in toto initiate the local budding of the endosomal membrane with sorted cargo; subsequently, ESCRTIII is mobilised to bring about

protein deubiquitination and drive vesicle scission. The subsequent formation of ILV-loaded MVBs may be destined to undergo two distinct fates :(a) fusion with lysosomes leading to the discharge and digestion of their ILVs within the internal confines of lysosomes and (b) fusion with the plasma membrane and consequent release of exosomes into the extracellular space. TSG101 and VPS4, principal components of the ESCRT machinery, were also shown to be succour in the direct budding of small EVs at the plasma membrane. Alternatively, the ESCRT-independent machinery mediated by lipids, tetraspanins and small GTPases has also been ensnared in the regulation of exosome biogenesis. Lipid metabolites, enzymes and metabolic products, such as neutral sphingomyelinase (nSMase) and ceramide, and phospholipaseD2 (PLD2) and phosphatidic acid, were shown to initiate and catalyse cargo sorting, inward budding of ILVs and exosome secretion. Evidence that overexpression of the tetraspanin CD9 or CD82 induced secretion of exosomal β-catenin and depletion of CD63 affected the size of ILV evince the roles of tetraspanins in cargo sorting and exosome formation. Several Rab proteins, including RAB11, RAB35, RAB7 and RAB27A/B, were suggested to partake in endosome maturation and exosome secretion. Another small GTPase, ARF6, together with its effector PLD2, was shown to affect the budding of ILVs into MVBs and its overexpression caused downstream depolymerization of the actin cytoskeleton, which triggered the release of vesicles at the plasma membrane.[4]

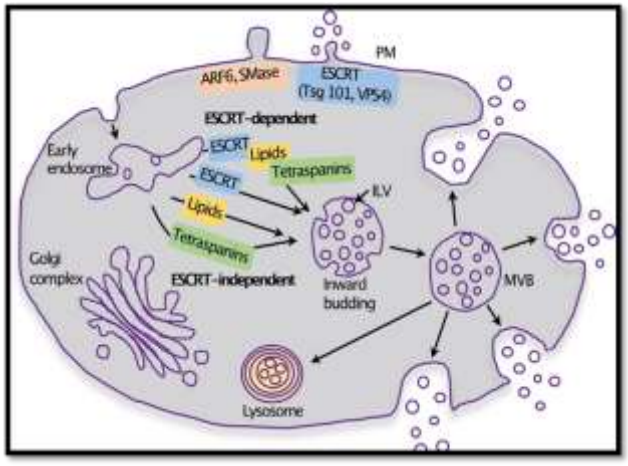


Figure 1: The exosome secretory apparatus.

DECODING THE MOLECULAR CONNUNDRUM

Exosomes harbour both cell type–dependent contents, reflecting their genealogy and secretion conditions, and cell type–independent contents, compositions that are enriched in exosomes and reflect the uniting front of commonality amidst diversity among exosomes

from diverse origins. As a testimony to the same, several lipid species, cholesterol, sphingomyelin (SM), phosphatidylserine (PS) and saturated fatty acids, were more profound in exosomes compared with the total cell membrane. In addition, exosomes also possess cell type-independent proteins, some of which are often involved intertwined in the astute machinery of exosome biogenesis: (1) transmembrane proteins such as (i) tetraspanins CD63, CD9, CD81 and CD82, (ii) integrins and (iii) lipid raft-bound proteins (i.e flotillin and annexins); (2) cytoskeleton proteins (i.e actin); and (3) cytosolic proteins such as (i) components of the ESCRT machinery (ie TSG101 and ALIX), (ii) GTPase , Rabs, (iii) heat shock 70-kDa protein (HSC70) and (iv) proteasomes. Owing to their perpetual presence in exosomes independent of its diverse pedigree, these proteins often serve as exosomal markers. Exosomes also house bioactive RNA, particularly miRNA and mRNA, and a regiment of non-coding RNAs, including vault RNA, Y RNA and tRNA. Current studies have also unsheathed the astounding presence of DNA inside or on the surface of exosomes. In addition to the cell type-independent contents, a growing number of studies have demonstrated that exosomes secreted by different cell types or by cells undergoing various differentiation stages or those residing in various environmental conditions, that is pathological or healthy conditions, carry specific contents, thereby characterising their parental lineage. The cell type–dependent exosomal contents are vividly implicated in the therapeutic application of exosomes, that is in tissue regeneration, and the potential discovery of exosomes as biomarkers for diseases.[4]

