Review article

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FROM ORIGIN TO THERAPEUTIC POTENTIAL OF EXOSOMES IN WOUND HEALING: A MINI-REVIEW

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Wound healing, particularly the management of chronic wounds, remains a major global health challenge due to its complexity and the limitations of current treatments. Exosomes, lipid-bound vesicles and the smallest subpopulation of extracellular vesicles (EVs), have emerged as highly promising therapeutic tools. These vesicles can be classified by their origin as natural-derived (animal or plant-derived), modified, or artificial. They exert a wide range of biological effects essential for tissue regeneration, including anti-inflammatory, immunomodulatory, and antioxidant properties, as well as promoting angiogenesis, intercellular communication, and extracellular matrix remodeling. Furthermore, the capacity of exosomes for targeted drug delivery and epigenetic regulation positions them as versatile candidates for treating various disorders. This review discusses and summarizes the characteristics of exosomes from diverse origins, providing an overview of their primary roles in wound healing and tissue regeneration.

Keywords: exosomes, wound healing, regenerative medicine

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OD POREKLA DO TERAPIJSKOG POTENCIJALA EGZOZOMA U ZARASTANJU RANA: MINI-PREGLED

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Zarastanje rana, a naročito lečenje hroničnih rana, predstavlja značajan globalni zdravstveni izazov zbog svoje kompleksnosti i ograničenja postojećih terapijskih opcija. Egzozomi, lipidne vezikule i najmanja subpopulacija ekstracelularnih vezikula (EV), postali su veoma perspektivno terapijsko sredstvo. Prema poreklu, ove vezikule se klasifikuju na prirodne (životinjskog ili biljnog porekla), modifikovane i sintetičke (veštačke). One ispoljavaju širok spektar bioloških efekata neophodnih za regeneraciju tkiva, kao što su anti-inflamatorna, imunomodulatorna i antioksidativna svojstva, uz stimulaciju angiogeneze, međućelijske komunikacije i remodeliranja ekstracelularnog matriksa. Pored toga, sposobnost egzozoma za ciljanu isporuku lekova i epigenetsku regulaciju čini ih svestranim kandidatima za lečenje brojnih poremećaja. Ovaj rad analizira i sumira karakteristike egzozoma različitog porekla, uz poseban osvrt na njihovu ulogu u procesima zarastanja rana i regeneracije tkiva.

Ključne reči: egzozomi, zarastanje rana, regenerativna medicina

INTRODUCTION

The skin is the largest organ of the human body, accounting for approximately 16% of total body mass, and serves as a key barrier between internal tissues and the external environment. Therefore, preserving skin integrity is essential for maintaining health, as skin plays a vital role in homeostasis, protection against infections, and the prevention of fluid loss (1). Skin injuries or wounds can seriously threaten the body's functionality, cause disability and psychological distress, and pose a significant challenge to healthcare systems worldwide (2).

The skin healing process is mediated by coordinated cellular responses, extracellular matrix remodeling, and the action of various growth factors, which restore the functional and anatomical integrity of the skin (3). However, numerous factors can disrupt this process, leading to significant delays; consequently, wounds often become resistant to standard therapeutic approaches (2). The prevalence of acute and chronic wounds, often described as a "silent epidemic", is a major global health challenge, as it impairs the functional and structural integrity of the skin, affecting more than 4% of the world's population due to various pathologies. In Europe, more than 10 million patients are affected, and the annual economic burden on healthcare systems for their treatment exceeds 4 billion euros (4).

Despite intensive research and efforts to enhance wound treatment methods, therapy remains a significant clinical challenge, due in part to the complexity of assessing wound status and managing the healing process. Consequently, developing innovative strategies to accelerate and improve wound healing is crucial for further advances in medical practice and global health (5). In this context, exosomes, as extracellular lipid-bound vesicles generated by eukaryotic cells or engineered, carrying nucleic acids, proteins, lipids, and metabolites, have emerged in recent years as a promising therapeutic option, attracting considerable attention from researchers and clinicians due to their regenerative properties (6).

This review analyzes the therapeutic potential of exosomes from various sources in the wound healing process, with a specific focus on the mechanisms by which these vesicles contribute to tissue regeneration. Furthermore, in this review the characteristics of exosomes of different origin are summarized and discussed.

THE DISCOVERY OF EXOSOMES

Initially, intercellular communication was described solely as resulting from direct cell-to-cell contact or the release of soluble molecules, such as cytokines and hormones. However, the discovery of extracellular vesicles (EVs) revealed an additional mechanism of cellular communication (7). "Extracellular vesicles" is an umbrella term encompassing various subtypes. EVs are a family of nanoparticles that includes exosomes, microvesicles, and apoptotic bodies, the latter being released during the cell death process. Secreted by nearly all cell types, EVs are present in various body fluids, making them highly promising candidates for biomarker detection (8).

