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REVIEW NON-SURGICAL AESTHETIC

Exosomes in the Real World of Medical Aesthetics: A Review

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Abstract

Background Exosomes are cell-derived nanovesicles that transport proteins, nucleic acids, and lipids and play a significant role in almost every physiological process in the human body. They have generated great interest, especially in the field of tissue regeneration. Studies in the last decade support their great regenerating and rejuvenating potential. However, the lack of standardized procedures, limited knowledge regarding their action mechanism, and little clinical evidence impair their implementation and approval in the medical setting. This review aimed to identify published studies and clinical trials using exosomes in human patients for clinical treatments in aesthetic medicine.

Materials and Methods A systematic search was conducted in the PubMed database using the search term "exosomes" and 25 terms related to aesthetic medicine treatments in human patients. Additionally, a search was conducted in the ClinicalTrials.gov database for interventional clinical trials using exosomes for aesthetic treatments in adults 18 to ≥ 65 years of age.

Results Nine articles were selected after debugging the initial list of published articles in which exosomes were related to Aesthetic Medicine (633 articles). Nine studies were identified from the initial search on ClinicalTrial.gov (104 trials with exosomes).

Conclusions There is no doubt about the scientific basis of exosome regenerative potential and the growing interest in exosomes in Aesthetic Medicine. However, companies must spend more on research to develop standardized and reliable procedures to obtain exosomes for their approval and application in clinical practice.

Level of Evidence III This journal requires that authors assign a level of evidence to each article. For a full description of these Evidence-Based Medicine ratings, please refer to the Table of Contents or the online Instructions to Authors www.springer.com/00266.

- This review highlights the large amount of published research on exosomes related to aesthetic medicine and, at the same time, the lack of products approved by regulatory agencies.
- Several issues have been suggested to elucidate a response, such as the need for standardized protocols and more knowledge to ensure safe treatments.
- It also highlights the few clinical trials conducted to evaluate exosome properties in aesthetic medicine treatments.

Keywords Exosomes · Medical aesthetics · Extracellular vesicles · Regenerative medicine · Skin · Clinical trials

Introduction

Exosomes and microvesicles are extracellular vesicles (EVs). Exosomes are cell-derived nanovesicles that transport proteins, nucleic acids, and lipids and play a significant role in almost every physiological process in the human body. Cells and platelets release exosomes that can communicate with neighboring or distant cells [1] facilitating a coordinated tissular response to virtually any stimulus. Exosomes possess unique physicochemical properties, such as low antigenicity and a great capability

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to cross tissue barriers or escape from mononuclear phagocytic cell systems [2]

Exosomes offer: (i) high long-term stability; (ii) high targeting capacity that can improve drug efficacy; (iii) high efficiency and diverse loading capacity for nucleic acids, proteins, or other small molecules; (iv) low immunogenicity; (v) the ability to effectively increase drug solubility and facilitate drug release; and (vi) the ability to achieve synergistic therapeutic results [3–5].

In the last decade, there has been growing evidence supporting their potential in regenerative medicine, as demonstrated by the increasing number of scientific articles on the subject [6]. Exogenous exosomes, including those derived from stem cells, offer innovative treatment options for repairing, regenerating, and rejuvenating skin tissues. Exosomes can be derived from bone marrow, placenta, adipose tissue, blood, umbilical cord, and many other tissues, including animal or plant sources. The plant-derived exosome-like particles advantages are that can be involved in intercellular communications, have a lipid membrane that can protect the cargo from the external agent, can pass through some of the human body barriers (blood-brain barrier and the placenta), are tolerated by the immune system, are suitability for industrial applications [7] non-toxic, and have long-term availability [8]. Animal-derived exosomes, such as animal-derived cord blood, may have the most compatible protein structure with humans and have advantages over human cord blood, which is not an easily obtainable source, and obtaining authorities' approval for research can be challenging. For this reason, obtaining exosomes from newborn animal tissue facilitates large-scale production and makes obtaining purer content possible [9]

They have great potential for skin rejuvenation, aging prevention, scarring reduction, regulating pigmentation, promoting hair growth, increasing fat graft survival, and serving as carriers for therapeutic drugs in aesthetic treatments [1, 2]. Furthermore, it has been studied if the youth of exosomes influences their effectiveness to demonstrate if the more youthful the exosome, the more effective it might be for treatment. A study found that exosomes from neonatal serum had a better effect on the wound-healing process, further underlining the importance of exosomes from young individuals in maintaining stem cell functions [10]. For example, in treating myocardial infarction, the researchers found that MSCs from younger donors were more efficacious than older donors [11]. Another study found that locally delivering the exosomes secreted by young hypothalamic stem cells slowed down the aging of mice [12].