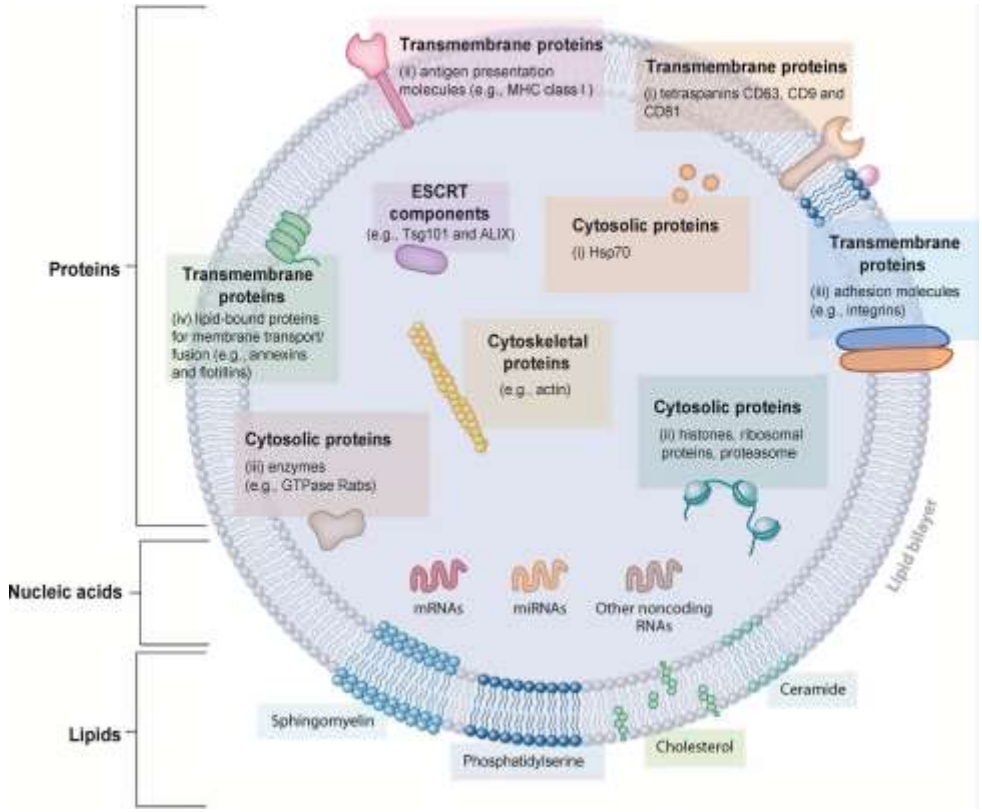


Figure 2: Composition of exosomes.

PROPERTIES

Exosomes are secreted by a wide range of mammalian cell types the conglomeration of which include mesenchymal stem cells, B cells, cytotoxic T cells, neurons, cancer cells, oligodendrocytes, platelets, epithelial cells, DCs, and mast cells. They are ubiquitous in body fluids - saliva, blood, bile, urine, semen, cerebrospinal fluid, ascitic fluid, amniotic fluid, and colostrum. The morphology of these exosomes bear a striking semblance to a cup or saucer as designated by observation under transmission electron microscope.

They float in a sucrose gradient and have a density ranging from 1.13 g/mL (B-cell-derived exosomes) to 1.19 g/mL (intestinal cell-derived exosomes). B-cell exosomes exhibit the greatest homogeny in terms of size (60-80 nm).

The volume of exosomes measures up to an exiguous one millionth that of MSCs, whilst also possessing a stable structure translating to dearth of any perplexities clouding production and storage. While stored at -20˚C, they are bereft of any afflictions for a week and their activity remains unperturbed during long-term storage at -80˚C. Their propensity to cause immune rejection following allogenic administration is trifling owing to the scant membrane bound proteins they possess. Also, as their proliferative prowess is limited, this nullifies the

possibility of tumor seeding. Owing to the possession of these sublime properties they veritably possess safety standards surpassing those of mesenchymal stem cells as far as safety for clinical application goes.[7]

