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Application of Extracellular Vesicles in Tendon Repair

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Abstract: Tendon injuries are often accompanied by damage to the surrounding soft tissue and muscle ligaments, resulting in a loss of muscle strength, flexibility, and other functions in the lower limbs. At present, non-surgical treatment and surgical treatment are mainly used clinically. However, non-surgical treatment may lead to loss of tendon drift and sliding, and ultimately lower ankle movement and function. Surgical treatment may cause risks such as infection and nerve damage. Extracellular vesicles are various types of membrane vesicles secreted by cells, which are rich in a variety of bioactive substances, and are characterized by low immunogenicity, strong tissue penetration and so on. It is a mediator of intercellular communication that plays an important role in tissue regeneration, transmitting information to recipient cells by paracrine effects and affecting various cellular functions. In this review, we summarize the mechanisms of MSC-derived EVs and platelet-derived EVs in tendon repair, providing new directions for the future treatment of tendon repair, and further advancing the study of EVs in sports medicine.

Keywords: Extracellular vesicles, Low immunogenicity, Tissue regeneration, Tendon repair.

1. Introduction

Tendon injuries are often accompanied by damage to the surrounding soft tissue and muscle ligaments, resulting in a loss of muscle strength, flexibility, and other functions in the lower limbs[1] .Severe cases may cause tendon rupture, putting a significant burden on patients, showing functional deficits or pain, and many athletes fail to return to their pre-injury activity levels[2-5]. Furthermore, after trauma, tendon healed slowly and vascular circulation at the site of injury is poor[6].At present, non-surgical treatment and surgical treatment are mainly used clinically. Nonsurgical treatment makes tendons repair by the formation of scar tissue, which may lead to loss of tendon drift and slip, and ultimately reduces ankle movement and function[7].The most common surgical treatment of tendon tears is direct repair with a suture, which allows patients to recover early, and the incidence of rerupture may be reduced[8].However, surgical repair is associated with the risk of infection, nerve injury, and focal adhesion formation[9].Subsequently, direct surgical repair was replaced with both autologous and allogeneic transplant methods. Autograft was rapidly remodeled around the ankle joint without an immune response. However, this option also increases the operative time and carries a risk of morbidity in the donor site. Furthermore, autograft-repaired new tendons have low tensile strength[7].At the same time, the risks of using allogeneic transplantation include immune rejection, infection, disease transmission、limited availability and the inability of artificial tendons to replicate the fibrous tissue or the anatomical structure of the attachment site is easy to form a foreign body granuloma etc[10].Therefore, how to accelerate tendon healing and develop new materials remains a major challenge in clinical practice[11,12].

Stem cells are a class of multipotential cells with extremely strong replication and renewal capacity, and under certain conditions, they can differentiate into a variety of functional cells.Stem cell therapies play a role in tissue regeneration because of their pluripotency, self-renewal, and their ability to promote regenerative cytokine secretion[13,14].For example, the MSC promotes tendon healing by either injecting the MSC directly into the site of injury or by implementing an MSC-loaded stent during surgical repair[14-16].However, direct cell therapy has long faced severe challenges, including immune safety, potential tumorigenicity, cell expansion and culture costs, and transportation etc[17].It is reported that stem cells mainly promote tissue regeneration through paracrine effects, and stem cells gradually enter a wider field of medical research[18-20],which has become another way to treat diseases such as wound injury [21], skin aging [22], and traumatic brain injury [23].

Extracellular vesicles (EVs) are various types of membrane vesicles secreted by cells into three subtypes: exosomes (30 – 150 nm), microparticles (100-1000 nm), and apoptotic bodies (> 1000 nm).And it is a medium of intercellular communication, mainly transmitting information to recipient cells through paracrine effects and affecting various cellular functions[24-27].These nanovesicles are collectively known as extracellular vesicles.Studies have demonstrated that a variety of EVs participate in various studies of cell proliferation and migration related to skin repair, such as:EVs in adipose-derived stemcells[28] ,BMSC-derived EVs [29], EVs of their origin in bovine milk[30] and platelet-derived EVs etc[31].In contrast to cell therapy, EVs provide a cell-free treatment modality, addressing the challenges of cell therapy, including tumorigenicity, immunocompatibility, infection etc.Secondly, EVs are easy to store, mass production from certified cell lines, and shortened cell amplification time and cost, and are expected to become raw materials for drug synthesis in the future[32].

