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MSCs-exosomes in regeneration medicine: Current evidence and future perspectives

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Abstract: Exosomes, especially from mesenchymal stem cells, have attracted extensive attention in regeneration medicine. Mesenchymal stem cells derived exosomes (MSCs-exosomes) have shown anti-inflammatory, anti-oxidant, anti-apoptosis and tissue regeneration effects in a variety of tissue injury repair models. MSCs-exosomes hold many excellent properties such as low immunogenicity, biocompatibility, and targeting capability. With the in-depth study on the generation and function of exosomes, MSCs-exosomes are considered to be the bright stars in the field of regenerative medicine. However, there are still many obstacles to overcome in terms of exosomes isolation, clinical trials and safety evaluation. In this article, what we should focus on about MSCs-exosomes in regeneration medicine will be discussed.

Introduction

Exosomes are nanosized vesicles with a diameter of 30–120 nm and can be secreted by most types of cells. These vesicles are widely distributed in various body fluids, such as blood, urine, milk, and saliva (Cho *et al.*, 2021; Duca *et al.*, 2021; Jiang *et al.*, 2021; Wu *et al.*, 2021c). Despite small size, exosomes play important roles in mediating intercellular communication, regulating immune response, and participating in the formation of microenvironment (Chen *et al.*, 2021; Hou and Chen, 2021). Exosomes contain abundant cargos, such as protein, RNA, and lipid, which are considered to be the key molecules for exosomes to function. These cargos in exosomes and other types of extracellular vesicle can be transferred to the recipient cells and alter the function of the cells (Tkach and Théry, 2016).

In recent years, mesenchymal stem cells derived exosomes (MSCs-exosomes) exhibit treatment promise in multiple tissue repair models via various signaling pathways. Considering the abundant sources of exosomes, such as bone marrow, umbilical cord, adipose tissue, placenta, et al. (Burkova *et al.*, 2021; Li *et al.*, 2021; Pomatto *et al.*, 2021; Tan *et al.*, 2021), MSCs-exosomes are becoming the future star in the field of regeneration medicine. Here we provide a concise viewpoint at recent advances in MSCs-exosomes based regeneration medicine therapy, with a focus on treatment benefit and the main challenges in this field.

MSCs-Exosomes in Regeneration

In past decades, MSCs-exosomes are applicated in a variety of tissue injury repair models. Zhang *et al.* (2015) showed that MSCs-exosomes mediated-Wnt4 activated the skin β-Catenin signal thus promoted the repair in cutaneous wound healing and restored the normal accessory structure of skin. In addition, MSCs-exosomes could attenuate unilateral ureteral obstruction induced rat renal fibrosis and cisplatin induced acute renal injury by regulating CK1δ/β-TRCP-mediated YAP degradation and enhancing autophagy (Jia *et al.*, 2018; Ji *et al.*, 2020). Jiang *et al.* (2018) found that MSCs-exosomes showed benefit in CCl4 induced liver injury through antioxidant effect. In intestinal diseases, research demonstrated that MSCs-exosomes carrying miR-326 inhibited neddylation to relieve inflammatory bowel disease in mice (Wang *et al.*, 2020). In terms of myocardial injury repair, MSCs-exosomes could alleviate acute myocardial infarction, and also promote the repair of myocardial injury caused by viral myocarditis and myocardial ischemia-reperfusion (Gu *et al.*, 2020; Li *et al.*, 2020). Circular RNA 0001273 and AMPK/mTOR-mediated autophagy hold an

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important role in this process. In chronic wound including diabetic foot ulcers, adipose-derived MSCs-exosomes were able to enhance skin collagen production, angiogenesis and reduced inflammation in skin lesions (Zhao *et al.*, 2021). What's more, Tsai *et al.* (2021) demonstrated that MSCs-exosomes could rescue the hearing loss by promoting tissue remodeling and repair. Wu *et al.* (2021b) showed adipose-derived MSCs-exosomes could promote *in vivo* hair follicle regeneration. The therapeutic effects of MSCs-exosomes on multiple organs injury are shown in Fig. 1.

To address these shortcomings of natural exosomes such as weak treatment effect, insufficient targeting, researchers have developed engineering strategies to enhance the functionality of MSCs-exosomes. Compared with exosomes of natural origin, genetic or non-genetic methods modified exosomes showed better therapeutic effects. For example, Akt-modified umbilical cord mesenchymal stem cells derived-exosomes were more effective in myocardial infarction by activating PDGF-D to promote angiogenesis (Ma *et al.*, 2017). Yao *et al.* (2021) demonstrated that MSCs-exosomes overexpressing miR-29a-3p via miR-29a-3p-specific agonist could amplify the treatment effects of MSCs-exosomes on tendon healing. Besides, engineering exosomes via magnetic nanoparticles showed enhanced targeting ability to injury tissue under magnetic field, thus to promote wound healing and bone regeneration (Wu *et al.*, 2021a). Moreover, the combination of MSCs-exosomes and new nanomaterials including new hydrogels, cardiac scaffolds and functional nanoparticles can provide great therapeutic effects. Zhang *et al.* (2021b) demonstrated that hyaluronic acid hydrogel encapsulated umbilical MSCs-exosomes could accelerate bone repair via enhancing angiogenesis, which potentially regulated through the miR-21/NOTCH1/DLL4 pathway.