Although EVs research has expanded rapidly over the last decade, EVs were not immediately embraced by the cell biology community upon their discovery in the 1980s. Early studies suggesting that cells release EVs as a mechanism for debris removal were met with skepticism (9,10), and these vesicles were often dismissed as experimental artifacts (11). EVs possess a lipid bilayer that encapsulates cellular cargo, including proteins, DNA, and various RNA molecules. This lipid membrane protects these molecules from degradation, facilitating their functional transfer between cells. Notably, while it has been demonstrated that EVs-shuttled miRNAs can be functional in recipient cells, their endogenous concentration remains remarkably low - approximately one miRNA molecule per 100 vesicles (12-14). Nevertheless, the vast diversity of biomolecules within EVs suggests a broad functional spectrum, and it is now established that EVs participate in numerous physiological and pathological processes (15).

Three main groups of EVs are currently recognized: exosomes, microvesicles, and apoptotic bodies. While microvesicles and apoptotic bodies form through direct budding from the cell membrane, exosomes originate within multivesicular bodies (MVBs) and are released when MVBs fuse with the cell membrane (16). Exosomes represent the smallest vesicle population (15,16). As a subset of extracellular vesicles, they are endosomal in origin and range in size from 30 to several hundred nanometers, depending on the cell source and the isolation methods employed (17). Due to their unique biogenesis, molecular complexity, and functional versatility, they play a crucial role in various cellular processes. Although the term "exosome" was first used in 1981 to describe vesicles secreted from the cell surface, it was not until 1983 that they were formally discovered (18). A pivotal moment for the field was the 2013 Nobel Prize in Physiology or Medicine, awarded for the discovery of vesicle transport mechanisms (19). This early potential has been realized over the past three decades through the explosive growth of exosome biology, leading to the formation of various specialized societies (such as the International Society for Extracellular Vesicles and the American Society for Exosomes and Microvesicles), a dedicated journal (Journal of Extracellular Vesicles), as well as various international symposia and congresses, and thousands of publications. Today, exosomes are recognized not only as biomarkers but as active participants in disease pathogenesis, diagnosis, and therapy. Their transition from basic science to clinical application is the result of significant advancements in understanding exosome biology and the development of modern analytical technologies (9).

EXOSOME ISOLATION TECHNIQUES

Various methods have been developed to isolate exosomes from diverse sources. The most widely utilized technique is ultracentrifugation, which enables the recovery of high yields of isolated exosomes (20,21). This method is based on differences in particle density and size, offering a relatively simple and cost-effective approach (22). Ultrafiltration is a rapid and convenient method for separating exosomes by size, using membranes with specific pore sizes. However, the primary limitations of this technique include membrane pore clogging and potential vesicle damage (23). Additionally, chromatographic methods and polymer-induced precipitation are frequently employed (23,24). Furthermore, innovative strategies based on the physicochemical properties of exosomes have emerged, including selective capture using biopolymers, microfluidic techniques, and size-based and hydrodynamic separation. Other advanced approaches involve hybridizing exosomes with liposomes to enhance capture efficiency or delivery (24).

ORIGIN OF EXOSOMES

Exosomes exhibit considerable heterogeneity, stemming from differences in their cellular origin and source. Although a single standardized classification is lacking, most studies categorize exosomes into natural, modified, and artificial types (20). Natural exosomes are endogenous nanomaterials secreted via exocytosis from a wide array of cells, including epithelial and endothelial cells, mesenchymal stem cells, macrophages, dendritic cells, tumor cells, neurons, oligodendrocytes, reticulocytes, mast cells, platelets, B and T lymphocytes, and astrocytes. Furthermore, they can be isolated from plant cells and tissues and that is why natural exosomes are usually divided into animal-derived exosomes and plant-derived exosomes (25). They are present in most body fluids, such as plasma, serum, urine, breast milk, seminal fluid, saliva, nasal secretions, lymph, amniotic fluid, ascites, and cerebrospinal fluid (26). Due to their superior biocompatibility and relatively straightforward, cost-effective isolation processes compared with other sources, plant- and animal-derived exosomes are increasingly being considered as potential natural therapeutic agents (27).