Currently, the treatment of EVs has been gradually applied in regenerative medicine.However, there are few studies in sports medicine, such as tendon repair, ligament repair, and muscle recovery.In this review, we summarize the mechanisms of MSC-derived EVs and platelet-derived EVs in tendon repair, providing new directions for the future treatment of tendon repair, and further advancing the study of

EVs in sports medicine.

2. Biological Properties of the Extracellular Vesicles

Extracellular vesicles, when secreted from the parental cells, information transfer can be performed through internalization into the recipient cells[33].Extracellular vesicles have the intrinsic ability to cross the tissue and cellular barriers and have certain targeting properties, and exhibit tropism for specific types of cells or tissues[34]. Moreover, compared with traditional nanomaterials, extracellular vesicles have good biocompatibility and low immunogenicity, which are more suitable as drug delivery carriers[35].

2.1. Structure and composition of the extracellular vesicles.

EVs are lipid bilayer nanospherical structures secreted by most cells to the extracellular and contain rich proteins, nucleic acids, miRNA, cholesterol and sphingolipids etc[36] (Fig 1).They can activate signaling cascades that can alter the physiological state of the receptor cells[37].Moreover, EVs surface molecules have important functional importance.For example, the EVs membrane is rich in the tetra-transmembrane protein family CD63, CD81 and CD9 involved in the transport of EVs etc[38].These EVs surface molecules are capable of the recognition, affinity isolation, and molecular classification of EV, and their use as biomarkers[39].

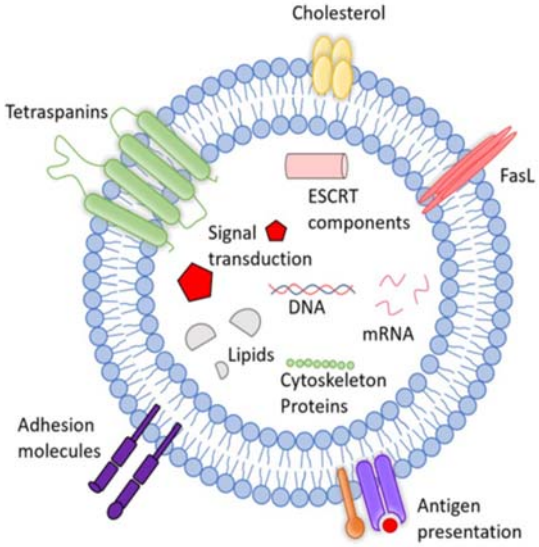

Figure 1. Structure and composition of the extracellular vesicles [36]

2.2. Biogenesis of extracellular vesicles

Investigation of extracellular vesicle biology is crucial for its application in Achilles tendon repair.The EVs are divided into three major subtypes based on their biological origin, morphology, and biochemical properties: exosomes, microvesicles, and apoptotic bodies[40].(i) Exosomes form luminal vesicles through early endosome inward germination, producing multivesicular bodies (MVBs), which fused with the lysosomes or the plasma membrane, leading to the secretion of exosomes[41].(ii) Microvesicles are shed by becoming outward budding and dividing the plasma membrane[42].(iii) Apoptotic bodies are produced through the plasma membrane vesicles of apoptotic cells[43].All three isoforms of EVs are able to transport their cargo to the recipient cells, functioning either by binding to the receptor on the receptor cells or through internalization by EVs into the recipient cells to play a role[40,44,45].(Fig2)

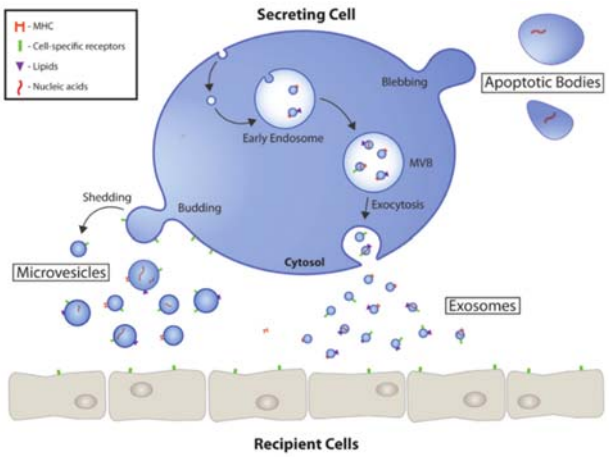

Figure 2. Biogenesis and secretion of extracellular vesicles (EVS). Schematic representation of the production and release of EVs by eukaryotic cells [40].

