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Review article / Обзорная статья

Exosomes in aesthetic medicine and dermatology: A review and clinical experience

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Abstract

Exosomes are microscopic particles secreted by various plant and animal cells, including human cells. They carry valuable biologically active substances such as proteins, lipids, nucleic acids, and metabolites that exert beneficial effects on various structures and tissues. In aesthetic medicine and dermatology, exosomes have attracted attention due to their ability to enhance collagen synthesis, eliminate inflammation, and improve the skin's protection against harmful external factors. To date, considerable clinical experience has been gained in the use of exosome products in the treatment of skin diseases such as rosacea, acne, pigmentation disorders, and alopecia, as well as for aesthetic correction and skin rejuvenation. When using exosomes, it is important to have an understanding of the origin of a particular product, including the manufacturer and the production and analysis standards used, due to the lack of international standardization in this area. For the most effective implementation of exosome-based treatments, adherence to official directions for use is critical. With no unified application protocols available, the evaluation of specialists' practical experience in this area is particularly valuable. This article presents a general overview of exosomes, discusses their production and analysis on an industrial scale, and describes the current state of their application in clinical practice. In addition, the experience of exosomal therapy in dermatology and aesthetic medicine is presented.

Keywords: extracellular vesicles, small extracellular vesicles, exosomes, mesenchymal stem cell-derived exosomes, plant-derived exosomes, mesenchymal stem cells, adipose tissue, intercellular interaction

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Экзосомы в эстетической медицине и дерматологии: обзор и опыт клинического применения

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Резюме

Экзосомы представляют собой микроскопические частицы, секретируемые различными клетками растений и животных, в том числе человека. В их составе имеются ценные биологически активные вещества, такие как белки, липиды, нуклеиновые кислоты и метаболиты, благодаря которым они могут оказывать положительное воздействие на различные структуры и ткани. В области эстетической медицины и дерматологии экзосомы привлекли к себе внимание благодаря своей способности усиливать синтез коллагена, устранять воспаление и улучшать защиту кожи от внешних неблагоприятных факторов. К настоящему времени накоплен достаточно большой опыт применения продуктов с экзосомами в лечении кожных заболеваний, таких как розацеа, акне, нарушения пигментации, алопеция, а также для устранения косметических дефектов

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и омоложения кожи. При практическом применении экзосом важно понимание происхождения конкретного продукта, включая производителя и используемые им стандарты производства и анализа, что связано с отсутствием международной стандартизации в этой области. Для достижения максимальной эффективности применения экзосом необходимо следовать официальным инструкциям. В условиях отсутствия унифицированных протоколов применения особую значимость приобретает изучение и анализ практического опыта специалистов в данной области. В статье представлены фундаментальные характеристики экзосом, рассмотрены методы их получения и анализа в промышленном масштабе, а также описано текущее состояние их применения в клинической практике. Помимо этого, представлен опыт применения экзосомальной терапии в дерматологии и эстетической медицине.

Ключевые слова: внеклеточные везикулы, малые внеклеточные везикулы, экзосомы, экзосомы из мезенхимальных стволовых клеток, растительные экзосомы, мезенхимальные стволовые клетки, жировая ткань, межклеточное взаимодействие

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INTRODUCTION

Extracellular vesicles (EVs) were first described in the 1940s, when experiments by Chargaff and West demonstrated the ability of platelet-free plasma to promote coagulation due to the presence of particulate components [1]. The vesicular nature of these particles was later confirmed using electron microscopy [2]. Subsequently, EVs were identified in various biological fluids, tissues, and structures, including cartilage matrix [3]; however, they were largely regarded as cellular artifacts [4]. In 1983, Pan and Johnstone demonstrated that maturing mammalian reticulocytes release EVs containing transferrin receptors that are no longer required by the cell [5]. Based on this observation, EVs were initially considered cellular waste disposal vesicles, involved in the elimination of unwanted cellular components. In the 2000s, with advances in analytical technologies, it was established that EVs contain a wide range of substances, including proteins, lipids, metabolites, and nucleic acids [6, 7]. This led to a paradigm shift in understanding their biological role, and EVs came to be recognized as key mediators of intercellular communication and vehicles for horizontal transfer of genetic material. Since then, research into EVs has expanded rapidly, driven by their potential applications in the development of novel diagnostic biomarkers, therapeutic agents, drug delivery systems, and anticancer vaccines. Various EV-based products are currently undergoing different phases of clinical trials, and some have already entered clinical practice.

EXTRACELLULAR VESICLES

EVs are nanosized, membrane-bound spherical structures delimited by a lipid bilayer, released by various cell types into the extracellular space and lacking the capacity to replicate on their own [8]. The EV population is heterogeneous and comprises several subgroups, among which the most extensively studied are the so-called classical EVs: exosomes, microvesicles (also termed ectosomes), and apoptotic bodies [9]. Initially, this classification of EVs was based on vesicle size and biogenesis pathways. Exosomes are the smallest EVs (50–200 nm) [10] and, in eukaryotic cells, are formed via the endosomal pathway. Ectosomes are larger (100–1000 nm) and are generated by outward budding of the plasma membrane followed by vesicle release. Apoptotic bodies vary widely in size (50–5,000 nm) and represent fragments of dying cells (*Fig. 1*) [11]. However, due to current technical limitations in EV isolation and characterization, the International Society for Extracellular Vesicles (ISEV) recommends classifying these particles based on size into small EVs (sEVs, <200 nm in diameter) and large EVs (>200 nm in diameter) [8].

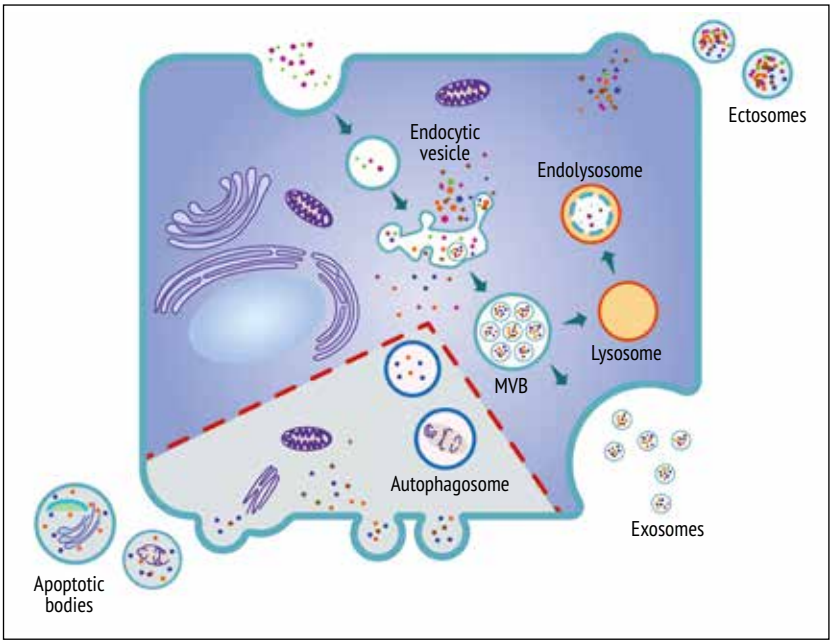
In addition to size and biogenesis, EVs differ in the conditions under which they are released and in their cell of origin. EV release into the extracellular space may be constitutive or induced following cellular activation, as well as in response to various stress factors, including hypoxia, oxidative stress, apoptosis, and senescence [12]. EVs can originate from human, animal, plant, and even bacterial cells, as they represent a universal mechanism of intercellular communication across all domains of life [13]. These differences determine the composition, biological properties, and functions of EVs. Among them, exosomes are currently of particular interest in aesthetic medicine and dermatology.