Animal-derived exosomes

Exosomes are generated through the endosomal pathway. They are formed with the invagination of the plasma membrane and may subsequently fuse with vesicles originating from the Golgi apparatus and the endoplasmic reticulum (ER). Precursors to exosomes, known as intraluminal vesicles (ILVs), are formed by the inward budding of the endosomal membrane, resulting in the formation of multivesicular bodies (MVBs) (28,29). The Endosomal Sorting Complex Required for Transport (ESCRT) comprises approximately 30 proteins organized into four major subcomplexes: ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III. These subcomplexes play a pivotal role in sorting proteins into ILVs inside the MVBs, thereby facilitating exosome formation (8). Following their formation, MVBs follow one of two fates: they either fuse with lysosomes for cargo degradation or fuse with the plasma membrane. Upon fusion with the plasma membrane, ILVs are released into the extracellular space and are formally termed "exosomes" (30). Exosomes are derived from a vast array of cellular sources (31). Animal-derived exosomes are primarily secreted by immune cells (such as lymphocytes, erythrocytes, platelets, dendritic cells, and tumor cells), and are present in various biological fluids (including urine, milk, and plasma) (32).

Plant-derived exosomes

The endocytic pathway in plant cells is not as clearly characterized as its animal counterpart. Proteins are internalized through the invagination, budding, and formation of transport vesicles on the plasma membrane, which are directed as early endosomes toward the trans-Golgi Network (TGN). A subunit of the TGN subsequently matures into MVBs (33). Most of the ESCRT complexes responsible for the formation and release of intraluminal structures within MVBs are highly conserved in plants and perform analogous functions. Despite a few distinctions, such as the absence of ESCRT-0 and the presence of TOL proteins along with the unique FREE1 protein, the mechanism of exosome formation in plants primarily occurs through the activity of ESCRT protein complexes involved in the sorting and maturation of MVBs (16,34). Plants, however, also employ a distinct pathway for exosome production that involves double-membrane autophagosome-like structures termed EXPOs (EXocyst-positive organelles). Although EXPOs share certain similarities with autophagosomes, they do not follow the typical endocytic pathway, nor do they fuse with lytic compartments. Instead, they fuse directly with the plasma membrane, releasing single-membrane vesicles into the cell wall. These vesicles are considered exosomes generated via the EXPO pathway, distinguishing them from animal exosomes, which are derived exclusively from MVBs (35). Plant exosomes can be isolated from a diverse range of sources, including roots, leaves, fruits, and seeds.

Modified exosomes

Researchers have made significant efforts to integrate exosomes into clinical practice. Concurrently, the engineering of artificial exosomes is advancing rapidly, effectively replicating the functional properties of natural exosomes (36). Various strategies are employed to create modified exosomes, including biological, chemical, and physical techniques. Biological methods involve the genetic engineering of exosome-secreting cells, whereby cells are genetically modified to produce exosomes with desired characteristics (37). Chemical methods rely on the conjugation of diverse chemical substances onto the exosomal surface, enabling the targeting of specific molecules or cell types. Physical techniques, such as sonication, electroporation, extrusion, freeze-thaw cycles, cell membrane permeabilization, and hypotonic dialysis, are utilized to load specific "cargo" into the exosomes (38). The genetic engineering of exosomes, aimed at regulating their formation, secretion, and intercellular communication, holds significant potential to enhance their efficacy and effectiveness across various applications. This approach involves the genetic modification of cells, exosome precursors, and the exosomes themselves, enabling the expression of functional molecules on the exosomal membrane or their encapsulation within the exosome (39).

Artificial exosomes

While offering numerous advantages, including biocompatibility, biological origin, and inherent functionality, natural exosomes also present significant challenges, such as low yields, costly and inefficient isolation procedures, and high structural complexity. These limitations have prompted researchers to develop artificial exosomes as a viable alternative (40). Artificial exosomes primarily include hybrid exosomes and exosome-mimics. Hybrid exosomes are formed by fusing exosomes with liposomes, resulting in a chimeric structure that leverages the benefits of both systems. In contrast, exosome-mimics constitute a distinct category of artificial exosomes developed as alternatives to their natural counterparts (41). Although chemical conjugation and exosome-liposome fusion offer innovative engineering pathways, they raise concerns regarding potential toxicity. Furthermore, the inherent variability in size, composition, and bioactivity remains a significant hurdle in achieving the standardization required for seamless clinical application of artificial exosomes (40).

COMPARATION OF EXOSOMES OF DIFFERENT ORIGIN

Considering the differences in their origin, composition, functionality, and associated risks, the comparative characteristics of natural and artificial/engineered exosomes are summarized in Table 1, facilitating a comprehensive assessment of their respective advantages and limitations within therapeutic applications.

