Mesenchymal Stem Cell-Derived Extracellular Vesicles

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Mesenchymal stem cells (MSCs) have recently emerged as promising candidates for treating a variety of age-related conditions, including ageing frailty. MSCs can differentiate into different cell lineages and secrete extracellular vesicles (EVs), such as exosomes and microvesicles, that contain bioactive molecules, such as proteins, nucleic acids, and lipids. These EVs can deliver cargo to target cells and influence cellular processes, such as inflammation, apoptosis, and angiogenesis, promoting tissue repair and regeneration.

mesenchymal stem cell extracellular vesicles ageing

1. Introduction

The global demographic pattern involves a growing number and proportion of elderly persons in the population. The proportion of people 65 years of age and older worldwide is predicted by the United Nations (UN) to double by 2050, reaching almost 1.5 billion elderly people globally [1]. Age-related frailty is a major public health concern globally as the global geriatric population rises, especially in nations with the longest life expectancies [2]. Frailty is defined as an age-related decline in the functional reserve of multiple body systems that results in a reduced ability to cope with acute or external stressors [3]. Frailty is indicated by easy exhaustion, diminished libido, emotional disruption, accelerated osteoporosis, impaired muscle strength, and vulnerability to illness [4]. A person is more likely to progress up the Clinical Frailty Scale as they age, which correlates to illnesses that have higher morbidity and mortality rates [5]. Several age-related conditions involving oxidative stress, such as cardiovascular diseases (CVDs), chronic obstructive pulmonary disease (COPD), chronic kidney disease (CKD), neurodegenerative diseases, and cancer, including sarcopenia and frailty, are more common in the elderly [6]. The prevalence of these chronic degenerative diseases will rise over time, placing a significant strain on the global healthcare industry to manage the diseases.

Meanwhile, skeletal muscle ageing frailty, which is defined as a loss in muscular mass, strength, and function, is a prevalent condition among older persons [7]. As a substantial clinical syndrome linked to an elevated incidence of falls, depression, and disability, which increases mortality, skeletal muscle ageing fragility is of increasing importance [8]. As a result of ageing, muscle mass declines naturally, beginning in the late twenties and accelerating in the fifties [9]. Sarcopenia, an age-related gradual loss of muscle, is one way that muscle loss can appear.

Many of the health issues that arise as people age are linked to chronic illnesses, especially degenerative illnesses, and can be avoided or delayed by adopting healthy behaviours. Indeed, both physical activity and a healthy diet have a significant positive impact on one's health and wellbeing [10]. Pharmacological therapies can be used to effectively control other health issues and capacity deficits, especially if they are caught early [3]. There is, however, still no known treatment for this illness. The use of stem cells to treat a variety of illnesses and disorders has recently shown promising outcomes. Since frailty is also linked to stem cell depletion and exhaustion, where the stem cells' activity is characterised by decreased survival, proliferation, differentiation, and homing capacity [11][12], cell-based therapy represents a viable strategy to be able to treat or prevent the development of frailty [13].

Mesenchymal stem cells (MSCs) have recently emerged as promising candidates for treating a variety of age-related conditions, including ageing frailty. MSCs can differentiate into different cell lineages and secrete extracellular vesicles (EVs), such as exosomes and microvesicles, that contain bioactive molecules, such as proteins, nucleic acids, and lipids. These EVs can deliver cargo to target cells and influence cellular processes, such as inflammation, apoptosis, and angiogenesis, promoting tissue repair and regeneration [14].

2. Mesenchymal Stem Cell-Derived Extracellular Vesicles (MSC-EVs)

In recent years, researchers have focused on the indirect use of MSCs, which is based on extracellular vesicles (EVs) derived from these cells [15]. Apoptotic bodies, microvesicles (MVs), and exosomes are three types of EVs that differ in size, content, and formation [16][17]. Apoptotic bodies are 50–4000 nm in size and are typically produced by apoptotic cells in the final stage of apoptosis. These EVs are diverse, containing membrane components (such as phosphatidylserine), nuclear material, and cellular organelles [18]. Microvesicles, unlike apoptotic bodies, shed directly from the membrane of healthy cells. These EVs, like the apoptotic body, have a heterogeneous morphology and range in size from 100 to 1000 nm. Microvesicles can influence gene expression by sending miRNA to neighbouring cells. Furthermore, because MVs are not released from the cell via endocytosis, they lack endocytosis-related proteins [19]. Exosomes are the smallest EVs, measuring 30–120 nm in size, and are formed during late endosome membrane inward invagination and the formation of multiple vesicular bodies (MVBs) [20]. Exosomes are formed inside MVBs and secreted to the extracellular environment via endocytosis by the MVB membrane fusing with the cell membrane [21]. Exosomes are now classified into three types based on their size: large exosomes (exo-L, size is between 90 and 120 nm), small exosomes (exo-S, size is between 60 and 80 nm), and exomers (35 nm) [22].