EXOSOMES

Exosomes are a subgroup of EVs that, based on their size (50–200 nm), are generally classified as sEVs. They are released by virtually all cell types and have been identified in a wide range of biological fluids, including blood plasma, saliva, cerebrospinal fluid, amniotic fluid, seminal fluid, urine, breast milk, and bile [14, 15]. From a biogenetic perspective, exosomes originate from the endosomal system and are formed through a multistep process. Initially, invagination of the plasma membrane leads to the formation of vesicles containing cytoplasmic

material, which subsequently develop into early endosomes. Following partial recycling of membrane proteins back to the plasma membrane, inward budding of the endosomal membrane and molecular sorting processes give rise to intraluminal vesicles (ILVs) containing various biomolecules (the contents of EVs are commonly referred to as cargo) [16]. This process results in the formation of multivesicular bodies (MVBs), which can interact with intracellular organelles, including the Golgi apparatus, endoplasmic reticulum, mitochondria, and phagosomes, thereby modulating the molecular composition of ILVs [17–19]. Upon maturation, MVBs undergo one of two processes: they either fuse with lysosomes, leading to degradation of their contents, or f use with the plasma membrane, releasing ILVs into the extracellular space. Following their release, ILVs are referred to as exosomes [20] (*Fig. 1*).

Figure 1. Biogenesis of three major subclasses of extracellular vesicles
Рисунок 1. Биогенез трех основных подгрупп внеклеточных везикул



MVB, multivesicular body.

Following release, a portion of exosomes is cleared by macrophages, whereas the remaining exosomes may act on the parent cell (autocrine signaling), neighboring cells within the same tissue (paracrine signaling), or enter the circulation and exert systemic effects (endocrine signaling). Importantly, exosomes have been shown to cross biological barriers, including the blood–brain barrier [20]. Exosomes interact with target cells through several mechanisms: 1) direct binding to receptors on the plasma membrane via surface proteins (e.g., tetraspanins); 2) fusion with the plasma membrane; or 3) internalization via endocytosis. Exosome–target cell interaction occurs via two main pathways. In the case of direct fusion with the plasma membrane of target cells, exosomal cargo is released into the cytoplasm. Following endocytosis, exosomes enter the endosomal system but may avoid degradation and still deliver their cargo into the cytoplasm of the target cell [21].

A critical determinant of exosome–target cell interaction is the composition of the exosomal lipid bilayer, which governs targeting and cellular uptake. Key membrane-associated proteins include tetraspanins (e.g., CD9, CD63, CD81), cell adhesion molecules (e.g., integrins), signaling proteins (e.g., GTPases), and antigen-presenting molecules (major histocompatibility complex, MHC class I and II) [22].

In addition, the exosomal lipid bilayer contains various glycoproteins and lipids, including cholesterol, ceramides, and sphingomyelin, which influence cargo sorting, vesicle release, structural integrity, and signal transduction [23]. Another important function of the exosomal membrane is the protection of luminal cargo from degradation in the extracellular environment, as the biological effects of exosomes are largely determined by their cargo. The intraluminal content of exosomes typically includes heat shock proteins, cytoskeletal proteins, components of the endosomal sorting complex required for transport (ESCRT), which are involved in ILV formation and cargo loading, growth factors (e.g., transforming growth factor beta, TGF-β), cytokines (e.g., tumor necrosis factor alpha, TNF-α), and nucleic messenger RNA (mRNA) and small acids, including oncoding RNAs (e.g., microRNAs) [22,24] (Fig. 2). It should be noted that some components are common to most exosomes, whereas others may vary substantially depending on their cellular origin.

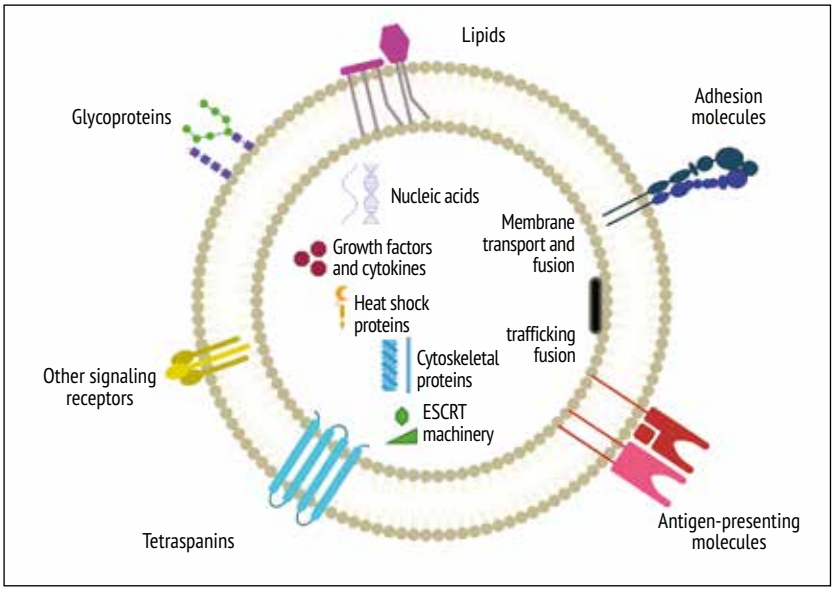
A key factor determining the uniqueness of exosome composition, and consequently their biological effects, is their source, i.e., the cell of origin. In their work, O. Janouskova et al. [25] propose the following classification of exosome sources: conventional (exosomes derived from humans and other mammals) and nonconventional (exosomes derived from other sources, including non-mammalian animals, microorganisms, and plants).

Exosomes can be isolated from biological fluids (e.g., blood plasma, milk, plant sap) or from cell culture systems. Currently, a wide range of cell types are being actively investigated as sources of exosomes, including human mesenchymal stem cells (MSCs), immune cells, neural stem cells, fibroblasts, human umbilical vein endothelial cells, Schwann cells, and others.

Mesenchymal stem cell–derived exosomes

In dermatology and aesthetic medicine, particular interest has been directed toward exosomes derived from human MSCs (MSC-derived exosomes). MSCs are multipotent stromal cells capable of self-renewal and differentiation into various specialized cell types, including osteoblasts, chondrocytes, myocytes, adipocytes, and connective tissue cells such as dermal fibroblasts [26], which contribute to the formation of corresponding tissues and their

Figure 2. Composition of exosomes
Рисунок 2. Состав экзосом



ESCRT, endosomal sorting complex required for transport.

components (e.g., bone, cartilage, collagen). Currently, MSCs are considered a promising option for cell-based therapy, including in the treatment of various skin conditions; however, their use is associated with certain limitations and concerns, including immunobiological risks and potential risk of tumorigenicity. In this context, MSC-derived exosomes may offer an opportunity to achieve some of the beneficial effects of cell-based therapy, potentially avoiding these limitations, through the delivery of bioactive molecules to target cells, with relatively low immunogenicity and a favorable safety profile [22, 27].