A limited understanding restricts the procurement and implementation of allogeneic therapies, which are relegated to cosmetic products in the best-case scenario. They only benefit a small number of patients compared to the broader impact of conventional medicinal batches. Besides, regenerative medical procedures are usually manual-based and require highly skilled operators [13], determining very limited yield and high production costs. Since biological materials must be procured from and readministered to a human being, medical facilities and specialized personnel requirements do not constitute a minor issue. The significant initial expenses of implementing these therapies in a private medical consultation had to be evaluated thoroughly. Understandably, the overall costs of shifting from conventional to regenerative therapies resulted in delayed implementation of aesthetic regenerative treatments. The lack of a thorough understanding of the action mechanism and the absence of legal regulations added two more barriers that restricted the implementation of aesthetic regenerative therapies and stood between the patient interest and physician treatment eligibility.

Additional aspects must be considered when facing the utilization of exosomes in aesthetic regenerative medicine since processing procedures and techniques (grafting, isolation, purification, optimization, administration) are not fully agreed upon. It would be beneficial to develop standardized quality control procedures and a more comprehensive understanding of the molecular communication between exosomes and their target cells [14]. To date, numerous researchers and health centers have already developed their own manufacturing processes. Still, the effective transference of these achievements to the clinical field remains elusive. Therefore, further clinical trials are required for regulatory clearance since no exosome-based injectable products or therapies are, to our knowledge, currently approved [15]

This review aimed to identify published studies and clinical trials using exosomes in human patients for Aesthetic Medicine (AM) clinical treatments.

Material and Methods

A systematic search was conducted in the PubMed database using the search term "exosomes" and others related to AM. The search was performed in English. Most AM-related terms were extracted from the American Academy of Aesthetic Medicine website's list of procedures. The search terms used to associate with exosomes were "aesthetic," "dermatology," "neurotoxin injection," "dermal filler," "chemical peel," "cosmetic," "microdermabrasion," "body contouring," "cellulite treatment," "nutrition," "hair transplantation," "hair reduction," "fat grafting," "platelet-rich plasma," "laser," "intense pulsed light," "scar," "vein treatment," "cosmetic gynecology," "rejuvenation," "light-emitting diode," "cryolipolysis," "acne," "rosacea," and "wrinkle." Articles were filtered by "human" and the date of the earliest published article on exosomes in humans

in the PubMed database until the search date. Terms were adjusted according to the terminology for this search type, and articles related to cancer and infectious diseases were excluded (Supplementary Table 1).

After obtaining the isolated searches by term, they were crossed with the search for "exosomes" to identify articles containing both terms. Following the new search for identifying relevant articles, the database was manually cleaned to remove duplicates and articles with titles that did not align with the study's objective. Inclusion criteria were limited to human studies on medical aesthetic treatments involving exosomes. Animal studies, laboratory-only studies, treatments performed during surgery, or applications targeting other medical fields were excluded. Using the debugged database data, we generated a year-by-year graph of published articles and a pie chart displaying the percentage of articles published by search term. Finally, a comprehensive manual review was conducted to identify articles that described studies on human subjects and exosome treatments in AM. Additionally, we conducted a search on the ClinicalTrials.gov database, filtering for adults aged 18 to $\geq$ 65, to identify unpublished interventional clinical trials for treatment with exosomes.

Results

The search for "exosomes" yielded 16,742 articles. The search was restricted to human adults and old adults from 1986 (the date of the first article in PubMed about exosomes) to May 26, 2023 (the search date). After crossing the search of all 25 selected terms related to AM with that for exosomes, a total of 633 articles were obtained. For nine terms ("chemical peels," "microdermabrasion," "body contouring," "cellulitis treatment," "hair reduction," "intense pulsed light," "cosmetic gynecology," "cryolipolysis," and "rosacea"), no articles related with exosomes were found. After the initial database debugging by article title, which removed duplicates and articles with titles indicating that they were out of scope, the number of articles selected for final debugging was 339. Figure 1 shows the number of these articles by publication date, and Figure 2 shows the percentage of articles by search term and exosomes. Table 1 shows the initial number of articles found per search term and the number after the first debugging of the database. After the second screening, in which the main text was deeply analyzed, nine articles were selected, involving around 250 patients: one article analyzed the use of exosomes for tissue regeneration; two, for local skin inflammation in atopic dermatitis patients treated with dupilumab; one, for melasma; one, skin rejuvenation; one, for sensitive skin; one, for melanin synthesis; one, for skin brightening; and one, as adjuvant therapy after application of fractional CO2 laser for acne scars [16–24] (Table 2) (Figure 3).