2.1. Isolation of MSC-EVs

MSCs produce more exosomes than other cells, making them clinically viable for exosome separation and therapy [23]. For example, tetraspanins (CD63, CD9, CD81, CD82), fusion-involved proteins (flotillins, CD9, annexin, GTPases), adhesion molecules, gap junction related proteins (Connexins-43) [24], heat shock proteins (HSC70 and HSC90), MHC-1, MHC-2, membrane transporters (GTPases), Rab proteins [25], lysosomal proteins (Lamp2b), and proteins involved in multivesicular body biogenesis (Alix and TSG101) [26][27].

Exosomes are separated using various methods, including ultracentrifugation, density gradient centrifugation, pegylation-based methods, and kit use. There are several relatively efficient protocols available, such as 100,000× *g* ultracentrifugation of complete medium (or serum after at least 1:4 dilution) for at least 18 h [28], centrifugation at higher speeds (e.g., 200,000× *g* [29]) for shorter periods of time, or tangential flow filtration or other forms of ultrafiltration [30]. A few hours of ultracentrifugation at around 100,000× *g* without dilution will not eliminate all EVs or EV-associated RNA [31][32][33].

2.2. Characterisation of MSC-EVs

Exosomes can be used for therapeutic purposes after being characterised using various methods, such as dynamic light scattering (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and ELISA. Exosomes do not pose a risk of genetic instability or immunosuppression after allogeneic administration in in vivo models. Exosome therapy has been shown in studies to be a new strategy for overcoming stem cell therapy deficiencies [34].

2.3. Therapeutic Effects of MSC-EVs

Exosomes are cellular communication vesicles that are paracrine. A lipid bilayer membrane can transport cytokines, chemokines, growth factors, various enzymes, various signalling molecules, miRNAs, lipids, and transcription factors. According to research, cargos present in MSC exosomes include ATP synthesis enzymes (glyceraldehyde 3-phosphate dehydrogenase (GAPDH)), phosphoglycerate kinase (PGK), phosphoglucomutase (PGM), enolase (ENO) [35], angiogenesis stimulating enzymes (VEGF, inducer extracellular matrix metalloproteinase (EMMPRIN), and MMP-9) [36], various transcription factors (transcription factor with Octamer 4 (Oct-4), HoxB4, and Rex-1) [34], tumour growth inhibitory miRs (miR-23b, miR-214, miR-451, miR-223, MiR-31, miR-24, miR-125b, and miR-122) [37], and inflammation regulating miRs (miR-155 and miR-146) [38]. Hence, bilayer lipids protect nucleic acids and proteins from extracellular degradation, allowing for efficient transport.

Exosomes are smaller and less complex than their parent cells, and their membranes contain less protein. As a result, they are easier to separate and store, and they are less immunogenic than cell therapy [39]. Exosomes are also less likely to become trapped in the lungs or liver. Exosomes can communicate information in a variety of ways, including juxtacrine and solution signalling [40]. Exosomes have several advantages over their source cells: (1) Their use prevents the transfer of cells containing immunogenic molecules as well as mutated or damaged DNA; (2) Exosomes are nano-sized and can easily enter and move within any organ, whereas cells are larger and cannot migrate to the site of injury through capillaries; (3) Exosomes can migrate to different parts of the body due to the presence of homing molecules on their surface; and (4) Because exosomes are native to the body, their surface has biochemical properties similar to those of their derived cells, allowing them to avoid phagocytosis, cell membrane fusion, and lysosomal fusion [41].

Because of the mentioned characteristics, MSC-derived exosomes have emerged as one of the most dynamic fields in regenerative medicine. One of the most common causes of function loss in many chronic degenerative

diseases is tissue destruction. The function of these tissues can be rejuvenated if treated with a therapeutic agent. MSC-derived exosomes have been shown to be therapeutic in heart, kidney, lung, skin, brain, liver, autoimmune, and musculoskeletal diseases [42]. Type 1 diabetes, macular degeneration, chronic kidney disease, ischemic stroke [43], Alzheimer's [44], multiple sclerosis [45], sepsis, hepatitis [46], chronic liver disease [39], and skin disease [47] can all be treated with these exosomes.

