THE POTENTIAL OF PLURIPOTENT STEM CELL-BASED THERAPY AND EXTRACELLULAR VESICLES IN PROMOTING TISSUE REGENERATION

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ABSTRACT

Stem cell research has paved the way for revolutionary regenerative therapies targeting damaged and diseased tissues. Beyond traditional cell transplantation, current evidence suggests that therapeutic benefits are primarily mediated through paracrine effects. Extracellular vesicles (EVs), which can traverse biological barriers and deliver bioactive molecules, represent a promising avenue for cell-free therapy. Tissue engineering, as the second-generation regenerative innovation, integrates biodegradable 3D scaffolds with cells to mimic natural extracellular matrices, enhancing therapeutic outcomes. This study examines the potential of EVs across diverse applications. In ocular regeneration, neural progenitor-derived EVs preserve photoreceptor cells and mitigate retinal inflammation in retinitis pigmentosa. For skin repair, EVs derived from mesenchymal stem cells (MSCs) support key phases of wound healing by modulating macrophage polarization and activating molecular pathways like RAC-alpha and Notch signaling. In cardiovascular therapy, EVs contribute to heart tissue recovery, reduce myocardial apoptosis, and combat fibrosis through targeted gene modulation. Skeletal muscle regeneration benefits from EVs enhancing myogenic differentiation, decreasing fibrosis, and addressing excessive extracellular matrix accumulation common in disorders like muscular dystrophy. The ability of EVs to emulate paracrine signaling processes expands the horizons of regenerative medicine, offering a scalable and efficient alternative to cell-based therapies. Literature highlights the critical role of high-quality, large-scale production under stringent standards to ensure therapeutic consistency. These findings underscore EVs as potent, cell-free agents capable of driving tissue repair and regeneration. Further investigations are encouraged to optimize production, application, and integration with advanced biomaterials for clinical efficacy.

Keywords : Regenerative therapy; extracellular vesicles; tissue engineering

INTRODUCTION

Significant advancements in stem cell research have provided a solid foundation for the application of regenerative therapies in damaged or diseased tissues. Recent studies indicate that the therapeutic benefits of stem cell-based approaches are largely associated with paracrine effects rather than relying on long-term cell engraftment or the survival of implanted cells. Due to the ability of extracellular vesicles to cross biological barriers and facilitate the transfer of bioactive molecules between cells, they are currently being studied as a potential option in cell-free therapy.

Approaches continue to be developed to meet the needs of large-scale production while also discovering the most optimal formulations to enhance therapy effectiveness (M'Barek & Monville, 2019). Tissue engineering technology has now become an innovation in the second generation of cell-based regenerative therapies by combining biodegradable 3D compounds that mimic the

extracellular matrix with various types of cells (Sun et al., 2014; Ghareeb et al., 2020).

In addition to providing direct therapeutic effects through their presence, some benefits arise from indirect mechanisms, meaning the direct presence of cells may not always be required (Morizur et al., 2020). Extracellular vesicles (EVs) are now recognized as key mediators in paracrine signaling processes and have the capability to perform those functions (Wiklander et al., 2019).

In regenerative medicine, the raw materials for cell- and tissue-based products play a crucial role. To enable widespread use of cell-based products, the ability to produce cells on a large scale with a high level of consistency at every stage of production is required, ensuring that the quality and effectiveness of the therapy are uniform for all patients (Jarrige et al., 2021). This discussion will outline various cell sources with potential for use in cell therapy and their applications in specific pathological conditions. Additionally, it will address the progression from cell suspension-based therapy to more complex and advanced tissue-engineered products.

MATERIALS AND METHODS

The research method using a literature review begins with defining a specific and relevant research focus aligned with the field of study. This focus serves as a guide to selecting appropriate literature, providing clear boundaries and direction for the review. Next, the author needs to design detailed criteria for literature selection, which typically include publication period, relevance to the research question, type of methodology, and credibility of the literature sources.

Once the selection criteria are established, the next step involves conducting a systematic search for literature across various academic databases such as PubMed, ScienceDirect, or Scopus. During this search process, the author strategically uses keywords, leveraging logical operators like "AND" or "OR" to ensure that the results encompass all relevant studies. The search results form an initial collection of literature that requires further screening to identify materials most pertinent to the topic.

The subsequent stage involves thoroughly reading and understanding the selected literature. This process includes detailed analysis to identify patterns, gaps, or contradictions in prior research. The information gathered is then synthesized into a coherent and comprehensive narrative. This narrative not only covers topic developments but also offers a critical perspective on what is already known and highlights aspects requiring further exploration.

