Introduction and Disclaimer

These mock examination questions span diverse disciplines and are designed for your practice in preparation for the International Research Olympiad (IRO) 2024. Endeavor to answer them to the best of your ability, utilizing this opportunity to enhance your skills and knowledge. For additional practice, it is advisable to engage in extensive reading of various papers; such efforts will contribute to a more comprehensive and nuanced understanding of the subject matter.

Try your best, and good luck! -International Research Olympiad 2024

Biomaterials constructed for MSC-derived extracellular vesicle loading and delivery—a promising method for tissue regeneration

Abstract:

Mesenchymal stem cells (MSCs) have become the preferred seed cells for tissue regeneration. Nevertheless, due to their immunogenicity and tumorigenicity, MSC transplantation remains questionable. Extracellular vesicles (EVs) derived from MSCs are becoming a promising substitute for MSCs. As a route of the MSC paracrine, EVs have a nano-sized and bilayer lipid-enclosed structure, which can guarantee the integrity of their cargoes, but EVs cannot obtain full function in vivo because of the rapid biodegradation and clearance by phagocytosis. To improve the efficacy and targeting of EVs, methods have been proposed and put into practice, especially engineered vesicles and EV-controlled release systems. In particular, EVs can be cell or tissue targeting because they have cell-specific ligands on their surfaces, but their targeting ability may be eliminated by the biodegradation of the phagocytic system during circulation. Novel application strategies have been proposed beyond direct injecting. EV carriers such as biodegradable hydrogels and other loading systems have been applied in tissue regeneration, and EV engineering is also a brand-new method for higher efficacy. In this review, we distinctively summarize EV engineering and loading system construction methods, emphasizing targeting modification methods and controlled release systems for EVs, which few literature reviews have involved.

Key Terms:

- **Mesenchymal Stem Cells (MSCs):** Multipotent stromal cells in regenerative medicine.
- **Extracellular Vesicles (EVs):** Small membrane structures for cell communication.
- **Immunogenicity:** Ability to provoke an immune response.
- **Biodegradation:** Breakdown of a substance by biological processes.
- **miRNA:** MicroRNA, regulating gene expression.
- **M2 Macrophage:** Macrophage with anti-inflammatory properties.
- **ROS:** Reactive Oxygen Species, involved in oxidative stress.
- **ATP:** Adenosine Triphosphate, carrying energy within cells.

Paper:

Extracellular vesicles (EVs) were first discovered in the 1960s and were previously described as cellular excrements; in recent decades, they have been recognized as intracellular communication mediators. EV refers to all lipid bilayer-enclosed extracellular particles, which were derived from cells and cannot self-replicate, as Minimal Information for Studies of Extracellular Vesicles (MISEV) defined in the latest guideline. EVs' nanoscale size and capacities for transporting cellular components have attracted the attention of researchers and their important roles in physiological and pathological processes are gradually being revealed, especially in immunoregulatory and cancer processes. In addition to that, researchers have long focused on the regenerative effect EVs possess.

Mesenchymal stem cells (MSCs) have become preferable seed cells for tissue regeneration and because of their multi-lineage differentiation potential and secretory function, they can facilitate multiple tissue repair through their proliferation, homing, and paracrine function. However, a study showed that MSCs implanted in subjects withered in 48 h, which showed that the by-products rather than MSCs act as determinants in tissue regeneration. Moreover, MSC implantation can lead to local inflammation and undirect cell differentiation, and recipients suffer from undesired teratoma.

In recent years, EVs derived from MSCs (MSC-EVs) have attracted extensive attention. Owning to their nano-size, EVs were verified to realize key molecules' targeted delivery via the lipid bilayer membrane and transmembrane ligand, which facilitates targeted tissue repairing. However, the mechanisms of tissue repair in vivo mediated by MSC-EVs are complicated and under study, including local immuno-environment modulation, angiogenesis enhancement, inhibition of apoptosis, and reduction of fibrosis. The high capacity of tissue repair makes MSC-EVs a promising part of tissue regeneration therapies, especially in biomaterial construction. The reasons include 1) EVs, as an endogenetic biological agent, have innate host affinity and can deliver easy-to-deactivate and -degrade substances to target cells. 2) The nano-size of EVs is suitable for traveling through the circulatory system and barriers, which provides the possibility for distant delivery to promote specific organ regeneration. Nevertheless, as experiments processed, the hollow nanospheres showed a high clean-up ratio by the liver and kidney; they cannot maintain effective concentration in the tissue nor the circulation system, and local injection was mainly limited by their unsatisfying retention ratio. To solve this problem, researchers have established multiple biomaterial carriers for EV control-releasing, which are capable of continuous and effective functioning, such as bio-macromolecular hydrogel, electrostatic spinning scaffold, and membrane and polymerized sponge. They could retain vesicles in local tissues for a longer time with varying degrees of design and manufacture. Beyond that, researchers are attempting to improve EV targeting by constructing engineered vesicles. By altering transcripts of the donor cells, the membrane proteins enriched on the surface of vesicles can be artificially manipulated, for example, CD47 on EV surfaces can be increased

to attenuate the degradation and inactivation of vesicles by the mononuclear phagocytic system (Chiangjong et al., 2021; Du et al., 2021).