METHODS OF ISOLATION

Though there exists various options, there is no consensus regarding a singular standardised method for isolation of exosomes. A pan-continental survey spearheaded by the International Society for Extracellular Vesicles (ISEV) revealed ultracentrifugation methods to be the most sought after as far as isolation goes. Recent years have seen the rise of alternative methods to overcome the challenges entwined in ultracentrifugation such as requirement of an ultracentrifuge, low-throughput of samples, and potential damage to EVs caused by high-speed centrifugation. The alternative methods encompass filtration/ultrafiltration, size exclusion chromatography, immunoprecipitation, and precipitation with reagents such as polyethylene glycol.[7]

THE ELIXIR FOR REGENERATION

Exosomes derived from mesenchymal stem cells at heterogeneous stages of osteogenic differentiation harbor microRNAs specific to its stage while those cells in the later stages of differentiation are particularly rich repertoires of osteogenesis-related microRNAs. To facilitate the induction of osteogenic differentiation and mineralization the exosomes of the stem cells perched higher up in the ladder of differentiation are primed with pro-osteogenic microRNAs like miR-10b and miR-21 whilst they harboured depleted levels anti-osteogenic microRNAs miR-31, miR-144 and miR-221. The exosomes further augmented the expression of pro-osteogenic and pro-angiogenic miRNAs, miR-2861 and miR-210, respectively, in recipient MSCs, which in turn effected an escalated expression of VEGF and the osteogenic master transcription factor RUNX2 which in turn paved way to accrued osteogenic differentiation. Exosomes create a milieu potentiating osteogenic differentiation by acting through multifarious signalling pathways, particularly the Wnt signalling pathway, P13/Akt pathways, AKT, ERK and AMPK signalling pathways.

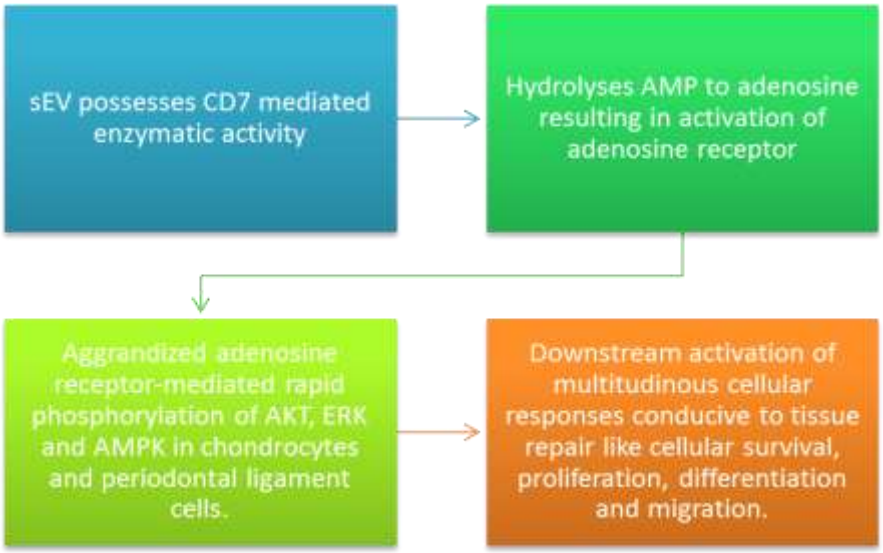
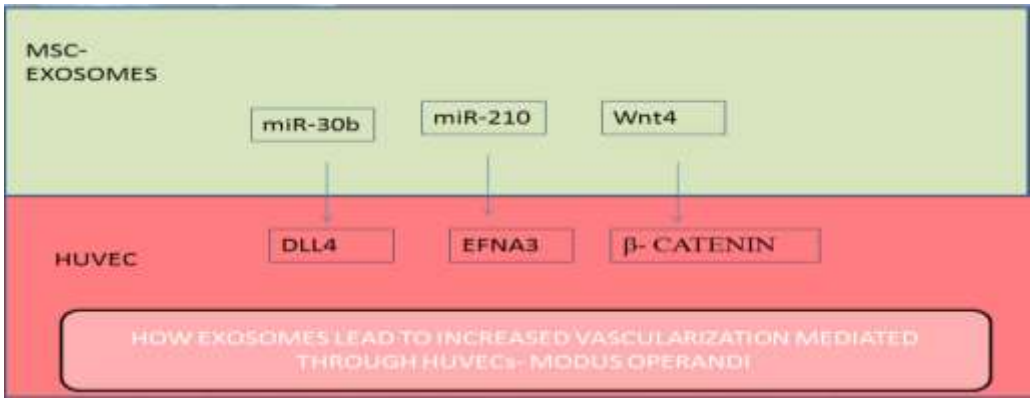


Figure 3: Exosomes - the role in regeneration.