Table 1. Comparative characteristics of natural and artificial exosomes (7, 34, 41)

Characteristics	Animal-Derived Exosomes	Plant-Derived Exosomes	Artificial / Engineered Exosomes
Particle Size	30–150 nm	50–500 nm	30–200 nm (depending on modification)
Composition	Proteins: Targeted fusion proteins; Heat shock proteins; Membrane transporters; ALIX, TSG101, CD9, CD63	Proteins: Actin; Proteolytic enzymes; Aquaporin; Reticulin heavy chain; Heat shock proteins	Proteins: Modified fusion proteins; targeting ligands; supplemental therapeutic proteins or enzymes
	Lipids: Cholesterol; Sphingomyelin; Glycosphingolipids; Ceramides	Lipids: Digalactosyl diacylglycerol; Phosphatidylethanolamine; Phosphatidic acid	Lipids: Similar to natural exosomes; optional lipid modification for stability or targeted delivery
	Nucleic Acids: mRNA, miRNAs, lncRNAs	Nucleic Acids: miRNAs	Nucleic Acids: Specific therapeutic miRNAs, siRNAs, mRNAs, or other genetic material
Origin	Mammalian cells (MSCs*, immune cells) and biofluids (plasma, milk)	Edible plants Ginger - *Zingiber officinale* Grapefruit - *Citrus* × *paradise* Grapes - *Vitis vinifera*	Synthetically engineered (lipids + proteins) or cell-derived hybrids
Functionality	Facilitates efficient delivery of hydrophobic pharmaceuticals; regenerative properties	Immunomodulatory and anti-inflammatory effects	Precise cell/tissue targeting; enhanced therapeutic efficacy

Risks	Immunogenicity; potential viral transmission	Lower risks compared to animal-derived exosomes	Potential immunogenicity based on modifications; possible adverse interactions with target tissues; challenges in standardization and stability

*MSCs – mesenchymal stem cells

THE ROLE OF EXOSOMES IN TISSUE REGENERATION AND WOUND HEALING

In recent years, the role of exosomes in the wound healing process has garnered increasing interest (42). These versatile vesicles play a key role in intercellular communication and in regulating target-cell functions by transporting proteins and nucleic acids. For tissue regeneration, exosomes offer several distinct advantages, including high stability, a low risk of immunogenicity, targeted delivery, and the potential for controlled dosage (43). Current research highlights the efficacy of exosome-based therapy across all stages of wound healing, actively accelerating the regenerative process (42).

Since they comprise proteins, nucleic acids, lipids, and growth factors, exosomes modulate pivotal cellular processes in wound healing, including inflammation, angiogenesis, cell proliferation, and extracellular matrix remodeling (44). Existing research suggests that exosomes influence the secretion of dermal fibroblasts, enhancing the synthesis and release of collagen and elastin, which in turn facilitates re-epithelialization (45-47). Furthermore, exosomes regulate inflammation and promote macrophage polarization toward the M2 phenotype. They also stimulate angiogenesis, direct cell migration and proliferation, collagen synthesis, and tissue remodeling to minimize scarring (43,48). Numerous studies have demonstrated the therapeutic potential of exosomes across various stages of wound healing. During the inflammatory phase, exosomes modulate the inflammatory microenvironment by inhibiting immune response, thereby reducing inflammation while simultaneously promoting the survival and regeneration of damaged cells (6). Possible biological pathways underlying exosome effects include immune modulation, reduction of oxidative stress, and prevention of apoptosis and necrosis in damaged cells, supporting their survival and repair. In the proliferation phase, exosomes accelerate wound closure by activating endothelial cells and fibroblasts, thus initiating angiogenesis and extracellular matrix (ECM) deposition (6,50). Angiogenesis is vital to the wound-healing process, as it facilitates nutrient transport, maintains oxygen homeostasis, and supports tissue regeneration. This complex process involves vascular endothelial cells and various angiogenesis-related factors (51,52). Exosomes are recognized as potent enhancers of endothelial cell proliferation and migration, as well as angiogenesis, via multiple signaling pathways, significantly improving local vascular regeneration of damaged areas (53). Finally, during the remodeling phase, exosomes balance the ratio between matrix metalloproteinases and their inhibitors, leading to optimized wound resolution (6,52).

The comparative characteristics of key natural and artificial exosomes investigated for use in wound healing purposes are summarized in Table 2.