The search on October 19, 2023, in the ClinicalTrials.gov database for interventional clinical trials in adults (18–64 years) and older adults (> 65 years) for treatment with exosomes yielded 104 trials in various pathologies and stages. Of the 104 trials, 99 included women, and 97 included men. Twenty-four were completed, but results were available from only four. From this initial search, nine studies were selected with treatments related to AM, one completed but without available results (Table 3).

Discussion

Our review and clinical trials search results showed that very few clinical studies have been performed to determine the potential of exosomes in AM strongly. This fact can also be translated to other medical disciplines. Few clinical trials have been carried out on skin improvement, scarring, aging, pigmentation, and hair or weight loss. Our findings indicate the need for more efforts in translational and clinical research in exosomes and AM.

However, in vitro and empirical studies in basic science showed the potential superiority of exosomes compared to stem cells in clinical therapies, mainly due to their potential lack of inherent risk [25], minimal risk of malignant transformation or replicant potential [26], or immunogenic response toward infections and cancer [25, 27], and their targeted action at the site of interest with multiple delivery routes [27]. However, there is not enough clinical data to make convincing arguments about what is better therapy. Taken together, exosomes, such as those derived from adipose-derived stem cells (ASCs), MSCs, or platelets, are attractive for next-generation products for regenerative aesthetics. They can act on the epidermis (keratinocytes), the dermis (fibroblasts, inflammatory cells, and hair follicles), and the hypodermis (subcutaneous fat), making them promising candidates for new therapies [28]. New lines of research are based on the combination of MSCs and exosome therapy to obtain a synergistic effect. Stem cells can provide a source of regenerative cells, and exosomes can deliver therapeutic cargo to target cells and modulate the immune response. Preclinical studies have shown that this combination enhances wound healing [29].

Exosome Clinical Application in AM: Current Status

Despite considerable progress in this field, therapies based on exosomes, secretomes, or extracellular vesicles have not been approved by regulatory authorities, such as the Food and Drug Administration (FDA) or the European