Vascularization is sine quo non for tissue healing and regeneration to occur and the mesenchymal stem cells by virtue of possession of these exosomes potentiate this cardinal event through their interaction with blood vessel-forming cells such as HUVECs which manifests as enhanced proliferation and migration of the same, upregulation in tube formation capacity and expression of angiogenesis associated genes akin to VEGF and HIF-1α.



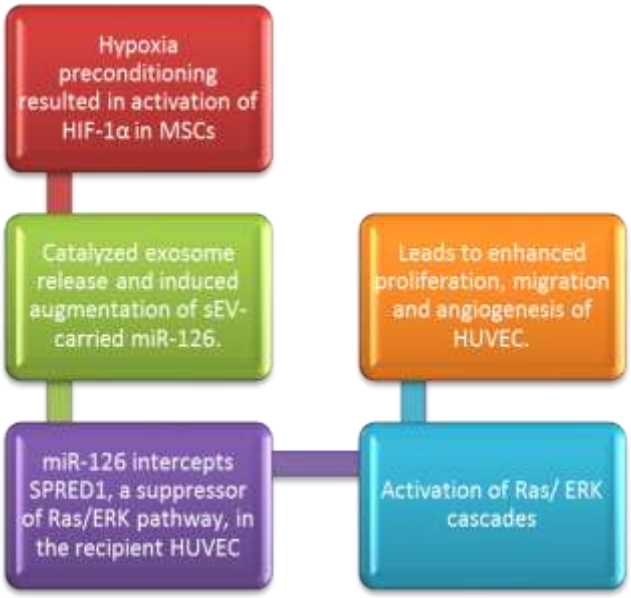
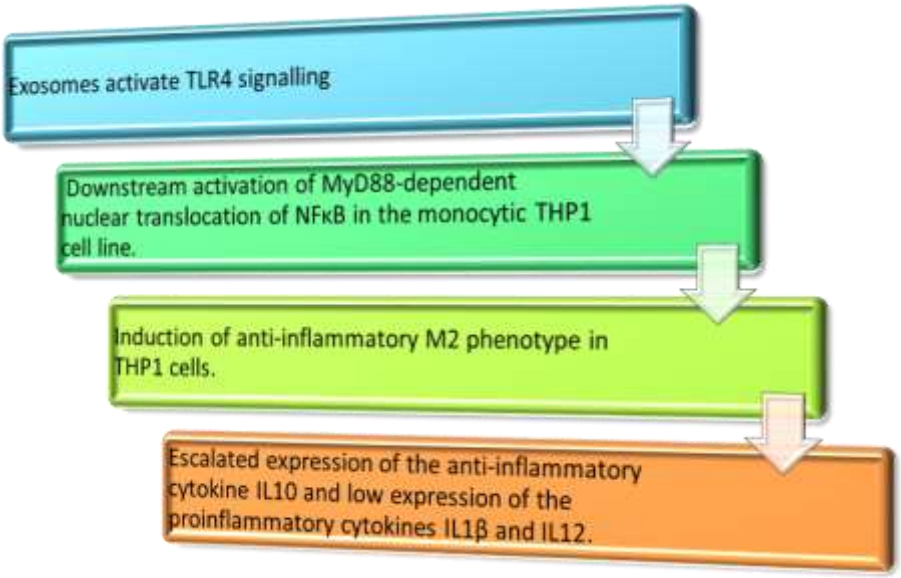