These studies showed the benefit of MSCs-exosomes in multiple tissue injury models. Various exosomal cargos such as proteins, miRNAs, lncRNAs and lipids are considered as key components in the regeneration process (Zhao *et al.*, 2020; Moghadasi *et al.*, 2021). Put together, the employment of MSCs-exosomes in the treatment of damage disease models offers a novel therapeutical direction based on a new era of cell-free therapy. However, there are still many challenges in the application of exosomes in regeneration medicine.

Challenges

The lack of clinical data is an obvious problem. Most of the experiments only stay at the level of animals and cannot truly reflect the effects of clinical application. The value of MSCs-exosomes in the field of tissue repair remains to be conclusively determined. Of course, this is also related to the complicated and strict approval of clinical trials. But it is undoubtedly correct to be cautious on issues involving clinical trials. At present, most of the industrialized products involving exosomes are concentrated in the aesthetic medical fields. Kwon *et al.* (2020) found adipose MSCs-exosomes had achieved a significantly greater improvement on acne scars than the control sides in a 12-week prospective, double-blind, randomized, split-face trial. A prospective nonrandomized open-label cohort study highlighted the capacity of bone marrow MSCs-exosomes to restore oxygenation, downregulate cytokine storm, and reconstitute immunity in severe COVID-19 (Sengupta *et al.*, 2020). MSCs-exosomes are promising therapeutic candidate for improving patients' clinical status with COVID-19. We look forward to more clinical trials on MSCs-exosomes to gain more understanding of the efficacy and safety of exosomes treatment.

The lack of large-scale and clinical-grade exosomes or extracellular vesicle isolation methods is another major

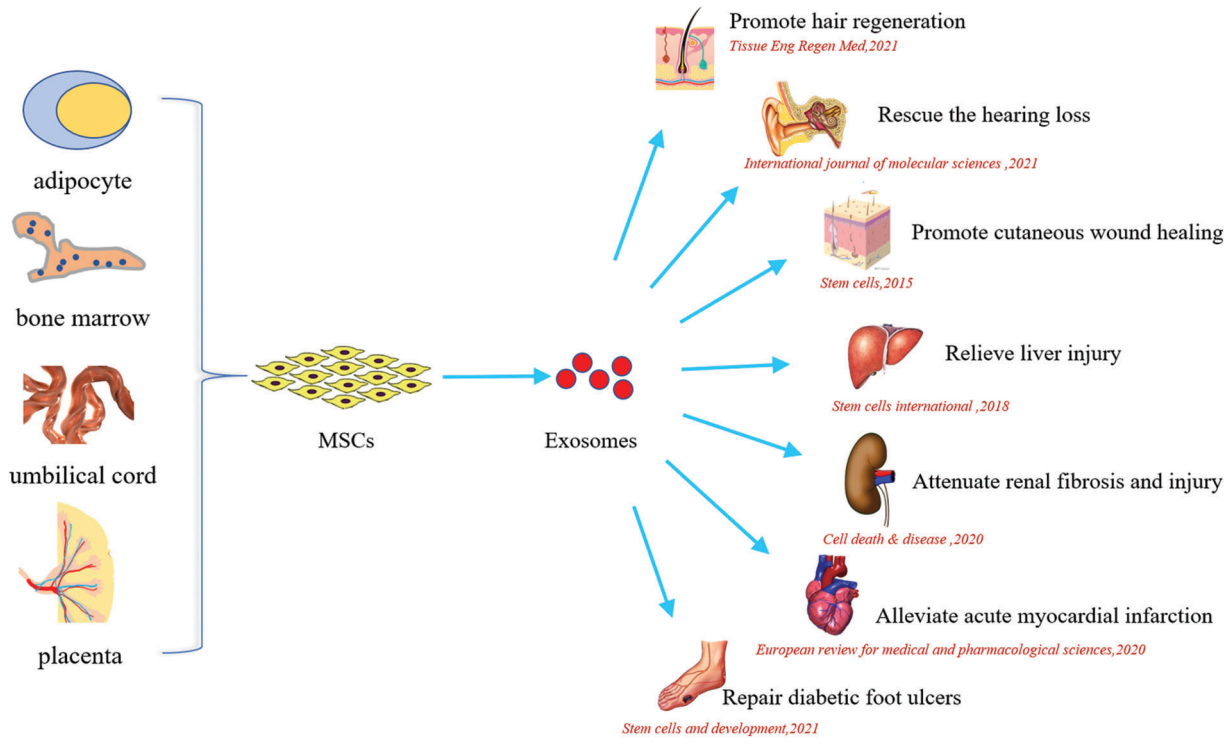

FIGURE 1. The beneficial effects of MSCs-exosomes on multiple organs injury.