3. Mechanisms of Extracellular Vesicles in Tendon Repair

Tendons are composed of highly ordered type I collagen fibers as their main extracellular matrix (60-85% dry weight) and a relatively small number of cellular components (mainly tendon stem cells) [46]. Extracellular vesicles contribute to tendon repair mainly by promoting the proliferation and migration of tenocytes and the synthesis of collagen I fibers [11,47]. Below, we specifically explore the roles and regulatory mechanisms of MES - enchymal stem cell-derived EVs and platelet-derived EVs in tendon repair.

3.1. Mechanisms of mesenchymal stem cell-derived EVs in tendon repair

Stem cell-derived extracellular vesicles are rich in active substances such as proteins and multiple miRNAs, which contribute to tendon repair by promoting the proliferation of tendon stem cells [47], reducing blood vessel density [48], improving collagen fiber alignment [49], and enhancing type I collagen expression [50].

Studies have shown that adipose mesenchymal stem cell-derived exosomes (a subtype of EVS), via Smad2 / 3 and Smad1 / 5 / 9 signaling pathways, promote their tendon stem cell proliferation, migration and tendon differentiation, thus alleviating early inflammation and promoting tendon healing [47] (Fig 3a). In addition, it was found that adipose mesenchymal stem cell-derived exosomes also reduced fat infiltration, increased histological scores, increased more fibrocartilage, and improved biomechanical properties at the tendon bone junction [52] (fig3b). Umbilical cord stem cell-derived exosomes regulate PTEN / mTOR / TGF by delivering mir-29a-3p- β 1 pathway, enhancing the expression of type I collagen, thereby promoting tendon healing [50] (fig3c, d). Adipose mesenchymal stem cell-derived EVs promote tendon regeneration by upregulating tendon specific markers in surgically repaired tendons, improving the mechanical properties of repaired tendons, and regulating collagen synthesis [11]. Besides that, HCPT EVs induced extracellular vesicles (HCPT EVs) derived from human

umbilical cord stem cells were more effective in improving tendon adhesion in a rat model of tendon injury and had anti adhesive potential for tendon injury therapy [53] (fig3e). However, the study of stem cell-derived extracellular vesicles in Achilles tendon repair is still at the theoretical research stage, which still needs to be supported by standardized and reproducible clinical trials before stem cell-derived extracellular vesicle preparations can be applied in the clinic.

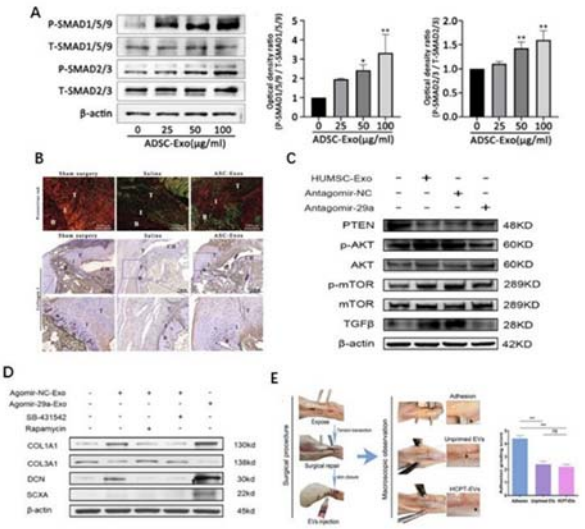

Figure 3. EVs from mesenchymal stem cells promote the repair of tendon injury. (A) Western blot analysis of p-SMAD2/3 and p-SMAD1/5/9 protein levels induced by ADSC Exos at different concentrations [47]. (B) The micrograph of tendon bone interface repaired by picroside staining and collagen I staining [52]. (C) Representative PTEN, TGF- β 1. Western blot images of p-AKT, AKT, p-mTOR and mTOR. (D) Representative western blot images of COL1A1, COL3A1, DCN and SCXA. [50] (E) Representative images show the operation process and naked eye observation of the repaired tendon and the accompanying adhesion and adhesion score (n=5/group) for the macroscopic score of tendon adhesion [53].

3.2. Mechanism of platelet derived EVs in tendon repair

Platelets are the first cells gathered at the wound site. They provide a wide range of biochemical signals and structural elements to rebuild the homeostasis in the tissue and coordinate the highly complex microenvironment in wound healing [54]. Compared with EVs derived from mesenchymal stem cells, platelet-derived EVs can be directly generated from collected platelet concentrates. Advantages such as the GMP devices are not required for in vitro cell expansion.[55].