Exosomes derived from MSCs have been shown to modulate the immune system, stimulate cell proliferation, promote angiogenesis, prevent apoptosis, and suppress oxidative stress [48]. These exosomes aid in the maintenance of homeostasis and cell repair by providing and transporting active enzymes that restore normal cell activity [49]. Proteomic studies of MSC exosomes revealed the presence of over 200 immunomodulatory molecules [50]. These exosomes also promote cell proliferation and prevent apoptosis by activating the Ras/Raf/MEK/ERK and PTEN/PI3K/AKT/mTOR signalling pathways [51]. Aside from their therapeutic potential, MSC-derived exosomes can migrate to lesion sites. Exosome surface molecules can also be modified to migrate more and better to the site of injury [52]. This feature of exosomes makes them an excellent vehicle and transport system for delivering drugs directly to the site of the disease [53]. Intravenous, intraperitoneal, or subcutaneous exosome injections result in the rapid clearance of exosomes from the bloodstream and accumulation in the liver, spleen, lungs, and gastrointestinal tract [54][55]. Furthermore, regardless of the injection route, the majority of systemically injected exosomes are quickly taken up by macrophages in the reticuloendothelial system and eliminated from the body [56]. As a result, the biological distribution of exosomes following systemic administration can be classified into two stages: (1) Rapid distribution in the liver, spleen, and lungs 30 min after administration, and (2) Exosome removal via hepatic and renal processing 1 to 6 h after administration [57]. Exosomes administered topically (such as the skin surface and ocular surface) have a shorter half-life due to fluid cleansing (sweat and tears) and exposure to external factors [58].

References

1. World Population Ageing, 2019; Department of Economic and Social Affairs: New York, NY, USA, 2019.
2. Crimmins, E.M.; Beltrán-Sánchez, H. Mortality and Morbidity Trends: Is There Compression of Morbidity? J. Gerontol. Ser. B 2011, 66, 75–86.
3. Florea, V.; Bagno, L.; Rieger, A.C.; Hare, J.M. Attenuation of frailty in older adults with mesenchymal stem cells. Mech. Ageing Dev. 2019, 181, 47–58.
4. Bryon, A.T.; Ana Marie, L.; Victoria, F.; Makoto, N.; Angela, C.R.; Wayne, B.; Ivonne Hernandez, S.; Joshua, M.H. Allogeneic Mesenchymal Stem Cells as a Treatment for Aging Frailty. In Frailty and Sarcopenia; Yannis, D., Ed.; IntechOpen: Rijeka, Croatian, 2017; Chapter 11.
5. Church, S.; Rogers, E.; Rockwood, K.; Theou, O. A scoping review of the Clinical Frailty Scale. BMC Geriatr. 2020, 20, 393.