obstacle. The ultracentrifugation method is currently considered the gold standard, but there are problems with time-consuming and high equipment cost (Witwer *et al.*, 2013). The immunological separation method can directly separate exosomes from the supernatant and body fluids, but it is not suitable for large-volume samples (Oksvold *et al.*, 2015). Commercial exosomes isolation kits provide efficient separation of exosomes from clinical samples, while avoiding exosomes being destroyed by long-term high-speed centrifugation. However, undesirable co-isolation of potential non-exosomal contents including polymer molecules limits its use for some quantitative examination (Taylor *et al.*, 2011). Thus, new isolation methods need to be developed to improve the yield and bioactivity of exosomes. Recently, Kim *et al.* (2021) developed a tangential flow filtration (TFF) system-based method to significantly increase the yield and purity of exosomes compared to ultracentrifugation, and exosomes had higher bioactivity in promoting wound healing (Kim *et al.*, 2021).

Moreover, the safety of MSCs-exosomes still needs more evidences. First, variations in effective dosing strategies are identified irrespective of the applied exosomes purification methods, which largely lead to the lack of stability between the current studies (Gupta *et al.*, 2021). And it leads to the uncertainty of the potential harm of different quality of exosomes on the body. Secondly, most of researches are only conducted in a short period of time. Due to the lack of research on the *in vivo* effects of long-term use of exosomes, we emphasize the potential side effects of exosomes in a long range of time. Besides, some studies reported the favorable effects of MSCs-exosomes on tumor progression (Luo *et al.*, 2020; Lyu *et al.*, 2021). And vivo imaging has shown that exosomes are widely distributed in various organs of the body including liver, lung and kidney after intravenous administration. Therefore, we emphasize the safety evaluation of system administration of exosomes compared with local application.

In addition to the obstacles mentioned above, there are still the following challenges about the application of exosomes in regenerative medicine. In a number of studies, lack of comparison of the therapeutic effects of "MSCs" *versus* "MSCs-exosomes" is a limitation. The heterogeneity of mesenchymal stem cells is also an urgent problem to be solved. Zhang *et al.* (2021a) found two populations of umbilical cord mesenchymal stem cells through single-cell RNA sequencing based on differentially expressed genes. Besides, the difficulty of detecting exosomes *in vivo* makes it difficult to carry out dynamic analysis of exosomes at the tissue level (Fig. 2).

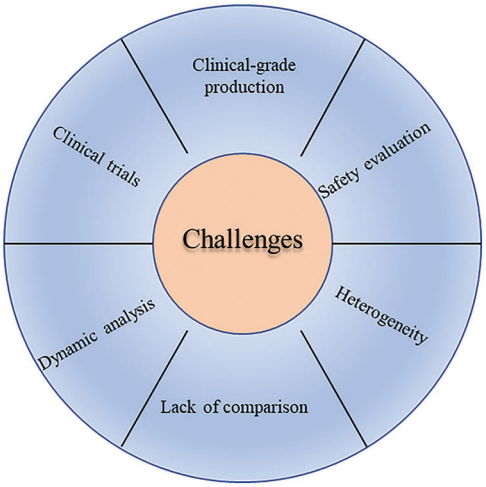

FIGURE 2. The main challenges of exosomes in the application of regeneration medicine.

Conclusion

To date, MSCs-exosomes therapy has been widely recognized and practiced in regenerative medicine based on multiple disease models. There is no doubt that MSCs-exosomes are bright stars in the field of regeneration medicine in the future. However, the complicated contents and signaling pathways involved in the regeneration process still remain unclear. Further research on the mechanisms of exosomes generation, isolation and function will help us to have a thorough understanding before further clinical trials. Exosomes are promising, but we still have a long way to go.

Materials and Methods

The relevant literatures and researches were searched in PubMed by using the keywords "mesenchymal stem cells", "exosomes", "injury repair" and "regenerative medicine".

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Authors' Contribution: Benshuai You conceived the work and wrote the first draft. Hui Qian critically reviewed the draft. All authors contributed to drafting the work, revised the final manuscript, and approved submission.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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