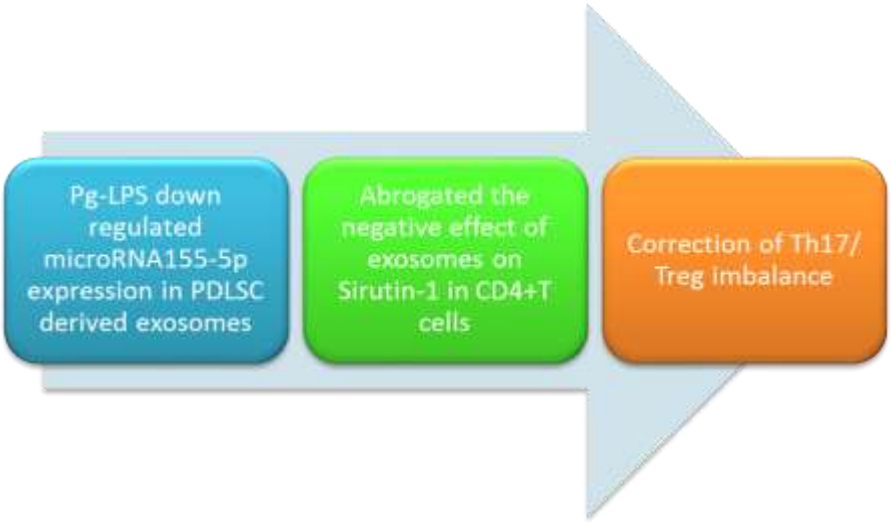
Figure 4: The assistance to angiogenesis.

Curtailing the inflammatory pandemonium is sapient for the genesis of a niche germane for repair and regeneration to blossom. The exosomes orchestrate this eventuality by virtue of their immunomodulatory properties which they exert upon interaction with immune cells, including monocytes/macrophages, B cells and T cells which are harbingers encompassing both innate and adaptive immune responses.



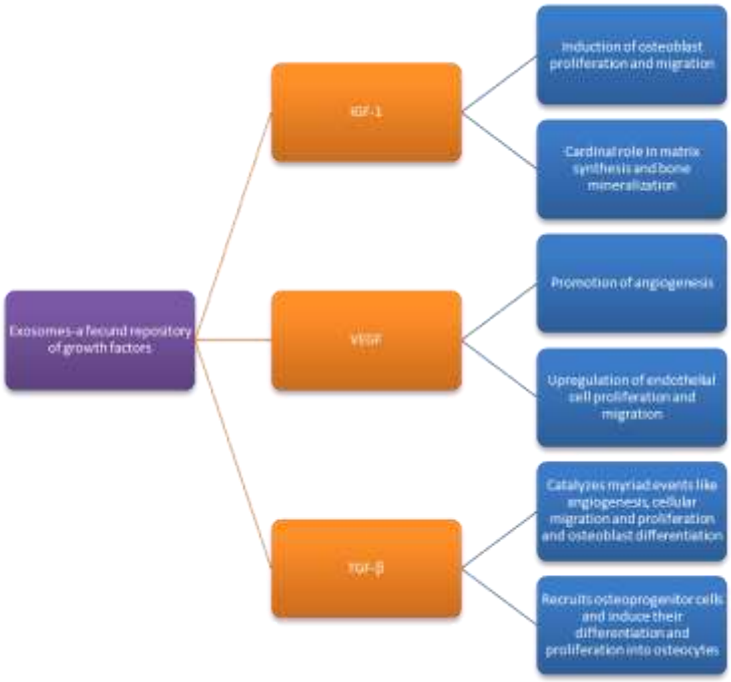
Lo Sicco et al promulgated that macrophage proliferation and polarization towards the M2 phenotype was promoted by exosomes derived from mesenchymal stem cells which was evident by the increased expression of M2 surface markers- CD36, CD51 and CD206, and a

diminished expression of M1 surface markers - Ly6C, CD11b, CD40 and CD86. Soon following the heels of the study, Cosenza et al revealed exosomes inhibited LPS- mediated activation of macrophages by terminating the teeming expression of M1 surface markers.[8]



While Chew et al evinced that transplantation of bone marrow mesenchymal stem cells derived exosomes facilitated periodontal regeneration in rat periodontal defects, Wu et al demonstrated the same but with exosomes of SHED origin.[9] Xu et al promulgated that P2-7 receptor gene modified periodontal ligament stem cells were a repertoire of exosomes in possession of the astounding ability to restore the compromised regenerative ability of adjacent cells mediated through their interaction with GREM-1 protein and upregulation of expression of miR3679-5p, miR-6515-5p and miR-6747-5p.[10]

Exosomes also don multiple roles to set a propitious stage conducive for periodontal healing to take place sans any perturbations. Primarily, transplantation of exosomes led to escalation in levels of anti-inflammatory cytokine – TNF-α and IL-10 while it clamped down on the production of pro-inflammatory mediators like IL-1β and COX-2. As quelling inflammation is incumbent for burgeoning of healing, the exosomes acting thus mediate periodontal regeneration in a nonpareil manner.[12] Succouring in the generation of a milieu conducive for optimal healing to proceed unabated is the distinguished ability the exosomes possess to quell the invading pathogens and hence extinguish the extraneous perturbations disturbing the host-microbe homeostasis. Pertaining to the same, Sundaram et al., undertook an extensive study and adeptly surmised that plant-derived exosomes from ginger could comprehensively enfeeble the pathogenicity of Porphyromonas gingivalis and thus abate its culpable role in the etiopathogenesis of periodontitis.[17]