At present, there are relatively few studies on platelet derived EVs. Systematic review and meta-analysis have not yet confirmed the significant efficacy of platelet rich plasma in the treatment of tendinosis [56, 57]. However, many studies have confirmed that platelet derived EVs contain rich active substances such as lipids, nucleic acids, growth factors and cytokines [58, 59], which play an important role in tissue regeneration and immune regulation [60-62]. Theoretically, their existence must be involved in the mechanism of tendon tissue healing and regeneration [63]. Moreover, compared with the treatment of platelet plasma, platelet derived EVs can be obtained from their own sources through simple blood extraction [55, 64, 65]. In addition, platelet-derived EVs can also be obtained from other discarded expired platelet units for clinical use on a large scale, and the difference between batches of growth factor composition is low [63, 66]. These advantages make platelet-derived EVs provide an unprecedented prospect in tendon repair.

Currently, studies have shown that platelet small extracellular vesicles (SEVs) have a positive effect on tendon injury recovery by increasing the expression of tenocyte markers and promoting ECM remodelling, with an increased type I / III collagen ratio, and that platelet extracellular vesicles (mevs) also increase the expression of anti-inflammatory cytokines, these illustrating that platelet EVs may be a promising therapeutic approach for tendon injury recovery [67] (fig4a-d). However, major possible limiting factors in the use of platelets as a source of EVs include the reliance on donors or blood sampling tissues for stable supply of starting material and the risk of pathogen contamination, and second, the variability that exists between platelet donors may affect platelet-derived EVs characteristics and performance [55]. Therefore, there is still a need for larger studies of platelet-derived EVs in tendon repair in the future.

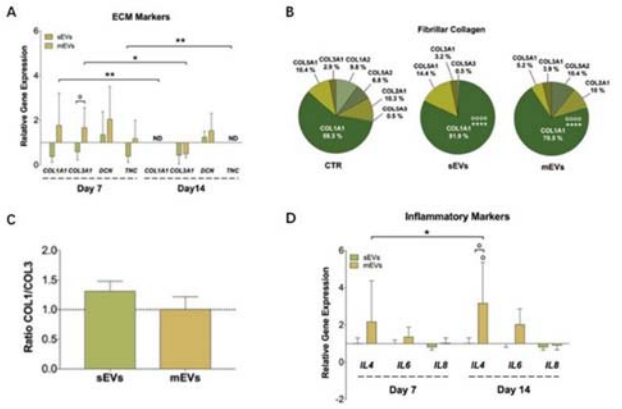

Figure 4. Platelet derived EVs contribute to tendon repair. (A) Tendon associated extracellular matrix (ECM) markers, collagen type I, after 7 and 14 days of (SEVs) and (mevs) formation α α 1 chain (COL1A1), type III collagen α Gene expression of the α 1 chain (COL3A1), decorin (DCN), and tenascin (TNC). (B) Proportion occupied by fibrillar collagen (n = 3). (C) Proteomic analysis of type I collagen and type III collagen (col1 / col3) ratio at 14 d of culture (n = 3). (D) After 7 and 14 days of culture, the amount of gene expression of inflammation related factors (interleukin-4; 6; - 8 (IL4, IL6, and IL8)). [67]

4. Conclusion and Outlook

EVs derived from mesenchymal stem cells and platelet-derived EVs have been gradually applied to the research of wound repair, cartilage repair and other tissue regeneration. However, the research on tendon repair is still in the initial stage and has not been fully verified clinically. This paper discusses the regulatory mechanism of stem cell derived EVs and platelet derived EVs in tendon repair, which provides a theoretical basis for the future application of EVs in tendon repair.

Then, the research on the function of EVs is still in its early stage, so it is necessary to conduct a comprehensive study on the absorption, distribution, metabolism and other functions of EVs. Secondly, the separation cost of EVs is high, the uncertainty and heterogeneity of EVs content obtained from each batch are strong, and the purity is low. In addition, the

wide application of EVs still has many challenges, and many aspects of exploration are needed in the future. First of all, the research on EVs in tendon repair is mainly focused on stem cell EVs, while the research on platelet derived EVs is relatively small, and it is still necessary to increase the research in this area in the future. Secondly, the value of many kinds of EVs, such as plant nano vesicles and microbial nano vesicles, in Achilles tendon repair needs to be further explored. In addition, as a drug delivery carrier, how to improve the efficacy is also worth exploring. At the same time, how to produce and preserve on a large scale is also worth studying.

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