The results of the literature review are written following the structure of a standard scientific article. In the introduction, the author explains the rationale for selecting the topic and the objectives of the literature review. The methodology, including how the literature was selected and analyzed, is described in detail to ensure the process is clear and credible. The results and discussion section focuses on the analysis of findings and identifying connections between studies. The conclusion summarizes the main findings and provides recommendations for future research to continue or deepen the review.

RESULT AND DISCUSSION

Since the discovery, human pluripotent stem cells (hPSCs) have been regarded as a potential cell source for applications in regenerative therapy. The ability of hPSCs to self-renew and differentiate into various types of human body cells makes

them highly valuable. These cells can be derived from embryos produced through in vitro fertilization (known as human embryonic stem cells or hESCs) or from reprogramming adult cells into a pluripotent state using specific combinations of factors (known as human induced pluripotent stem cells or hiPSCs) (Takahashi et al., 2007; Nakagawa et al., 2008). Additionally, hPSCs can be produced in large quantities under GMP-certified manufacturing facility standards, with their quality strictly controlled similar to pharmaceutical products.

Utilization of Extracellular Vesicle Particles as an Innovative Strategy in the Field of Regenerative Medicine

a. Regeneration Process in Eye Structures

Various studies suggest that the presence of cells may not be entirely necessary to achieve therapeutic effects. For example, subretinal implantation of human neural progenitor cells was shown to preserve vision in RP models through paracrine mechanisms. Similar effects were observed in mouse RP models and human patients using human fetal retinal progenitor cells. Since these transplants were performed before PR degeneration occurred, replacement of dead cells was not anticipated. Based on these findings, extracellular vesicles (EVs) derived from neural progenitor cells were injected subretinally into RP mouse models prior to vision loss. A single EV injection resulted in temporary improvement in visual function and PR survival (up to 28 days post-operation). The EVs were predominantly internalized by Iba1+ microglia, which had migrated from the inner retina to the subretinal space. These EVs also downregulated pro-inflammatory cytokines and inhibited microglial activity, where suppression of microglial activation contributed to improved PR survival in RP (Wang et al., 2008; Luo et al., 2014; Peng et al., 2014; Liu et al., 2017; Bian et al., 2020).

Electric vehicles (EVs) have the potential to affect retinal damage by modulating inflammatory responses. This mechanism mirrors the effects produced by implanted cells, indicating that the benefits of cell transplantation may primarily stem from paracrine activity, as observed in MSCs, retinal astrocytes, and human neural progenitors.

b. Regeneration Process in Eye Structures

Significant skin damage caused by severe burns, infections, or trauma requires medical intervention to ensure optimal healing (Chu et al., 2018). Therapeutic approaches focus on supporting the four key stages of skin wound healing hemostasis, inflammation, proliferation, and remodeling with the goal of accelerating tissue regeneration and repair (de Oliveira Gonzalez et al., 2016). These stages involve complex interactions between molecules and cells that progress in an organized sequence to restore damaged tissue. Extracellular vesicles (EVs) show potential in supporting all these stages and accelerating skin regeneration.

The transition from the inflammatory phase to the proliferative phase is a critical step in successful wound healing (de Fonseca Ferreira & Gomes, 2018; Ha et al., 2020). In the early inflammatory phase, most macrophages differentiate into the pro-inflammatory M1 phenotype. Over time, this dominance shifts toward the M2 phenotype, which plays a greater role in tissue remodeling and accelerating wound healing (Hesketh et al., 2017). EVs derived from mesenchymal stem cells (MSCs) preconditioned with lipopolysaccharides can promote M1 to M2 macrophage polarization, contributing to reduced inflammation and faster wound healing in diabetic mice through the transfer of let-7b miRNA (Ti et al., 2015). Additionally, MSC-derived EVs enhance skin wound healing in mice by regulating macrophage polarization via miR-223 (He et al., 2019).

Consistent with these findings, human umbilical cord-derived mesenchymal stem cells (hUCMSCs) significantly suppress the number of inflammatory cells and reduce pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6, while increasing the anti-inflammatory cytokine IL-10 in severe burn wounds in mice (Liu et al., 2014).

During the proliferative phase, processes such as re-epithelialization, wound contraction, and angiogenesis play crucial roles in restoring tissue structure. Early recruitment of keratinocytes and fibroblasts is critical, as disruptions in epidermal-dermal communication can decrease the efficiency of skin healing. EVs from hUCMSCs and MSCs are capable of activating key signaling pathways, including RAC-alpha serine/threonine-protein kinase (AKT) and Notch signaling, which are relevant to wound healing. Furthermore, EVs from human adipose-derived MSCs prevent fibroblast differentiation into myofibroblasts, increase the transforming growth factor-β3 (TGF-β3) to TGF-β1 ratio, and stimulate the expression of matrix metalloproteinase-3 (MMP3) in dermal fibroblasts through the activation of the ERK/MAPK pathway (Wang et al., 2017).

c. Heart

EVs have been explored as an innovative therapeutic approach for various cardiovascular disorders, including ischemic heart disease and heart attacks. The primary focus of this therapy is to enhance vascular repair mechanisms to minimize myocardial damage that can trigger cell death. Conditioned media from hESC-derived MSCs (hESC-MSC-CoM), collected through procedures suitable for clinical applications, have been found to contain factors that influence pathways related to cardiovascular health (Sze et al., 2007).