Numerous studies suggest that exosomes released by MSCs possess wound-healing, immunomodulatory, anti-inflammatory, and regenerative properties [28–30]. In addition, they may enhance fibroblast proliferation and migration, increase the production of type I and III collagen, and stimulate fibroblast secretion of extracellular matrix proteins and matrix metalloproteinases (MMPs) involved in tissue remodeling, as well as promote epithelialization [31]. These properties make MSC-derived exosomes a promising tool for therapeutic applications and skin rejuvenation. MSCs can be isolated from adipose tissue, umbilical cord tissue, placenta, bone marrow, and other sources. In this field, adipose tissue is considered one of the most promising and practical sources of MSCs and exosomes. This is due both to the relative ease of cell isolation and to the composition and biological properties of the exosomes derived from these cells. Adipose tissue is abundant, readily accessible, and contains a high number of stem cells [32]. In culture, adipose-derived MSCs proliferate more rapidly, exhibit stronger antiapoptotic potential, and produce larger amounts of exosomes [33, 34]. Exosomes derived from adipose tissue (commonly referred to as ADSC-Exos) in relatively more lipids, which may contribute to enhanced paracrine signaling activity. They may support tissue repair through more efficient interaction and fusion with target cells [35]. Adipose-derived exosomes contain anti-inflammatory cytokines, growth factors, and microRNAs, which may modulate the immune microenvironment, reduce inflammation, and stimulate angiogenesis and adipogenesis [36]. In addition, antifibrotic effects have been reported, including a reduction in scar formation [37]. ADSC-Exos may also support the development of hair follicles and sebaceous glands, promote collagen synthesis and organization, enhance tissue regeneration, and reduce hyperpigmentation [38, 39].

Plant-derived exosomes

Plants belong to the Eukaryota domain; therefore, their cells are capable of producing exosome-like vesicles referred to as plant-derived exosome-like nanoparticles (PELNs). PELNs share similarities with mammalian exosomes in terms of size, morphology, density, and molecular composition; however, they also exhibit distinct features related to their biogenesis and cargo [40]. Three main pathways of biogenesis have been described: 1) formation via MVBs; 2) formation via exocyst-positive organelles (EXPO), which are structurally similar to autophagosomes; and 3) formation via vacuolar pathways [40, 41]. The MVB-dependent pathway is considered the primary mechanism and is largely analogous to exosome biogenesis in mammalian cells, including humans [42].

PELNs contain three major classes of biomolecules: lipids, proteins, and nucleic acids [7]. Their membrane consists of a lipid bilayer rich in phospholipids and, in contrast to mammalian exosomes, lacks cholesterol [43]. Phospholipids contribute to vesicle stability and interactions with target cells and may be associated with antioxidant and anti-inflammatory effects [44]. Proteins within PELNs are generally less abundant and less diverse, primarily belonging to two groups: transmembrane and membrane-associated proteins [45]. Nucleic acids include mRNA, microRNAs, and other noncoding RNAs [43]. In addition, PELNs contain various small molecules and metabolites, the composition of which depends on the plant source. For example, ginger-derived exosome-like nanoparticles (GDENs) contain 6-gingerol and 6-shogaol, compounds with reported anti-inflammatory, antioxidant, and antitumor properties [46, 47]. Grapefruit-derived vesicles contain naringenin, which has demonstrated antineoplastic activity [48], whereas aloe vera–derived vesicles contain aloesin and β-sitosterol, known for their antioxidant properties [49].

The medical relevance of PELNs lies in their ability to interact not only with plant cells but also with cells from other biological kingdoms, including humans [50]. This phenomenon is referred to as cross-kingdom

communication. In aesthetic medicine and dermatology, PELNs demonstrate multifactorial effects. At the cellular level, they may regulate the functional activity of skin cells by reducing inflammation, promoting fibroblast proliferation, enhancing neocollagenesis, and modulating melanogenesis [51]. At the tissue level, they have been associated with hair regrowth [52], reduction in sebaceous gland activity and duct size, decreased wrinkle severity, increased dermal density, and improved skin hydration [53]. In addition, reparative effects have been reported, including accelerated wound healing [54]. PELNs are also characterized by favorable properties such as biocompatibility, low immunogenicity, and low toxicity [55]. They may offer advantages over conventional plant extracts [56].

PRODUCTION AND ANALYSIS OF EXOSOMES

The production of exosomes is a complex process that requires appropriate standardization, specialized expertise in biotechnological products, and rigorous analytical control. Currently, there are no unified international standards governing the production of exosome-based products. This is due both to the diversity of manufacturing and analytical methods and to the variability in the composition of exosomes derived from different sources. Nevertheless, a widely accepted approach is that exosome-based products are considered biological products, and their manufacturing and quality control are generally aligned with Good Manufacturing Practice (GMP) guidelines or similar regulatory frameworks. Based on these principles, standard operating procedures (SOPs) are developed within biotechnological manufacturing facilities.

An important aspect is the evaluation of the effects of exosomes on physiological functions [57]. Critical stages of exosome production include source selection, isolation and purification methods, quality control based on exosome characteristics, assessment of product purity, evaluation of stability, and determination of storage conditions. An example of the standardization of exosome production incorporating these aspects will be presented below, based on practices in South Korea.

Standardization of exosome production in South Korea: the example of Creabello EXO

South Korea has been one of the first countries to initiate the production of exosome-based products intended for use in aesthetic medicine and dermatology. The national regulatory authority in this field is the Ministry of Food and Drug Safety (MFDS), which considers such products as biological products. In the Republic of Korea, guidance has been developed by the Korean Society for Extracellular Vesicles (KSEV). This guidance provides recommendations on the conduct of nonclinical and clinical studies of EVs, as well as their quality control. The primary objective of this guidance is to support the development of effective and safe EV-based products. In December 2018, the National Institute of Food and Drug Safety Evaluation issued the Guideline on Quality, Nonclinical, and Clinical Evaluation of Extracellular Vesicle Preparations [57]. The extensive experience accumulated in South Korea in the production and clinical application of exosome-based products is increasingly being adopted by the international community. However, it should be emphasized that, at present, these products are classified exclusively as cosmetic products and do not have the status of medicinal products.