6. Liguori, I.; Russo, G.; Curcio, F.; Bulli, G.; Aran, L.; Della-Morte, D.; Gargiulo, G.; Testa, G.; Cacciatore, F.; Bonaduce, D.; et al. Oxidative stress, aging, and diseases. Clin. Interv. Aging 2018, 13, 757–772.
7. Marzetti, E.; Leeuwenburgh, C. Skeletal muscle apoptosis, sarcopenia and frailty at old age. Exp. Gerontol. 2006, 41, 1234–1238.
8. Soysal, P.; Stubbs, B.; Lucato, P.; Luchini, C.; Solmi, M.; Peluso, R.; Sergi, G.; Isik, A.T.; Manzato, E.; Maggi, S.; et al. Inflammation and frailty in the elderly: A systematic review and meta-analysis. Ageing Res. Rev. 2016, 31, 1–8.
9. Deschenes, M.R. Effects of Aging on Muscle Fibre Type and Size. Sport. Med. 2004, 34, 809–824.
10. O'Connell, M.L.; Coppinger, T.; McCarthy, A.L. The role of nutrition and physical activity in frailty: A review. Clin. Nutr. ESPEN 2020, 35, 1–11.
11. O'Driscoll, S.W.M.; Saris, D.B.F.; Ito, Y.; Fitzimmons, J.S. The chondrogenic potential of periosteum decreases with age. J. Orthop. Res. 2001, 19, 95–103.
12. Stenderup, K.; Justesen, J.; Clausen, C.; Kassem, M. Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. Bone 2003, 33, 919–926.
13. Oliva, A.A.; McClain-Moss, L.; Pena, A.; Drouillard, A.; Hare, J.M. Allogeneic mesenchymal stem cell therapy: A regenerative medicine approach to geroscience. Aging Med. 2019, 2, 142–146.
14. Varderidou-Minasian, S.; Lorenowicz, M.J. Mesenchymal stromal/stem cell-derived extracellular vesicles in tissue repair: Challenges and opportunities. Theranostics 2020, 10, 5979–5997.
15. Fitzsimmons, R.E.B.; Mazurek, M.S.; Soos, A.; Simmons, C.A. Mesenchymal Stromal/Stem Cells in Regenerative Medicine and Tissue Engineering. Stem Cells Int. 2018, 2018, 8031718.
16. Lai, F.W.; Lichty, B.D.; Bowdish, D.M.E. Microvesicles: Ubiquitous contributors to infection and immunity. J. Leukoc. Biol. 2015, 97, 237–245.
17. György, B.; Szabó, T.G.; Pásztói, M.; Pál, Z.; Misják, P.; Aradi, B.; László, V.; Pállinger, É.; Pap, E.; Kittel, Á.; et al. Membrane vesicles, current state-of-the-art: Emerging role of extracellular vesicles. Cell. Mol. Life Sci. 2011, 68, 2667–2688.
18. Turturici, G.; Tinnirello, R.; Sconzo, G.; Geraci, F. Extracellular membrane vesicles as a mechanism of cell-to-cell communication: Advantages and disadvantages. Am. J. Physiol. Cell Physiol. 2014, 306, C621–C633.
19. Koniusz, S.; Andrzejewska, A.; Muraca, M.; Srivastava, A.K.; Janowski, M.; Lukomska, B. Extracellular Vesicles in Physiology, Pathology, and Therapy of the Immune and Central Nervous

System, with Focus on Extracellular Vesicles Derived from Mesenchymal Stem Cells as Therapeutic Tools. Front. Cell. Neurosci. 2016, 10, 109.

20. Lai, C.; Breakefield, X. Role of Exosomes/Microvesicles in the Nervous System and Use in Emerging Therapies. Front. Physiol. 2012, 3, 228.
21. Hessvik, N.P.; Llorente, A. Current knowledge on exosome biogenesis and release. Cell. Mol. Life Sci. 2018, 75, 193–208.
22. Zhang, H.; Freitas, D.; Kim, H.S.; Fabijanic, K.; Li, Z.; Chen, H.; Mark, M.T.; Molina, H.; Martin, A.B.; Bojmar, L.; et al. Identification of distinct nanoparticles and subsets of extracellular vesicles by asymmetric flow field-flow fractionation. Nat. Cell Biol. 2018, 20, 332–343.
23. Vader, P.; Mol, E.A.; Pasterkamp, G.; Schiffelers, R.M. Extracellular vesicles for drug delivery. Adv. Drug Deliv. Rev. 2016, 106, 148–156.
24. Soares, A.R.; Martins-Marques, T.; Ribeiro-Rodrigues, T.; Ferreira, J.V.; Catarino, S.; Pinho, M.J.; Zuzarte, M.; Isabel Anjo, S.; Manadas, B.; Sluijter, J.P.G.; et al. Gap junctional protein Cx43 is involved in the communication between extracellular vesicles and mammalian cells. Sci. Rep. 2015, 5, 13243.
25. Pfeffer, S.R. Two Rabs for exosome release. Nat. Cell Biol. 2010, 12, 3–4.
26. Vilaça-Faria, H.; Salgado, A.J.; Teixeira, F.G. Mesenchymal Stem Cells-derived Exosomes: A New Possible Therapeutic Strategy for Parkinson's Disease? Cells 2019, 8, 118.
27. Yu, B.; Zhang, X.; Li, X. Exosomes Derived from Mesenchymal Stem Cells. Int. J. Mol. Sci. 2014, 15, 4142–4157.
28. Théry, C.; Amigorena, S.; Raposo, G.; Clayton, A. Isolation and Characterization of Exosomes from Cell Culture Supernatants and Biological Fluids. Curr. Protoc. Cell Biol. 2006, 30, 3.22.21–23.22.29.
29. van Balkom, B.W.M.; de Jong, O.G.; Smits, M.; Brummelman, J.; den Ouden, K.; de Bree, P.M.; van Eijndhoven, M.A.J.; Pegtel, D.M.; Stoorvogel, W.; Würdinger, T.; et al. Endothelial cells require miR-214 to secrete exosomes that suppress senescence and induce angiogenesis in human and mouse endothelial cells. Blood 2013, 121, 3997–4006.
30. Kornilov, R.; Puhka, M.; Mannerström, B.; Hiidenmaa, H.; Peltoniemi, H.; Siljander, P.; Seppänen-Kaijansinkko, R.; Kaur, S. Efficient ultrafiltration-based protocol to deplete extracellular vesicles from fetal bovine serum. J. Extracell. Vesicles 2018, 7, 1422674.
31. Shelke, G.V.; Lässer, C.; Gho, Y.S.; Lötvall, J. Importance of exosome depletion protocols to eliminate functional and RNA-containing extracellular vesicles from fetal bovine serum. J. Extracell. Vesicles 2014, 3, 24783.