There exist a plethora of scaffolds succouring the delivery of exosomes to aid in therapeutics which are enlisted in the table as follows

SCAFFOLDS	AUTHORS
β-TCP particles	Wu et al. 2019; Zhang et al. 2016; Qi et al. 2016
Hydrogel	Zhang et al. 2019;Chen et al. 2019
Collagen sponge	Chew et al. 2019; Takeuchi et al. 2019
Hydroxyapatite (HA), Bio-Oss® Collagen or PLGA	Liang et al. 2019; Sun et al. 2019; Li et al. 2018
Titanium oxide nanotubes	Wei et al. 2019
3D printed extracellular matrix/gelatin methacrylate scaffolds	Chen et al. 2019
Protein nanocages	Cho et al. 2018

THE ROAD AHEAD AND THE FRAILTIES TO EXTIRPATE

MSC-derived exosomes are in possession of the following unprecedented advantages endowing proficiency to their novelty thus expanding the scope of cell-free therapies for treating various human ailments

(1) MSC derived exosomes are intrinsically hypoimmunogenic[13]; (2) Exosomes avert any qualms regarding safety which is very much pertinent while resorting to stem cell transplantation(tumor seeding) and also possesses the additional virtue of posing a subtracted technical challenge as far as production and delivery goes[14]; and (3) Exosomes owing to

their exiguous size navigate with ease across the barriers (blood-brain barrier, capillaries).[15] They can be used as alternatives to liposomes to facilitate application of nano drug delivery and most profusely emulate the regenerative and immunomodulatory characteristics of stem cells.

However, on the downside certain limitations do impede its otherwise seemingly flawless potential

- One major concern is the qualms that exist regarding its ability to replicate this peerless success of invitro testing when transplanted in vivo.
- There exists no lucidity regarding its therapeutic dose and efficacy.
- It is not yet understood which **specific cargo contents** (lipids, proteins and nucleic acids) are responsible for the observed effects.
- **Non- specific targeting** – an ostensible double – edged sword.
- **Drug loading efficiency** is another challenge to overcome and there is an exigency to formulate the ideal quantity of drug which can be incorporated into exosomes without compromising their physical integrity and biological activity.
- To provide a robust therapy, **the scalable production of sEVs with high purity** is judged to be cardinal.
- Need to explore **optimal exosomes scaffolds and concentrations** to achieve the quintessential regenerative outcome.[4]

Regaling under the non-pareil and distinctive properties they possess, MSC-derived sEVs might be envisioned as riveting contrivances based on which avant garde biologically functionalized materials can be structured and engineered. Being empowered by this idea thus envisaged, exosomes have been immobilized onto titanium surfaces and the implantation of the same resulted in accelerated MSC adhesion , growth and subsequent osteogenic differentiation, all of which holistically translated to tasting credibly supreme success in the domain of healing and regeneration.[16]

CONCLUSION

It is only justified to confer the sobriquet of “the peerless regalia of regeneration” onto exosomes, the stupendous discovery which are going to steer the transmogrification of regeneration to its elusive pinnacle. Exosomes based approaches have flung the doors open to a refreshingly novel paradigm of cell-free therapies which in turn have emancipated the current clinical regimens from the constraints that cellular therapies come bearing. Housing a

retinue of prolific proteins, RNAs and an endless list of valuable cargo, they can seamlessly navigate the frontiers imposed by plasma membranes thus delivering the treasured elixirs into the target cells whilst being tolerated by the body while accomplishing their intended tasks. Exosomes thus unerringly propagate the regenerative and immunomodulatory characteristics of stem cells and it is veracious to state that they are indeed the uncrowned monarchs in the empire of regeneration.