Additionally, hESC-MSC-CoM has been shown to reduce myocardial apoptosis and oxidative stress levels in various ischemia-reperfusion injury models, including in pigs (Timmers et al., 2011). This media contains particles ranging in size from 50–100 nm, which have been identified and further processed as Evs (Lai et al., 2010). In an ex vivo mouse model of myocardial ischemia-reperfusion injury, EVs derived from hESC-MSCs were able to reduce infarct size. Both EVs and their parent cells were administered intramyocardially in a single dose. Gene profile analysis revealed that 927 genes were upregulated to a comparable extent in hearts treated with EVs from hPSC-CMs and their parent cells compared to the control group (Kervadec et al., 2016). Most of the biological processes associated with these genes are predicted to support cardiac tissue regeneration and inhibit fibrosis (Kervadec et al., 2016).

To ensure sustained delivery, EVs derived from hPSC-CMs were incorporated into collagen-based hydrogel patches.

d. Skeletal Muscle

Significant muscle damage and genetic disorders such as muscular dystrophy can lead to the death of myofibers. Various types of cells, both myogenic and non-myogenic, have been proposed as candidates for skeletal muscle regeneration. Cell transplantation-based therapeutic strategies have been extensively studied; however, their effectiveness is limited by the high mortality of donor cells and inefficient cell distribution following injection (Qazi et al., 2019). Furthermore, these approaches have not been able to fully reconstruct muscle structures, including adequate neural and vascular networks. Overall, cell therapy is more suitable for addressing injuries in small muscles rather than conditions affecting the entire muscular system.

Skeletal muscle cells actively produce extracellular vesicles (EVs) that play a role in muscle repair and regeneration. EVs produced during the differentiation process of human

skeletal myoblasts (HSkM) into myotubes contain biochemical signals that can promote myogenic differentiation in human adipose-derived stem cells (HASC) (Choi et al., 2016). Treatment of injured muscle areas with EVs from differentiated HSkM shows enhanced muscle regeneration, with more myofibers and lower levels of fibrosis compared to controls (Choi et al., 2016). EVs also help reduce excessive extracellular matrix (ECM) formation, which is crucial for optimal muscle recovery (Kharraz et al., 2014).

In conditions such as muscular dystrophy and severe injuries, excessive fibrogenic cell activity can lead to overproliferation, ultimately replacing muscle tissue with nonfunctional fibrotic tissue (Kharraz et al., 2014). This excessive accumulation of ECM not only impairs muscle function but also reduces the available area for regenerative therapy. Developing new anti-fibrotic strategies is therefore a key clinical step in improving muscle function in patients. During hypertrophic stimuli, satellite cells generate myogenic progenitor cells (MPCs) that secrete EVs containing miR-206. This molecule suppresses collagen production by fibroblasts through the regulation of ribosome-binding protein 1 (Rrbp1), preventing excessive ECM accumulation (Fry et al., 2017). These findings highlight the potential of EVs as promising anti-fibrotic therapeutic agents.

SUMMARY

Human pluripotent stem cells (hPSCs) are considered a potential source for regenerative therapy due to their ability to self-renew and differentiate into various cell types in the body. hPSCs can be derived from embryos (hESCs) or through the reprogramming of adult cells (hiPSCs) and can be mass-produced under high-quality standards.

Extracellular vesicles (EVs) are emerging as an innovative strategy in regenerative medicine, including the repair of eye, skin, heart, and skeletal muscle tissues. In retinal regeneration, EVs derived from human neural progenitors protect photoreceptor cells from degeneration through paracrine mechanisms, reducing inflammation and inhibiting microglial activation. In skin wound healing, EVs support the inflammatory and proliferative phases as well as tissue repair by regulating macrophage polarization and enhancing intercellular communication. For cardiovascular disorders, EVs aid in heart tissue regeneration, reduce apoptosis, and prevent fibrosis through various molecular pathways. In skeletal muscles, EVs produced by myoblast cells accelerate muscle regeneration by reducing fibrosis and promoting cell differentiation.

These findings highlight the potential of EVs in regenerative therapy as an effective alternative to cell transplantation.

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