32. Tosar, J.P.; Cayota, A.; Eitan, E.; Halushka, M.K.; Witwer, K.W. Ribonucleic artefacts: Are some extracellular RNA discoveries driven by cell culture medium components? J. Extracell. Vesicles 2017, 6, 1272832.
33. Wei, Z.; Batagov, A.O.; Carter, D.R.F.; Krichevsky, A.M. Fetal Bovine Serum RNA Interferes with the Cell Culture derived Extracellular RNA. Sci. Rep. 2016, 6, 31175.
34. Burrello, J.; Monticone, S.; Gai, C.; Gomez, Y.; Kholia, S.; Camussi, G. Stem Cell-Derived Extracellular Vesicles and Immune-Modulation. Front. Cell Dev. Biol. 2016, 4, 83.
35. Arslan, F.; Lai, R.C.; Smeets, M.B.; Akeroyd, L.; Choo, A.; Aguor, E.N.E.; Timmers, L.; van Rijen, H.V.; Doevendans, P.A.; Pasterkamp, G.; et al. Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. Stem Cell Res. 2013, 10, 301–312.
36. Vrijsen, K.R.; Maring, J.A.; Chamuleau, S.A.J.; Verhage, V.; Mol, E.A.; Deddens, J.C.; Metz, C.H.G.; Lodder, K.; van Eeuwijk, E.C.M.; van Dommelen, S.M.; et al. Exosomes from Cardiomyocyte Progenitor Cells and Mesenchymal Stem Cells Stimulate Angiogenesis Via EMMPRIN. Adv. Healthc. Mater. 2016, 5, 2555–2565.
37. Ono, M.; Kosaka, N.; Tominaga, N.; Yoshioka, Y.; Takeshita, F.; Takahashi, R.-U.; Yoshida, M.; Tsuda, H.; Tamura, K.; Ochiya, T. Exosomes from bone marrow mesenchymal stem cells contain a microRNA that promotes dormancy in metastatic breast cancer cells. Sci. Signal. 2014, 7, ra63.
38. Di Trapani, M.; Bassi, G.; Midolo, M.; Gatti, A.; Takam Kamga, P.; Cassaro, A.; Carusone, R.; Adamo, A.; Krampera, M. Differential and transferable modulatory effects of mesenchymal stromal cell-derived extracellular vesicles on T, B and NK cell functions. Sci. Rep. 2016, 6, 24120.
39. Lou, G.; Chen, Z.; Zheng, M.; Liu, Y. Mesenchymal stem cell-derived exosomes as a new therapeutic strategy for liver diseases. Exp. Mol. Med. 2017, 49, e346.
40. McKelvey, K.J.; Powell, K.L.; Ashton, A.W.; Morris, J.M.; McCracken, S.A. Exosomes: Mechanisms of Uptake. J. Circ. Biomark. 2015, 4, 7.
41. Riau, A.K.; Ong, H.S.; Yam, G.H.F.; Mehta, J.S. Sustained Delivery System for Stem Cell-Derived Exosomes. Front. Pharmacol. 2019, 10, 1368.
42. Joo, H.S.; Suh, J.H.; Lee, H.J.; Bang, E.S.; Lee, J.M. Current Knowledge and Future Perspectives on Mesenchymal Stem Cell-Derived Exosomes as a New Therapeutic Agent. Int. J. Mol. Sci. 2020, 21, 727.
43. Mendt, M.; Rezvani, K.; Shpall, E. Mesenchymal stem cell-derived exosomes for clinical use. Bone Marrow Transpl. 2019, 54, 789–792.