Table 2. Selected overview of research on the role of exosomes in tissue regeneration and wound healing, categorized by exosomal origin

ANIMAL-DERIVED EXOSOMES			
Characteristics	Contribute to wound healing by modulating inflammation and oxidative stress, stimulating cell proliferation and migration, promoting angiogenesis, and orchestrating extracellular matrix remodeling. Furthermore, they serve as intrinsic delivery systems for the targeted transport of bioactive molecules.		[53-55]
Examples and key findings	Deer Antler Stem Cell Exosomes	Accelerated wound healing and promotion of the regeneration of skin structures (facilitating the formation of collagen in a "basket-weave" orientation) by inhibiting the fibroblast transition into myofibroblast.	[56]
	Rat Mesenchymal Stromal Cell Exosomes	Accelerated wound healing achieved through combined effects on cell proliferation, migration, angiogenesis, and extracellular matrix remodeling.	[57]
	Rat Hair Follicle Stem Cell Exosomes	Accelerated wound healing mediated by bioactive molecules involved in cell migration and proliferation, as well as extracellular matrix remodeling	[58]
	Rat and Mouse Adipose-Derived Stem Cell Exosomes	Enhanced angiogenesis, epithelialization, and collagen deposition	[59, 60]
PLANT-DERIVED EXOSOMES			
Characteristics	Due to their unique structural properties, they can be efficiently internalized by cells, enabling their specific biochemical cargo to be delivered to target cells. They are inherently biocompatible and non-toxic, posing a minimal risk of immunogenic reactions, and offer a cost-effective and sustainable production platform compared to animal-derived or artificial alternatives The molecular components of plant exosomes, including proteins, lipids, and nucleic acids, can stimulate cell proliferation, migration, and differentiation, supporting the formation and repair of new tissue, ultimately accelerating the		[61-64]

	healing of chronic wounds.		
Examples and key findings	Common wheat - *Triticum aestivum*	Promoted wound healing by enhancing angiogenesis, epithelialization, and collagen deposition.	[65]
	Grapefruit – *Citrus × paradise*		[66]
	Goldenberry – *Physalis peruviana*		[67]
	Aloe Vera – *Aloe vera*		[68]
	Tomato – *Solanum lycopersicum*		[69]
	Indian mulberry - Morinda officinalis		[70]
ARTIFICIAL EXOSOMES			
Characteristics	Artificial exosomes enable the controlled and targeted delivery of bioactive molecules while promoting dermal fibroblast proliferation and migration, angiogenesis, and collagen organization, while reducing immunogenicity. A key advantage is their compatibility with various biomaterials, making them an effective and safe approach to advanced tissue regeneration and wound healing.		[71, 72]
Examples and key findings	Human Umbilical Mesenchymal Stem Cell Exosome Mimetics	Promotion of wound healing by stimulating the proliferation and migration of dermal fibroblasts.	[73]
	MiR146a-loaded Engineered Exosomes Released from Silk Fibroin Patch	Enhanced healing outcomes achieved through miRNA loading, which actively modulates inflammatory pathways and promotes tissue regeneration.	[74]
	Neutrophil-Derived Exosome Mimetics	Improved wound repair utilizing exosome mimetic-hydrogel hybrids	[75]

Despite functional variations arising from their diverse cellular origins, exosomes exert similar therapeutic effects on wound repair. Plant-derived exosomes are characterized by their inherent biocompatibility, non-toxicity, and minimal immunogenic risk. Animal-derived exosomes, such as those sourced from mesenchymal stem cells, demonstrate significant efficacy in accelerating wound healing by acting as cellular messengers that promote tissue repair and regeneration. Concurrently, engineered and artificial exosomes facilitate the targeted delivery of bioactive molecules, offering enhanced stability, precise cargo control, and the ability to integrate with biomaterials, thereby amplifying their therapeutic impact (42). Nevertheless, the clinical translation of exosomes, regardless of their origin, remains in its infancy. Critical challenges must be addressed before widespread implementation, including low yields, costly and time-consuming manufacturing processes, and rigorous quality control requirements that necessitate extensive clinical validation (36).

CONCLUSION

Exosomes exhibit remarkable heterogeneity in their origin and physicochemical properties, positioning them as great candidates for a wide range of applications in regenerative medicine. Both natural and artificial exosomes exhibit promising effects in wound healing by modulating various physiological processes, including the regulation of inflammation, induction of angiogenesis, stimulation of cellular proliferation and migration, synthesis of the extracellular matrix, and the reduction of scar formation. Despite the existence of substantial experimental and preclinical evidence of the efficacy of exosomes, further research is essential to precisely quantify their therapeutic potential, optimize production and isolation strategies, standardize protocols, and test their safety and efficacy in clinical settings. In the future, the development of scalable, stable, and functionally optimized exosome-based therapies could provide a perspective strategy for enhancing tissue regeneration and the overall quality of wound healing.

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