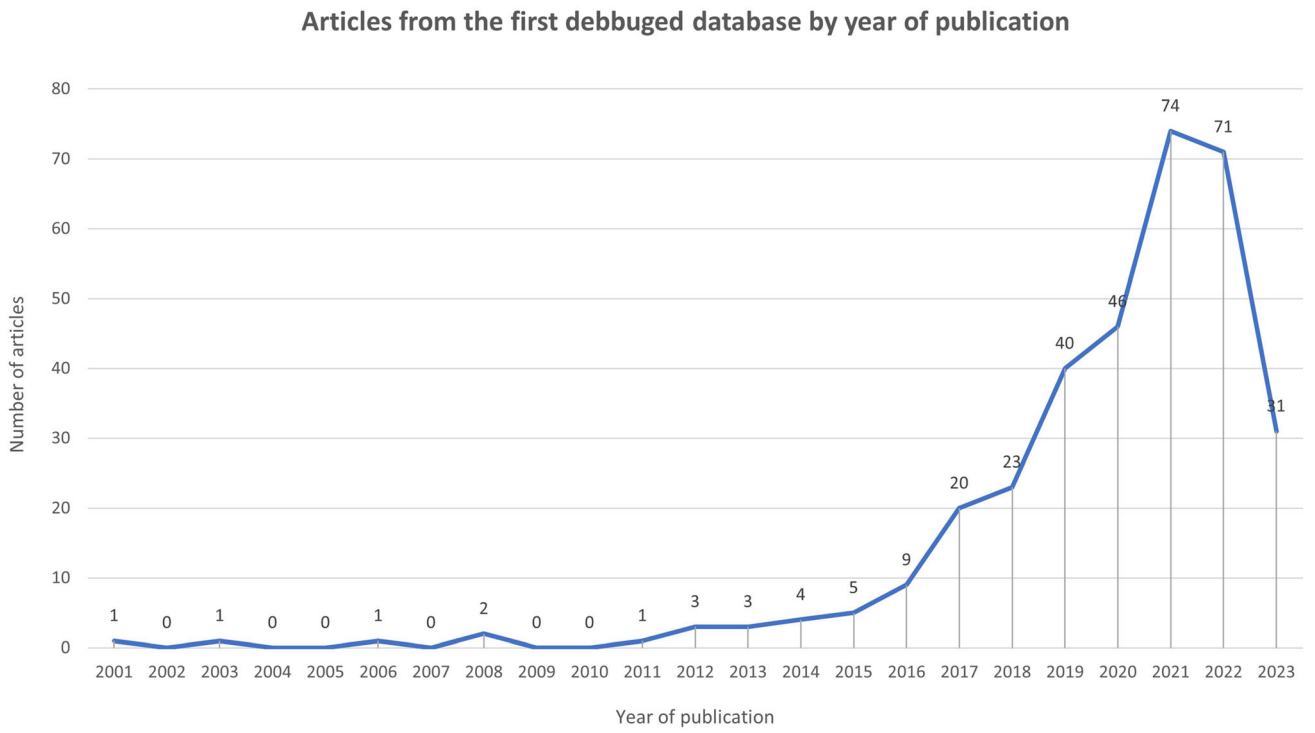

Fig. 1 Number of articles by publication date after initial debugging of the database

Medicines Agency (EMA), for use in Aesthetic Medicine. The reasons are multifactorial and include, as previously mentioned, a lack of standardization techniques and protocols for the isolation, purification, and optimization of exosomes, limitations in knowledge, regulatory challenges, and scalability problems.

Regulation, Legal Framework, and Gray Zone

Any new therapy or drug requires developing a standardized process for validation. Therapies that use exosomes are in the investigational new drug (IND) development phase and require approval from regulatory agencies before starting the clinical trial [30]. The FDA classifies all exosomes as Biological Products Filed Under Section 351 and requires that studies with exosomes demonstrate their safety and effectiveness, along with the purity of the product and its potency in treating the condition [31, 32]. In the European Union (EU) and Japan, EVs for dermatological treatments could be considered a new cosmeceutical product ingredient. However, in the EU, the USA, and Malaysia, using human-derived ingredients in cosmetics is prohibited due to concerns about the transmission of prions and viral diseases, so additional testing and monitoring will be required to confirm their safety [33].

Cosmeceuticals, defined as cosmetic products that provide medical benefits for skin enhancement, have become of interest to EVs. Due to their suitable biological properties and marketing claims, the cosmeceutical industry is eager to include exosomes and EVs in their preparations. However, due to the wide diversity of EVs, which depends on the type of cells from which they are obtained and the condition from which they are derived, among other factors, the term "exosomes" should not be used generically. The manufacturer or supplier of the active ingredient is responsible for providing this information, which should include its toxicokinetic and safety profile in the registry.

Under current regulations, the cosmetic industry can easily develop and market cosmetic products instead of the tough process that drugs/biologics must undergo for commercialization. As can be guessed, this implies fewer guarantees for consumer safety and product quality. A new and clear regulatory framework for cosmeceutical products commercialized and accurate advertising and communication would be useful to distinguish true cosmetics and highly regulated medicines/medical devices from useless and sometimes even illegal fashion claims.

The ability of a true cosmeceutical product to modify any cellular behavior must be restricted to the skin. Manufacturers must provide this information and demonstrate EV absorption and pharmacokinetics to guarantee systemic indemnity. On top of this, cosmeceutical products are not regulated the same way worldwide.

For these reasons, and due to the lack of data regarding efficacy and safety profiles, direct-to-consumer companies marketing exosome-based products might provide