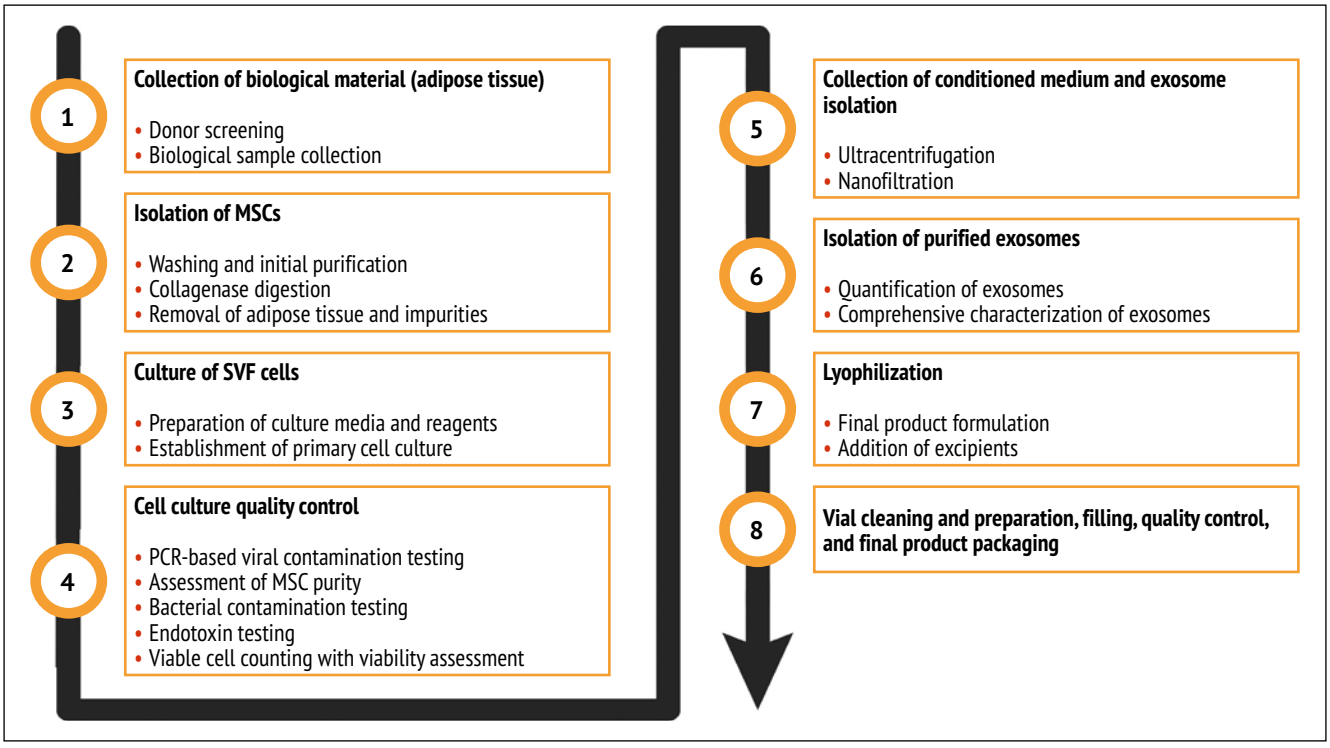
As an example of a standardized high-technology manufacturing process based on the principles described above, the production of the product Creabello EXO in South Korea is considered. This product is manufactured in dedicated facilities equipped with HEPA filtration, where ultraclean conditions are maintained (*Fig. 3*)[1].

At *the first stage*, biological material (adipose tissue) is collected in a clinical setting from young, clinically healthy female donors, taking into account data suggesting that exosomes derived from older donors may exhibit reduced efficacy [58]. Immediately prior to the procedure, donors undergo screening for infectious agents (including hepatitis B and C viruses, human immunodeficiency virus, human T-lymphotropic virus, cytomegalovirus, and pathogens causing syphilis, chlamydial infection, and gonorrhea). Following sample collection, data on the donor, the time and method of the procedure, as well as quantitative characteristics of the biological material, are recorded. These measures help minimize the risk of contamination and support the safety of the exosomes. At *the second stage*, MSCs are isolated. This involves washing and initial purification of adipose tissue followed by collagenase digestion. This process enables efficient removal of adipose components and impurities, resulting in the formation of the stromal vascular fraction (SVF). The obtained material undergoes rigorous multistep quality control. At *the third stage*, a primary culture of SVF-derived cells is established, followed by eight cycles of subculturing in a specialized culture medium, with additional purification performed after each passage. At *the fourth stage*, quality control of the cell culture is conducted, including PCR-based testing for viral contamination, assessment of MSC purity, monitoring of bacterial contamination, endotoxin testing (Limulus amebocyte lysate [LAL] assay), and viable cell counting with viability assessment.

If any deviations are detected, the cell culture is discarded, and the donor is excluded from further use in the manufacturing process. At *the fifth stage*, the conditioned medium is separated from the cells to obtain the supernatant, followed by exosome isolation. Ultracentrifugation, considered the gold standard for exosome isolation [59], is employed to avoid the use of additional chemical reagents that may contaminate the product. Ultracentrifugation is performed in four sequential steps, enabling the isolation of exosomes purified from cells, cellular debris, large vesicles, and non-exosomal vesicles larger than 200 nm. Nanofiltration is subsequently performed as an additional purification step. At *the sixth stage*, exosomes are further purified and concentrated

[1] Internal data of LOTOS UNITED.

Figure 3. Creabello EXO manufacturing process
Рисунок 3. Процесс производства Creabello EXO



MSCs, mesenchymal stem cells; SVF, stromal vascular fraction; PCR, polymerase chain reaction.

using tangential flow filtration (TFF), which facilitates the removal of smaller molecules and impurities. At this stage, comprehensive characterization of exosomes is also performed, including determination of vesicle concentration and size, assessment of purity, identification of specific markers/proteins using Western blotting, detection of contaminants, and visualization of vesicle morphology by transmission electron microscopy. At *the seventh stage*, MSC-derived exosomes are lyophilized, followed by the addition of excipients (including PELNs) and sterilization. PELNs are produced in a separate manufacturing unit from purified plant cell cultures using a standardized production process established by the manufacturer. At *the eighth stage*, vials are cleaned and prepared, followed by filling, quality control, and packaging of the final product. Upon completion of the eighth stage, a ready-to-use product is obtained, the composition and characteristics of which are described in the following section.

Composition and characteristics of Creabello EXO

Creabello EXO is supplied in two vials. One vial (EXO, 100.0 mg) contains the exosome-based product in the form of a lyophilized powder, whereas the second vial (EXO MIXER, 5.0 mL) contains a solvent intended to be mixed with the main product prior to use.

The lyophilized EXO powder (100.0 mg) contains conditioned medium from human adipose tissue-derived MSCs, MSC-derived exosomes, EVs from Centella asiatica, EVs from Aloe vera, mannitol, trehalose, and methionine. *The conditioned medium derived from human adipose MSCs* has undergone a full set of preclinical evaluations supporting its safety for medical use, including the absence of toxic, mutagenic, and irritant effects. It contains bioactive components, growth factors, and cytokines that may support skin regeneration and repair[2]. As noted above, *exosomes derived from human adipose MSCs* have been associated with various beneficial biological effects (see the section Mesenchymal Stem Cell–Derived Exosomes), including support of tissue architecture restoration and modulation of immune responses [60]. *EVs derived from Centella asiatica* exhibit anti-inflammatory properties, may stimulate collagen and hyaluronic acid synthesis, and have been associated with enhanced wound healing [53]. *EVs derived from Aloe vera* provide moisturizing and soothing effects on the skin, exhibit antioxidant activity, and contain bioactive components that may contribute to epidermal restoration [54, 61, 62]. *Mannitol* exerts osmoprotective effects and contributes to the formation of a moisture-retaining barrier in the stratum corneum [63], whereas *trehalose* forms a protective matrix that helps reduce transepidermal water loss [64]. *Methionine* is an essential sulfur-containing amino acid involved in phospholipid synthesis in cell membranes and contributes to the maintenance of epidermal barrier integrity.

The EXO MIXER solvent (5.0 mL) contains water and additional components (including peptides, panthenol, and others) that may complement the activity of exosomes and contribute to collagen synthesis, tissue regeneration, and reduction of inflammatory responses. Further details on the composition of the solvent are provided in the Creabello EXO instructions for use.