MSC-EVs could promote tissue regeneration by various mechanisms, and they carry and transfer distinct bio-cargoes, which mirror their parental cells' genomic and proteomic pools. But owing to the nano-scale of EVs, the cargoes they carry which significantly affect recipients are supposed to be the key genetic materials. As Akbari et al. reviewed, MSC-EVs act as a depot for the encapsulation of bioactive molecules to deliver them to the desired cells to function rather than enzymatic degradation. MSC-exosomes contain more than 150 miRNAs and 850 proteins, and the key molecule delivery through EVs to target cells can lead to favorable phenotype changes. For example, exosomes derived from MSCs bear several cytokines and growth factors, including interleukin (IL) -6, IL-10, and transforming growth factor (TGF) β1, which regulate the local immune microenvironment. In addition, EVs derived from umbilical cord mesenchymal stem cells (UMSCs) are verified to promote angiogenesis and prevent scar formation in skin wound recovery; TGF-β/SMAD signaling is validated to be blocked by miR-21, miR-23a, miR-125b, and miR-145 enriched in EVs. Also, the additional complement of MSC-EVs enhanced oligodendrogenesis, neurogenesis, and neural remodeling in the ischemic boundary region; EVs containing miR-133b and miR-17–92 clusters can be transferred to astrocytes and neuron cells and consequently contribute to neurite remodeling and promote recovery.

MSC-EVs may function in the following ways:

(1) MSC-EVs can modulate the autophagic flux of recipient cells to facilitate tissue regeneration.

As a nano-scale cellular by-product, MSC-EVs can be easily taken up by phagocytes and non-phagocytic cells. The foreign vesicles were likely to change the phenotypes of recipients, which may be induced by an autophagic flux change. Debnath et al. found that EVs were strongly lysosome-associated and they could correspondingly influence the autophagic flux of recipients. Either activation or inhibition of autophagy can be related to tissue regeneration. Kuang et al. proved that adipose mesenchymal stem cell (ADMSC)-EVs rescued neurons under oxygen and sugar deprivation and promoted regeneration by inhibiting autophagic flow. Their work focused on microRNA transmission (miR-25-3p) by AMSC-EVs through the p53-Bcell lymphoma 2–interacting protein 3 (BNIP3) signaling axis, which significantly promoted the recovery of neurological functions in mouse apoplexy models. In addition, Rong et al. elaborated that EVs derived from neural stem cells could reduce neuronal apoptosis, inhibit neuroinflammation, and promote functional recovery in spinal cord injury by increasing autophagic flux.

(2) MSC-EVs could reverse pro-inflammatory macrophages to anti-inflammatory phenotypes by transmitting anti-inflammatory substances to immune cells. Nakao et al. verified that EVs derived from gingival mesenchymal stem cells (GMSCs) enhanced M2 macrophage polarization and inhibited periodontal bone loss. They also found that the application of CD39/73-enriched MSC-EVs could enhance the polarization of macrophages toward the M2 phenotype, which directly alleviated the local inflammatory environment. The mechanism is that the CD39-CD73-adenosine axis is necessary for immunoregulation, which could inhibit the proliferation of CD4+ T cells and promote tissue remodeling activity. CD39 is associated primarily with endothelial cells and immune cell populations and it is known to be an ecto-nucleoside triphosphate diphosphohydrolase which could convert extracellular ATP into AMP. CD73 (ecto-5′-nucleotidase, Ecto5′NTase) commonly expressed on the cytomembrane of MSCs could dephosphorylate AMP into adenosine. AMP accumulation could induce local inflammation, and the AMP/adenosine ratio could significantly affect the inflammatory microenvironment.

(3) MSC-EVs may affect the energy metabolism of host cells. The phenomenon that the engulfed vesicles were mostly enriched in the mitochondrial area suggests that MSC-EVs can target the recipient cells' mitochondrial metabolism pathways. Proteomic and RNA-seq analyses demonstrated that it can be achieved by the modulatory effect of several contained miRNAs, proteins, enzymes, and kinases involved in glycolysis such as glyceraldehyde-phosphate dehydrogenase (GAPDH), glucose-6-phosphate isomerase, in the tricarboxylic acid cycle (2-oxoglutarate dehydrogenase), and electronic transport chain (ATPase). MSC-EVs have been reported to stimulate ATP production and the antioxidant defense of tubular epithelia cells through the activation of the KEAP1-NRF2 signaling pathway, and they could transfer miR-222 in mesangial cells and induce miR-21 downregulation, which correspondingly rescue the function of electron transport chain complex, and relieve mitochondrial disorders.