REFERENCES

1. Jiang, S., & Xu, L. Exosomes from gingival mesenchymal stem cells enhance migration and osteogenic differentiation of pre-osteoblasts. Die Pharmazie, 2020; 75(11): 576–580. https://doi.org/10.1691/ph.2020.0652.
2. Chew J, Chuah S, Teo K, Zhang S, Lai R, Fu J et al. Mesenchymal stem cell exosomes enhance periodontal ligament cell functions and promote periodontal regeneration. Acta Biomaterialia, 2019; 89: 252-264.
3. Gowen A, Shahjin F, Chand S, Odegaard KE and Yelamanchili SV Mesenchymal Stem Cell-Derived Extracellular Vesicles: Challenges in Clinical Applications. Front. Cell Dev. Biol., 2020; 8: 149. doi: 10.3389/fcell.2020.00149.
4. Wang, X., & Thomsen, P. Mesenchymal stem cell-derived small extracellular vesicles and bone regeneration. Basic & clinical pharmacology & toxicology, 2021; 128(1): 18–36. https://doi.org/10.1111/bcpt.13478.
5. Cooper LF, Ravindran S, Huang C-C and Kang M A Role for Exosomes in Craniofacial Tissue Engineering and Regeneration. Front. Physiol., 2020; 10: 1569. doi: 10.3389/fphys.2019.01569.
6. Ma Z, Yang J, Lu Y, Liu Z, Wang X. Mesenchymal stem cell-derived exosomes: Toward cell-free therapeutic strategies in regenerative medicine. World Journal of Stem Cells, 2020; 12(8): 814-840.
7. Tsiapalis D, O'Driscoll L. Mesenchymal Stem Cell Derived Extracellular Vesicles for Tissue Engineering and Regenerative Medicine Applications. Cells, 2020; 9(4): 991.
8. Zhou L, Liu W, Wu Y, Sun W, Dörfer C, Fawzy El-Sayed K. Oral Mesenchymal Stem/Progenitor Cells: The Immunomodulatory Masters. Stem Cells International, 2020; 2020: 1-16.
9. Iwasaki K, Peng Y, Kanda R, Umeda M, Ishikawa I. Stem Cell Transplantation and Cell-Free Treatment for Periodontal Regeneration. Int J Mol Sci., 2022 Jan 18; 23(3): 1011. doi: 10.3390/ijms23031011. PMID: 35162935; PMCID: PMC8835344.

10. Lee, B. C., Kang, I., & Yu, K. R. Therapeutic Features and Updated Clinical Trials of Mesenchymal Stem Cell (MSC)-Derived Exosomes. Journal of clinical medicine, 2021; 10(4): 711. https://doi.org/10.3390/jcm10040711.
11. Novello S, Pellen-Mussi P, Jeanne S. Mesenchymal stem cell-derived small extracellular vesicles as cell-free therapy: Perspectives in periodontal regeneration. Journal of Periodontal Research, 2021; 56(3): 433-442.
12. Lin, H., Chen, H., Zhao, X., Chen, Z., Zhang, P., Tian, Y., Wang, Y., Ding, T., Wang, L., & Shen, Y. Advances in mesenchymal stem cell conditioned medium-mediated periodontal tissue regeneration. Journal of translational medicine, 2021; 19(1): 456. https://doi.org/10.1186/s12967-021-03125-5.
13. E. Klyushnenkova, J. D. Mosca, V. Zernetkina et al., "T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression," Journal of Biomedical Science, 2005; 12(1): 47–57.
14. A. Marote, F. G. Teixeira, B. Mendes-Pinheiro, and A. J. Salgado, "MSCs-derived exosomes: cell-secreted nanovesicles with regenerative potential," Frontiers in Pharmacology, 2016; 7: 231.
15. H. Choi and D. S. Lee, "Illuminating the physiology of extracellular vesicles," Stem Cell Res Ther, 2016; 7(1): 55.
16. Wang X, Shah FA, Vazirisani F, et al. Exosomes influence the behavior of human mesenchymal stem cells on titanium surfaces. Biomaterials, 2020; 230: 119571. [PubMed] [Google Scholar].
17. Sundaram, K., Miller, D. P., Kumar, A., Teng, Y., Sayed, M., Mu, J., Lei, C., Sriwastva, M. K., Zhang, L., Yan, J., Merchant, M. L., He, L., Fang, Y., Zhang, S., Zhang, X., Park, J. W., Lamont, R. J., & Zhang, H. G. Plant-Derived Exosomal Nanoparticles Inhibit Pathogenicity of Porphyromonas gingivalis. iScience, 2019; 21: 308–327. https://doi.org/10.1016/j.isci.2019.10.032