[2] Internal data of LOTOS UNITED.

After mixing the lyophilized EXO powder (100.0 mg) with the EXO MIXER solvent (5.0 mL), a suspension of Creabello EXO is obtained with the following characteristics: a sterile, clear, odorless liquid containing exosomes at a concentration of 33 billion per mL[3].

Analysis of the composition and effects of Creabello EXO

To verify the morphological and structural characteristics of exosomes in Creabello EXO, several studies have been conducted in Russia[4]. At the National Research University "Moscow Power Engineering Institute", scanning electron microscopy (SEM) was used to analyze samples of both the lyophilizate and the suspension of Creabello EXO. The obtained images demonstrated a uniform distribution of particles with a regular spherical shape within a consistent size range (*Fig. 4A–C*). At the Bach Institute of Biochemistry, transmission electron microscopy (TEM) was used to examine the Creabello EXO suspension. The analysis indicated the presence of structures with a typical spherical morphology measuring 50–200 nm in diameter (*Fig. 4D–F*). At the Belozersky Institute of Physico-Chemical Biology, TEM analysis of both the lyophilizate and suspension revealed structures with a characteristic cup-shaped morphology in the lyophilizate and typical spherical vesicles with a visible lumen in the suspension (*Fig. 4G–I*). These findings support the presence of exosomes in Creabello EXO and their expected morphological characteristics [65].

To evaluate the effects of Creabello EXO on skin structures, several studies have been conducted in South Korea[5]. The following observations were reported:

1. Exosomes were observed to penetrate into the deeper layers of the dermis in substantial amounts, which may be associated with their small size, lipid bilayer structure, and high affinity for cellular membranes.

2. Within the dermis, more than 82% of exosomes were reported to interact with fibroblasts, which are key cells involved in in maintaining skin homeostasis and supporting regeneration.

3. Exposure to exosomes was associated with increased synthesis of type I collagen, elastin, and fibronectin, as well as with enhanced fibroblast proliferation and cytoskeletal organization.

4. Exosome activity was associated with increased levels of filaggrin, which may contribute to restoration of the skin barrier function and improvement in overall skin condition.

5. Exosomes were reported to promote proliferation of dermal papilla cells of hair follicles, potentially prolonging the active growth phase (anagen) and reducing the resting phases (catagen and telogen). They were also associated with an increase in the number of functional hair follicles and with stimulation of hair growth with improved follicular anchoring.

[3] Ibid.
[4] Unpublished data. Studies were conducted within the framework of the Creabello EXO research program in the Russian Federation.
[5] Internal data of LOTOS UNITED.

CLINICAL APPLICATIONS OF EXOSOMES

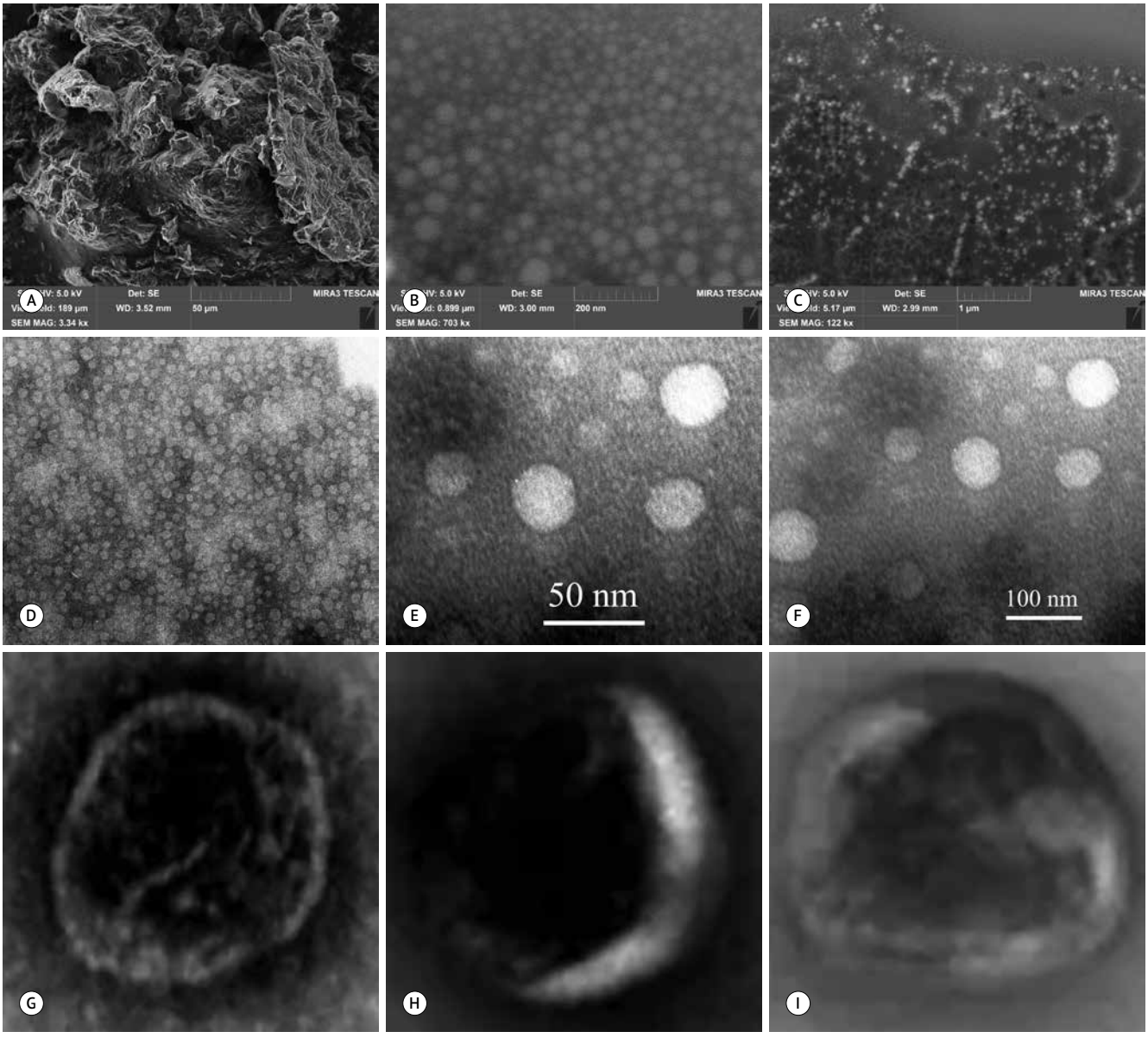
Exosomes have considerable therapeutic potential in the treatment of skin disorders, wound healing, and correction of age-related changes due to their ability to mediate intercellular communication, deliver bioactive molecules to target cells, and modulate immune responses. Evidence supporting the clinical utility of exosome-based therapies has been reported in various skin conditions, including wounds and burns [66–68], atopic dermatitis [69], rosacea [70], psoriasis [71], diffuse alopecia, alopecia areata, and androgenetic alopecia [72–74], scar formation [36, 75], post-acne changes [76], hyperpigmentation [39], and chrono- and photoaging of the skin [77, 78]. More detailed mechanisms of action of exosomes in these conditions are discussed below in the context of clinical observations.