However, the energy metabolic signal may not only be transferred by exosomal nuclei but also by exosomal organelles. In recent studies, Clair et al. proved that EVs released from stress-induced cells are enriched with oxidatively damaged mitochondria, which can induce a burst of ROS in cardiac tissue which protects the heart through hormesis. Meanwhile, Gentaro et al. verified that EVs enriched with mitochondria significantly improved post-MI cardiac function through the restoration of bioenergetics and mitochondrial biogenesis. To be specific, the EV-enclosed mitochondria could fuse with the recipients' endogenous mitochondrial network, retrieve ATP production, and improve contractile profiles of hypoxia-injured iCMs.

Paper 3: Medicine/Biomaterials

Question 1

Question: When looking at the study, the high capacity of tissue repair makes MSC-EVs a promising part of tissue regeneration therapies, especially in biomaterial construction. What reasons support this specific claim?

a.) EVs, as an endogenetic biological agent, have innate host affinity and can deliver easy-to-deactivate and -degrade substances to target cells.

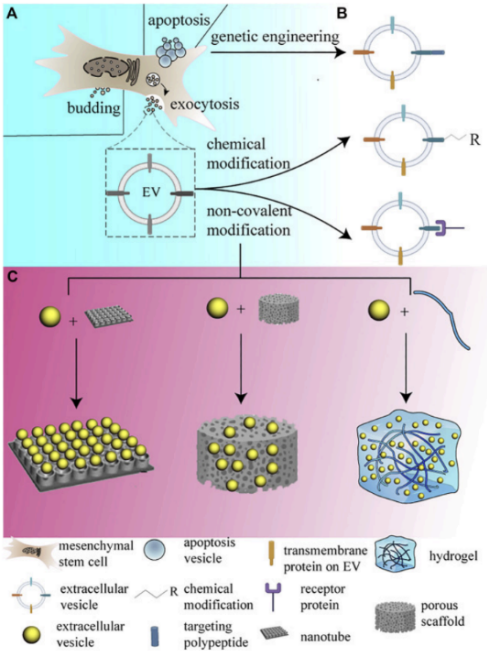
b.) The nano-size of EVs is suitable for traveling through the circulatory system and barriers, which provides the possibility for distant delivery to promote specific organ regeneration.

c.) The nano-size of EVs is not suitable for traveling through the circulatory system and barriers, which increases and provides the possibility for distant delivery to promote specific organ regeneration.

d.) EVs cannot maintain effective concentration in the tissue nor the circulation system, and local injection is mainly limited by their retention ratio.

Question 2

Question: According to the study we are examining a visual in specific, and we can see that all the steps from EV secretion to the final component are shown. What can be implied about Part C given only the information in the visuals?



a.) Certain EVs can only go into specifically tailored biomaterials.

b.) EVs can be loaded onto different forms of biomaterials.

c.) All EVs can only go into any biomaterials as long as it's porous.

d.) EVs cannot be loaded onto different forms of biomaterials, only one certain one.

Question 3

Question: As indicated by the study, MSC-EVs may function in the following way, which includes modulating the autophagic flux of recipient cells to facilitate tissue regeneration. In the context of this paper, what would best describe autophagic flux?

a.) The measure of the degradation activity of a certain pathway.

b.) The measure of the variability and disturbance of a certain pathway.

c.) The flow of MSC-EVs to recipient cells to facilitate tissue regeneration.

d.) The measure of the variability and disturbance of MSC-EVs to recipient cells to facilitate tissue regeneration.

Question 4

Question: Either activation or inhibition of autophagy can be related to tissue regeneration. Adipose mesenchymal stem cell (ADMSC)-EVs rescued neurons under oxygen and sugar deprivation promoted regeneration by inhibiting autophagic flow. What is this indicative of?

a.) After someone goes through an epilepsy, the postictal state is further elongated, and the timespan is longer than what it was previously.

b.) After someone goes through an epilepsy, the prodrome and aura state is further expedited, and the timespan is longer than what it was previously.

c.) After someone goes through an epilepsy, the prodrome and aura state is shortened, and the timespan isn't as long.

d.) After someone goes through an epilepsy, the postictal state is further expedited, and the timespan isn't as long.

Question 5

Question: In the studies outlined, it has been proved that EVs released from stress-induced cells are enriched with oxidatively damaged mitochondria, which can induce a burst of ROS in cardiac tissue which protects the heart through hormesis. Based on the paper, which of the following predictions is best supported?

a.) An increased spike of ROS would cause increased cell adaptation to stress or resilience, or balanced hormesis.

b.) An increased spike of ROS would cause no change in cell adaptation to stress or resilience, resulting in unchanged hormesis.

c.) A burst of ROS would cause decreased cell adaptation to stress or resilience, or unbalanced hormesis.

d.) A burst of ROS would cause decreased cell adaptation to stress or resilience, or balanced hormesis.