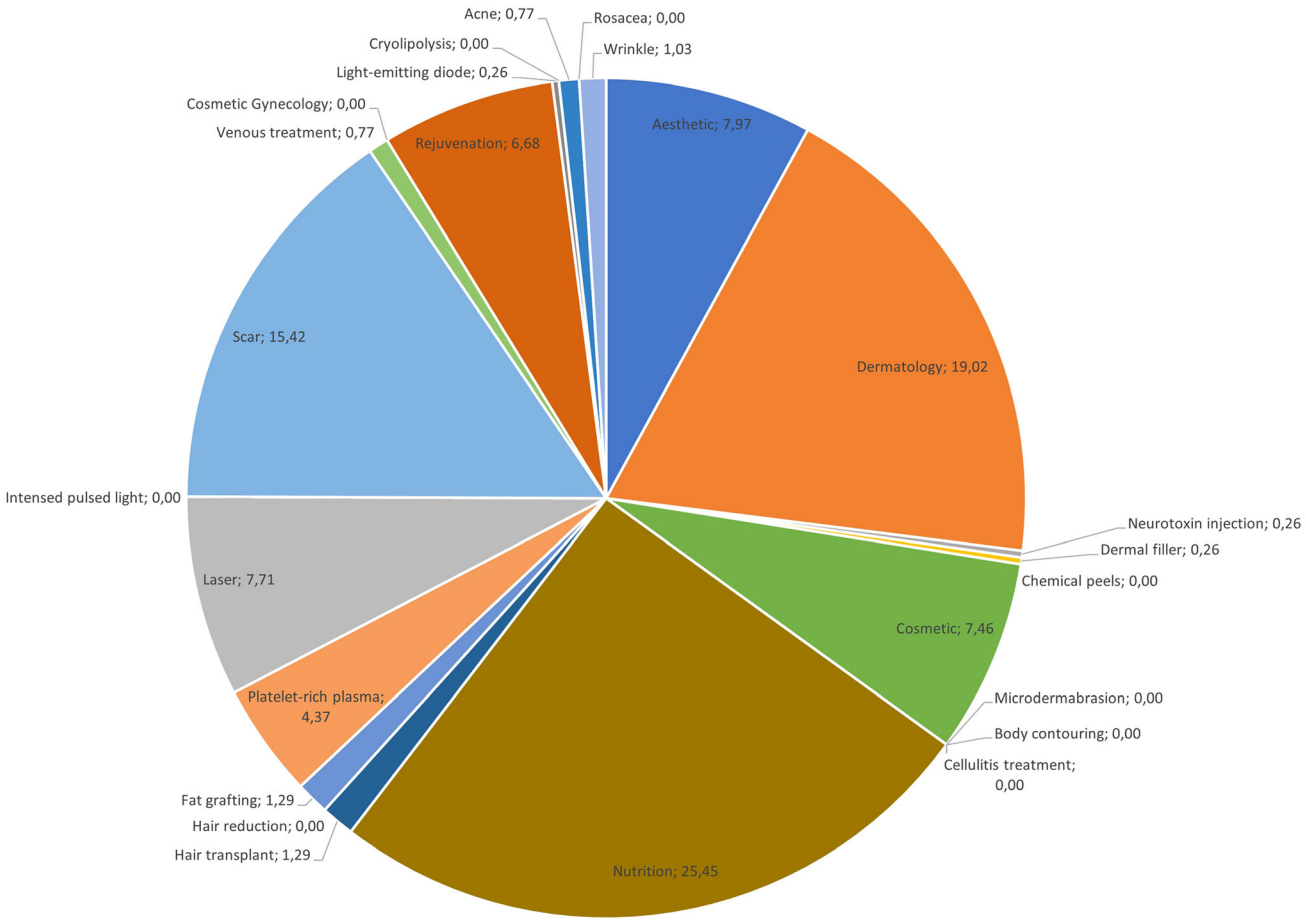

Fig. 2 Percentage of articles per search term from the refined database

unproven therapies. Many of them are primarily marketed online, and clinics operate outside the EU in locations with different and less strict medical regulations. Additionally, many of these companies are spending millions of dollars recruiting key opinion leaders and marketing and selling unapproved products. One study revealed that >30% of direct-to-consumer companies offering these treatments are in the US, where any novel treatment must be approved by the Food and Drug Administration (FDA), which, as mentioned, has not yet approved any [34].

In December 2019, the FDA published a safety notification on exosome products on its website to advise about multiple reports of serious adverse events experienced by patients in Nebraska who were treated with unapproved products marketed as containing exosomes (https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/public-safety-notification-exosome-products).