When considering the methodology of exosome-based therapy, it is important to distinguish between approaches used in dermatology and aesthetic medicine, with particular attention to the condition of the skin, which may be intact or compromised. In skin diseases and wounds, the barrier function of the skin is typically impaired, which may facilitate the penetration of exosomes into deeper skin layers and their interaction with target structures. In intact skin, exosomes may be applied topically or via transdermal approaches in combination with mechanical or device-assisted delivery methods. When applied topically to intact skin, exosomes may enter via endocytosis, micropinocytosis, and through hair follicles [79, 80], reaching the epidermis; however, the efficiency of this penetration is limited, as a substantial proportion of exosomes is retained within the stratum corneum and does not reach the dermis [81]. Therefore, in intact skin, exosome-based products are often used in combination with other techniques, such as microneedling or laser-assisted delivery, which create channels that may enhance exosome penetration [82].

A critical aspect of the use of exosome-based products, particularly those derived from human cell cultures, is the assessment of viral contamination and potential oncogenic risk. The issue of viral safety can be addressed through the application of standardized approaches that have been validated over decades in the manufacturing of biomedical products derived from human biological materials (such as blood components and their derivatives). These approaches include comprehensive donor screening with mandatory laboratory verification for viral agents, as well as the use of multistep purification and control systems, including nanofiltration, TFF, PCR-based screening, and other technological procedures. With regard to potential oncogenic risk, the following aspects should be considered:

1. Exosomes lack a nucleus and are not capable of replication, as reflected in the definition provided by the International Society for Extracellular Vesicles [8]. This implies that exosomes themselves do not undergo oncogenic transformation.

Figure 4. Structural analysis of lyophilized powder (A, G) and suspension (B-F, H, I) of exosomes by electron microscopy
Рисунок 4. Анализ структуры лиофилизированного порошка (A, G) и суспензии (B-F, H, I) экзосом методом электронной микроскопии



Experimental conditions. A, a set of analytical instruments based on the scanning electron microscope Tescan Mira LMU (Tescan, Czech Republic) was used. For analysis, liquid samples were applied onto polished silicon and, after drying, placed into the chamber of the electron microscope, where they were examined in backscattered electron (BSE) mode. The accelerating voltage and probe current during surface analysis were 5 kV and approximately 0.1–1 nA, respectively. B, transmission electron microscopy was performed using a JEM-100C microscope (JEOL, Japan) at an operating voltage of 80 kV. According to the standard protocol, 7 μL of the initial exosome suspension (or the suspension after mixing on a shaker) was applied to a copper TEM grid (200 mesh) with a diameter of 3.05 mm, containing multiple openings measuring 97 × 97 μm and coated with a Formvar support film. Negative staining was then performed using a 2% phosphotungstic acid solution. C, a JEM-1400 Flash electron microscope (JEOL, Japan) equipped with a Rio 9 digital camera (GATAN) was used. For sample preparation, copper electron microscope grids (1GC300, PELCO) coated with a collodion film and carbon layer were employed. A volume of 15 μL of the sample was applied to the grid. After incubation for 1 minute, excess liquid was removed using filter paper, followed by negative staining with a 2% uranyl acetate solution for 10 seconds, after which the excess liquid was again removed using filter paper.

2. Several studies have demonstrated that primary MSCs do not form tumors [83–85]. Accordingly, exosome-based products derived from MSCs are generally considered to have a favorable safety profile. This is supported by the study by T.T. Tan et al., which reported that MSC-derived exosomes did not promote tumor growth [86]. In this context, it is important to distinguish between reparative cell proliferation (e.g., of fibroblasts) and oncogenic transformation.

3. Blood and plasma transfusion are standard medical procedures that have been widely used since the 1940s and have saved millions of lives. Blood plasma contains exosomes at concentrations ranging from 100 million to 10 billion per mL [87], meaning that substantial amounts of exosomes are introduced into the recipient during transfusion. Notably, in a large study including more than 48,000 participants, no association was found between blood transfusion and an increased risk of malignant neoplasms [88].

CLINICAL CASES

This section presents the authors' experience with the use of the exosome-based product Creabello EXO.

Given the composition and properties of Creabello EXO, its potential areas of application, including in combination with device-based techniques, may include skin laxity, atonic skin, chronological aging, pigmentation disorders, scarring, rosacea, and dry skin. Based on the authors' experience, when applied to intact skin, the use of Creabello EXO in combination with mechanical or device-assisted techniques appears to be more effective.

Case 1. Alopecia

A 72-year-old female patient presented with complaints of hair loss in the frontoparietal region and decreased sensitivity in the same area. Three months prior to symptom onset, the patient had undergone a frontotemporal lifting procedure with dissection of the *galea aponeurotica* in the frontotemporal region. No preoperative scalp preparation aimed at strengthening hair was performed. On examination, diffuse hair loss extending to the vertex was observed, along with age-related thinning of hair in the frontoparietal region (*Fig. 5A*) and reduced cutaneous sensitivity in this area. Such complications are relatively common and predictable following this type of procedure (typically occurring within 1–1.5 months), particularly in the absence of preoperative hair strengthening.

The patient underwent a treatment course consisting of seven procedures over a total duration of 7 months. Procedure 1 included intradermal (ID) administration of polynucleotides (PDRN) at a dose of 20 mg, followed by application of Creabello EXO suspension using a dermaroller in the area of hair loss. Procedure 2 was conducted after 28 days: ID administration of PDRN (10 mg), a combined preparation of PDRN and hyaluronic acid (10 mg), followed by dermaroller-assisted application of Creabello EXO.

Procedure 3 was conducted after 30 days: ID administration of PDRN (2 mg), followed by dermaroller-assisted application of Creabello EXO. Subsequent procedures (procedures 4–7) were performed at 30-day intervals; each consisted of dermaroller-assisted application of Creabello EXO suspension. The dermaroller technique was as follows: after PDRN injections, each parting was treated with eight passes; after the 7th and 8th passes, Creabello EXO suspension was applied.

Treatment outcomes: depigmented (gray) vellus hair appeared 3 months after treatment initiation. By 5 months, increased hair density, thickening, and strengthening were observed (*Fig. 5B*, after hair coloring). These observations suggest a potential beneficial effect of Creabello EXO in the management of diffuse alopecia and support its use in combination with other injectable therapies.

Mechanism of action: during lifting procedures, tissue tension and impaired blood supply may occur. Combined with surgical stress, this may lead to a shift of a substantial proportion of hair follicles into the telogen phase, resulting in telogen effluvium [89]. Creabello EXO may contribute to stimulation of hair follicles, potentially reducing the duration of the telogen phase and supporting an increase in the number of functional follicles. It may also exert anti-inflammatory and antioxidant effects.