44. Nakano, M.; Kubota, K.; Kobayashi, E.; Chikenji, T.S.; Saito, Y.; Konari, N.; Fujimiya, M. Bone marrow-derived mesenchymal stem cells improve cognitive impairment in an Alzheimer's disease model by increasing the expression of microRNA-146a in hippocampus. Sci. Rep. 2020, 10, 10772.
45. Baharlooi, H.; Azimi, M.; Salehi, Z.; Izad, M. Mesenchymal Stem Cell-Derived Exosomes: A Promising Therapeutic Ace Card to Address Autoimmune Diseases. Int. J. Stem Cells 2020, 13, 13–23.
46. Harrell, C.R.; Jovicic, N.; Djonov, V.; Arsenijevic, N.; Volarevic, V. Mesenchymal Stem Cell-Derived Exosomes and Other Extracellular Vesicles as New Remedies in the Therapy of Inflammatory Diseases. Cells 2019, 8, 1605.
47. Ha, D.H.; Kim, H.-k.; Lee, J.; Kwon, H.H.; Park, G.-H.; Yang, S.H.; Jung, J.Y.; Choi, H.; Lee, J.H.; Sung, S.; et al. Mesenchymal Stem/Stromal Cell-Derived Exosomes for Immunomodulatory Therapeutics and Skin Regeneration. Cells 2020, 9, 1157.
48. Pashoutan Sarvar, D.; Shamsasenjan, K.; Akbarzadehlaleh, P. Mesenchymal Stem Cell-Derived Exosomes: New Opportunity in Cell-Free Therapy. Adv. Pharm. Bull. 2016, 6, 293–299.
49. Li, M.; Li, S.; Du, C.; Zhang, Y.; Li, Y.; Chu, L.; Han, X.; Galons, H.; Zhang, Y.; Sun, H.; et al. Exosomes from different cells: Characteristics, modifications, and therapeutic applications. Eur. J. Med. Chem. 2020, 207, 112784.
50. Lai, R.C.; Tan, S.S.; Teh, B.J.; Sze, S.K.; Arslan, F.; de Kleijn, D.P.; Choo, A.; Lim, S.K. Proteolytic Potential of the MSC Exosome Proteome: Implications for an Exosome-Mediated Delivery of Therapeutic Proteasome. Int. J. Proteom. 2012, 2012, 971907.
51. Steelman, L.S.; Chappell, W.H.; Abrams, S.L.; Kempf, R.C.; Long, J.; Laidler, P.; Mijatovic, S.; Maksimovic-Ivanic, D.; Stivala, F.; Mazzarino, M.C.; et al. Roles of the Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways in controlling growth and sensitivity to therapy-implications for cancer and aging. Aging 2011, 3, 192–222.
52. Shahabipour, F.; Banach, M.; Sahebkar, A. Exosomes as nanocarriers for siRNA delivery: Paradigms and challenges. Arch. Med. Sci. 2016, 12, 1324–1326.
53. Guy, R.; Offen, D. Promising Opportunities for Treating Neurodegenerative Diseases with Mesenchymal Stem Cell-Derived Exosomes. Biomolecules 2020, 10, 1320.
54. Smyth, T.; Kullberg, M.; Malik, N.; Smith-Jones, P.; Graner, M.W.; Anchordoquy, T.J. Biodistribution and delivery efficiency of unmodified tumor-derived exosomes. J. Control. Release 2015, 199, 145–155.
55. Yi, Y.W.; Lee, J.H.; Kim, S.-Y.; Pack, C.-G.; Ha, D.H.; Park, S.R.; Youn, J.; Cho, B.S. Advances in Analysis of Biodistribution of Exosomes by Molecular Imaging. Int. J. Mol. Sci. 2020, 21, 665.

56. Charoenviriyakul, C.; Takahashi, Y.; Morishita, M.; Matsumoto, A.; Nishikawa, M.; Takakura, Y. Cell type-specific and common characteristics of exosomes derived from mouse cell lines: Yield, physicochemical properties, and pharmacokinetics. Eur. J. Pharm. Sci. 2017, 96, 316–322.

57. Lai, C.P.; Mardini, O.; Ericsson, M.; Prabhakar, S.; Maguire, C.A.; Chen, J.W.; Tannous, B.A.; Breakefield, X.O. Dynamic Biodistribution of Extracellular Vesicles in Vivo Using a Multimodal Imaging Reporter. ACS Nano 2014, 8, 483–494.

58. Agrahari, V.; Mandal, A.; Agrahari, V.; Trinh, H.M.; Joseph, M.; Ray, A.; Hadji, H.; Mitra, R.; Pal, D.; Mitra, A.K. A comprehensive insight on ocular pharmacokinetics. Drug Deliv. Transl. Res. 2016, 6, 735–754.