Advancing Toward EV Clinical Application in AM

Progress toward EV commercialization and their safe widespread use in medical aesthetics is hindered by low performance, storage, short shelf life, insufficient clinical data, a lack of knowledge of exosomes cargo, appropriate doses, and action mechanisms. In addition to further research, for a better understanding of their effect on the skin, improved and standardized guidelines will be required [33]. The International Society for Extracellular Vesicles (ISEV) [35] and the European Network on Microvesicles and Exosomes in Health and Disease (ME-HaD) have formulated certain guidelines to promote the clinical usage of exosomes [36]: standard operative protocols for exosomes isolation, processing, testing, quality control, and manufacturing for clinical usage and to be utilized at appropriate standards for therapeutic usage

Table 1 Articles related to exosomes found by search term and the number of articles found after the first debugging of the database

Search term	Articles related to exosomes	
	Total articles	First debugging
Aesthetic	41	31
Dermatology	122	74
Neurotoxin injection	2	1
Dermal filler	1	1
Chemical peels	0	0
Cosmetic	31	29
Microdermabrasion	0	0
Body contouring	0	0
Cellulitis treatment	0	0
Nutrition	215	99
Hair transplant	7	5
Hair reduction	0	0
Fat grafting	6	5
Platelet-rich plasma	25	17
Laser	70	30
Intense pulsed light	0	0
Scar	138	60
Venous treatment	8	3
Cosmetic gynecology	0	0
Rejuvenation	33	26
Light-emitting diode	2	1
Cryolipolysis	0	0
Acne	3	0
Rosacea	0	0
Wrinkle	4	1
Total	633	339

[31, 37]The regulatory framework addresses the safety standards for microbial and viral contamination and Good Clinical Practice standards, such as Good Manufacturing Practice (GMP) [38], Good Laboratory Practices (GLP) [39], Good Distribution Practices (GDP) [40], Good Clinical Practice (GCP) [41], Good Scientific Practice (GSP) [41], or for the production and quality control of the corresponding therapeutics [42]; falling out of the possibilities of the daily work of aesthetic physicians but set the basis of safe and effective products, whether they are cosmeceuticals or not.

Current Technologies for Exosome Production

Currently, there are no standardized methods for isolating exosomes. Consequently, the yield and purity of exosomes vary depending on the method used, which is information that most companies do not provide. However, some techniques have been tested to obtain and produce exosomes for later application. Among these, photothermal biomodulation is getting much attention due to its remarkable clinical outcomes [43–46].

Isolation and Purification

The isolation and purification of exosomes at a large scale is one of the most important obstacles to the advancement of this technology [47, 48]. Various experimental methods have been developed, such as differential ultracentrifugation (UC), density gradient ultracentrifugation (DGUC), ultrafiltration (UF), size exclusion chromatography (SEC), precipitation and tangential flow filtration (TFF) [47]. EVs isolated by different methods show discrepant functions in endothelial cell migration [49]. Therefore, choosing the most appropriate method before their clinical translation is critical [50]. According to a report, UC would be the most used method to isolate exosomes from MSC–conditioned media, followed by protein precipitation using commercial kits [51]. Each technology has inherent advantages and limitations, making its application difficult. For example, ultracentrifugation is the least expensive and most mature, but EVs can be damaged by high-speed centrifugation. SEC does not have this risk based on EV molecular size or hydrodynamic volume; however, it requires specialized equipment. Microfluidic technology offers integration and high throughput advantages but is limited to treating large quantities [52]. TFF has been proposed as the most suitable method for industrial exosome manufacturing due to TFF systems' availability of GMP-compliant [53, 54]. Recently, methods and technologies such as microfluidics, nanolithography, electro-deposition, immunomagnetic beads, and covalent chemistry have been developed, significantly impacting exosome isolation [55]

Although various isolation and purification methods have been developed for exosomes, their suitability differs. By combining different methods to isolate and purify exosomes, it is possible to improve yield and purity [48].

Storage

After isolation, exosomes can be stored. The major preservation techniques include cryopreservation, freeze-drying, and spray–drying, which are involved in EV storage. Cryopreservation is based on using low temperatures (−80 °C or less) to maintain the operation of EVs. Long-