Case 2. Hyperpigmentation

A 52-year-old female patient presented with complaints of hyperpigmentation in the zygomatic region that developed after trauma followed by healing (*Fig. 6A*). On examination, a hyperpigmented area with an uneven

Figure 5. Alopecia areata before (A) and after (B) the treatment
Рисунок 5. Гнездная алопеция до (A) и после (B) лечения

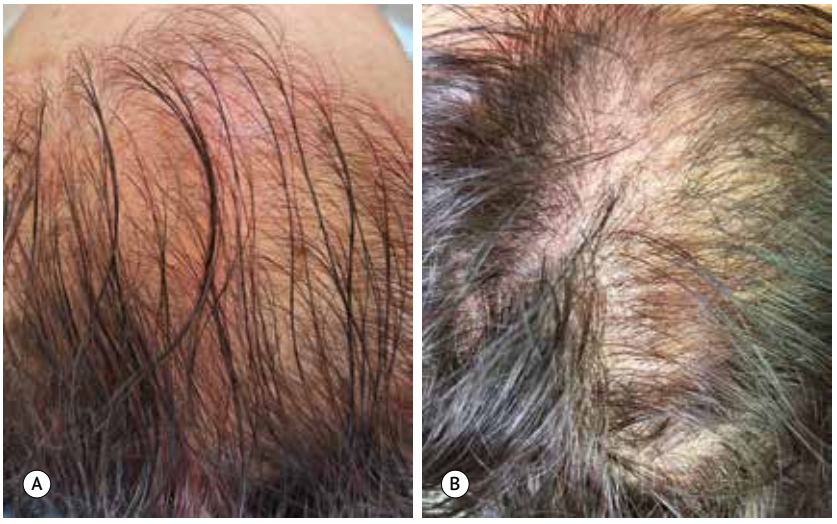
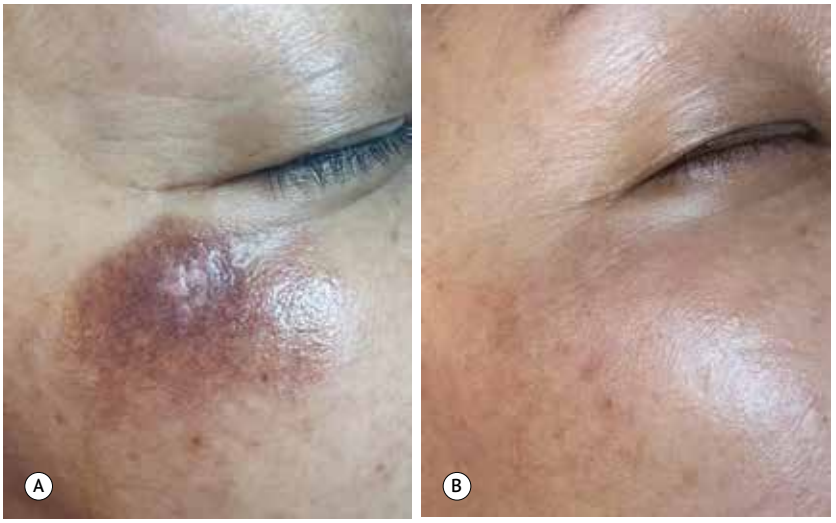


Figure 6. Hyperpigmentation in the malar area before (A) and after (B) the treatment
Рисунок 6. Гиперпигментация в скуловой области до (A) и после (B) лечения



skin surface was observed in the lateral part of the zygomatic region. A combined treatment course of 2 months was prescribed, consisting of four procedures. Within the first procedure, ablative fractional CO_2 laser resurfacing was performed in the area of hyperpigmentation, followed by topical application of Creabello EXO suspension. Procedure 2 was conducted after 1 month and included application of Creabello EXO suspension using the dry nappage technique in the hyperpigmented area. Two additional procedures of exosome-based therapy using the same technique, performed at 2-week intervals.

Treatment outcomes: complete resolution of hyperpigmentation and improvement in skin surface uniformity were observed (*Fig. 6B*). These observations suggest a potential beneficial effect of exosome-based therapy for the correction of hyperpigmentation, particularly when combined with device-based treatments.

Mechanism of action: any skin injury is associated with the development of an inflammatory response, which may be accompanied by postinflammatory hyperpigmentation. This is related to activation of melanocytes, increased melanin production, and its uneven accumulation in the epidermis, dermis, or (most commonly) both layers [90]. Exposure to exosomes has been associated with a reduction in intracellular melanin levels, which may contribute to decreased pigmentation intensity [39].

Case 3. Postsurgical scar

A 26-year-old female patient presented with a request to reduce the scar changes in the lower abdomen. The scar had formed following surgery performed 7 months prior to presentation. On examination, a moderately mature postsurgical scar was observed in the suprapubic region. The scar was linear, pink in color with areas transitioning to reddish, slightly depressed below the level of the surrounding tissues, and characterized by an uneven surface. The adjacent skin was also altered, with pale punctate marks corresponding to suture sites (*Fig. 7A*). The patient underwent treatment consisting of two procedures with Creabello EXO in combination with dermaroller application, performed at 2-week intervals.

Treatment outcomes: a reduction in the severity of the postsurgical scar was observed, including improved surface leveling, smoothing of skin texture, and normalization of color approaching that of the surrounding tissues.

The visibility of suture marks also decreased, with their color becoming closer to that of adjacent skin (*Fig. 7B*). These observations suggest a potential beneficial effect of exosome-based therapy for the correction of postsurgical scars, particularly when combined with microneedling techniques.

Mechanism of action: scar formation is a complex process involving fibroblasts and myofibroblasts, which synthesize extracellular matrix components and contribute to wound contraction, thereby supporting timely and effective re-epithelialization [91]. Studies have shown that conditioned medium derived from adipose tissue–derived MSCs may reduce excessive collagen fibers production [92] and suppress the proliferative capacity and migratory activity of fibroblasts in hypertrophic scar tissue [93]. Similarly, treatment with adipose-derived exosomes has been associated with inhibition of fibroblast proliferation and migration in hypertrophic scars, acceleration of wound healing, and reduction of excessive collagen deposition [94].

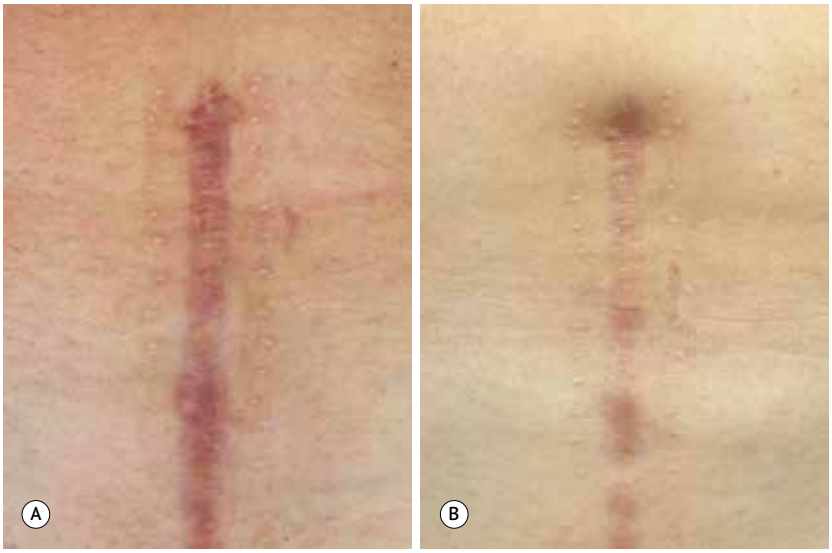
Case 4. Wrinkle correction

A 46-year-old female patient presented with complaints of wrinkles and unsatisfactory skin condition in the forehead area. On examination, the area revealed deep dynamic horizontal wrinkles, moderately expressed static horizontal wrinkles, a mild crease in the glabellar region and above the left eyebrow, as well as changes in skin texture accompanied by erythema in the glabellar region and above the eyebrows (*Fig. 8A*). The patient underwent a single procedure consisting of treatment of the forehead and glabellar region with a mesoroller, followed by application of Creabello EXO suspension.

Treatment outcomes: treatment efficacy was assessed 2 weeks after the procedure. A reduction in the severity of dynamic horizontal wrinkles to a moderate level was observed, along with near-complete resolution of static horizontal wrinkles in the forehead area. A substantial decrease in the depth and length of creases in the glabellar region and above the left eyebrow was noted, as well as improvement in skin texture. This was accompanied by resolution of erythema in the glabellar region and above the eyebrows,

Figure 7. Postoperative scar in the suprapubic area before (A) and after (B) the treatment

Рисунок 7. Послеоперационный рубец в надлобковой области до (A) и после (B) лечения



along with smoothing of the skin surface (*Fig. 8B*). These observations suggest a potential beneficial effect of exosome-based therapy for wrinkle correction and other age-related skin changes when combined with microneedling.

Mechanism of action: microneedling enables the formation of microscopic channels in the skin, facilitating more efficient delivery of exosomes through the stratum corneum into deeper skin layers. Exosomes may stimulate fibroblasts to produce collagen and elastin, which may contribute to reduced wrinkle severity and improved skin texture [95]. In addition, exosomes may modulate inflammatory responses, which may contribute to a reduction in erythema intensity [96].

Figure 8. Wrinkles in the forehead and glabellar area before (A) and after (B) the treatment

Рисунок 8. Морщины в области лба и межбровья до (A) и после (B) коррекции

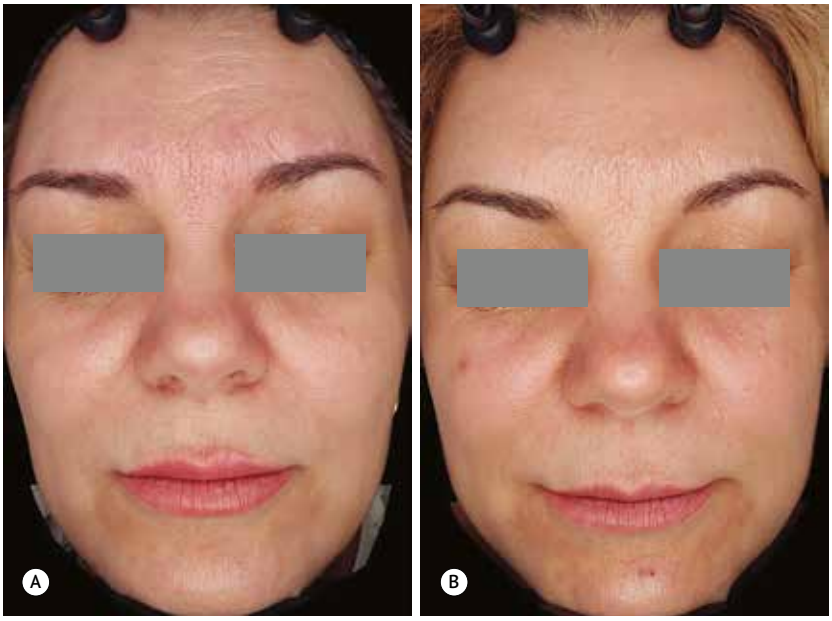
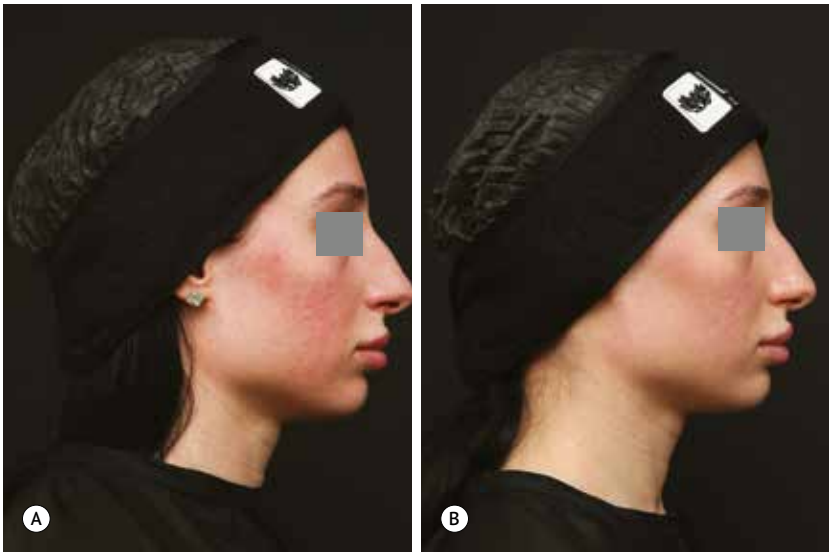


Figure 9. Rosacea before (A) and after (B) the treatment

Рисунок 9. Розацеа до (A) и после (B) лечения



CASE 5. ROSACEA

A 22-year-old female patient presented with persistent facial redness. On examination, persistent erythema ranging from bright pink to reddish was observed in the central facial region, accompanied by multiple telangiectasias on the cheeks and nasal alae, as well as mild skin edema (*Fig. 9A*). The diagnosis of rosacea, erythematotelangiectatic subtype, was established. In accordance with the diagnosis, treatment consisted of two procedures involving microneedling of the facial area with application of Creabello EXO suspension. The interval between procedures was 1 month. The patient was also advised to avoid trigger factors and to follow a gentle skincare regimen.

Treatment outcomes: treatment outcomes were assessed 2 weeks after the second procedure. A marked reduction in erythema was observed. A decrease in both the diameter and number of telangiectasias was noted, along with smoothing of the skin surface and reduction of edema to near-complete resolution (*Fig. 9B*). These observations suggest a potential beneficial effect of exosome-based therapy for the management of rosacea when combined with microneedling.

Mechanism of action: rosacea is a chronic inflammatory dermatosis characterized by facial erythema, papulopustular lesions, phymatous changes, and ocular involvement [97]. Its pathogenesis is multifactorial and includes vascular and immune dysregulation, alterations in dermal structure, impaired skin barrier function, and oxidative stress. Exosomes may act on multiple pathogenic pathways, including modulation of inflammatory responses [98], improvement of skin barrier function [99], reduction of oxidative stress [54,61,62], extracellular matrix remodeling, and potential improvement of microcirculatory disturbances [100].

CONCLUSION

Exosome-based therapy represents a promising approach in modern medicine. Accumulating clinical experience suggests potential benefits of exosomes both in the management of dermatological conditions and in aesthetic applications, including skin rejuvenation. The biological activity of exosomes is associated with their content of bioactive components, including proteins, lipids, nucleic acids, and metabolites. These components may contribute to stimulation of neocollagenesis, modulation of inflammatory processes, and support of skin barrier function.

Despite these prospects, the implementation of exosome-based therapy is associated with several limitations. Key challenges include the need for international standardization of production and analytical methods, as well as the development of application protocols based on data from randomized clinical trials.

Under current conditions, a comprehensive approach appears most appropriate, including adherence to instructions for use, evaluation of available evidence and clinical outcomes, and careful assessment of product origin, manufacturing technologies, and quality control methods. Such an approach may help optimize the safety and effectiveness of exosome-based therapies.

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