Table 2 Final list of articles selected from the PubMed search

Author year	Title	Application	Age Sex Patients recruited	Exosomes-Samples	Procedure	Measurements	Results
Greg Chernoff [16]	Combining topical dermal infused exosomes with injected calcium hydroxylapatite for enhanced tissue biostimulation	Tissue regeneration via angiogenesis, neocollagenesis, and elastin production	34–72 years 35 Females 5 Males 40	Exosomes Kimera Labs (Miramar, FL), a suspension in a saline solution of isolated and purified, c-section donated, placental, mesenchymal stem cell-derived extracellular vesicles.	Two groups: 20 EBI alone; 10 EBI followed by CaHA injection (5 to the face [dilution 1:1], and 5 to the neck [dilution 1:4]); 10 with CaHa injection (5 to the face [dilution 1:1], and 5 to the neck [dilution 1:4]) without exosomes. All groups received 1.0 cc of exosomes (1 million), 1.0 cc of BoNT-A (25 units) and 1.0 cc of HA. Dermal infusion was optimized through a TNO-G serum, salt exfoliation, CU, and LED treatment.	In all groups, pretreatment Quantificare 3D photo-documentation and skin analysis (Quantificare, France) was performed at 15 and 30 days after treatment.	All patients showed an improvement in the tone, quality, and texture of skin. Quantificare results showed consistent improvement in wrinkles, pores, skin evenness, vascularity, and a reduction in oiliness and unwanted pigment. When EBI was employed as a skin primer prior to CaHa injections, enhanced and more rapid results were seen.
Han, HS [17]	Adipose-derived stem cell exosomes for treatment of dupilumab-related facial redness in patients with atopic dermatitis prospective study 12-week	Atopic dermatitis with local skin inflammation seen in dupilumab-treated patients	>18 years 20	Exosomes from human adipose-derived stem cell-conditioned medium, using ExoSCRT^{TM} technology (ExoCoBio Inc., Seoul, Republic of Korea)	One vial of ASCE + SRLV-S was applied to each patient's entire face. Prism sonophoresis was used to ensure effective drug delivery. Topical applications weekly for five consecutive weeks.	Clinical photographs at each visit, and clinical and objective evaluations at baseline and every week PT and at weeks 8 and 12. Variables assessed at the forehead, chin, right and left cheek: IGA, CEAS, and SSS, SSHI, and TEWL. At baseline and week 8, stratum corneum samples were collected.	After exosomes treatment (week 2 to week 12), the average IGAS decreased. The average CEA score decreased from the first week. The average SSS slowly decreased over time. EI decreased in all four areas PT, fastest on the forehead. SSHI and TEWL decreased but not SS.

Table 2 continued

Author year	Title	Application	Age Sex Patients recruited	Exosomes-Samples	Procedure	Measurements	Results
Wang, T [18]	Stem cell-derived exosomes in the treatment of melasma and its percutaneous penetration	Treatment of melasma and the means to promote its percutaneous penetration.	60 patients	Umbilical cord mesenchymal stem cell-derived exosomes	Group A, NAFL combined with normal saline treatment. Group B with Microneedles, Group C with NAFL, and Group D with PBASMP; all combined with hUCMSC-Exos. Each patient received four treatments at 1-month intervals.	Degree of pain posttreatment, melasma area and severity score, improvement rate, PGAS, satisfaction, and complications.	Groups B, C, and D showed significantly improved therapeutic effect and PGAS ($p < 0.05$). There was no significant difference among B, C, and D groups ($p > 0.05$). Group B reported higher pain levels ($p < 0.05$). In Group D the treatment experience was better.
Proffer, SL [19]	Efficacy and Tolerability of Topical Platelet Exosomes for Skin Rejuvenation: Six-Week Results prospective, single-arm, non-randomized, longitudinal study 6 weeks	Safety and tolerability of novel human platelet-derived exosome product and its maximal effects on skin rejuvenation	–	Intensive Repair Serum (Rion Aesthetics, Rochester, MN)	Prospective, single-arm, non-randomized, longitudinal study.	Evaluation at baseline and 6 weeks included participant questionnaires and photo-documentation with VISIA-CR imaging.	Improvement in skin health score at 6 weeks ($P \leq 0.0001$), correlated to reduction in redness, wrinkles, and melanin production ($P = 0.005$, $P = 0.0023$, $P \leq 0.0001$, respectively), and luminosity and color evenness ($P \leq 0.001$).
Park, KY [20]	Exosomes derived from human adipose tissue-derived mesenchymal stem cells for the treatment of dupilumab-related facial redness in patients with atopic dermatitis: A report of two cases	Two patients with atopic dermatitis and refractory DFR	Patient 1: 33 years severe AD Patient 2: 28 years old AD	Exosomes from human adipose-derived stem cell-conditioned medium, using ExoSCRT™ technology (ExoCoBio Inc., Seoul, Republic of Korea)	One mL of ASCEs (concentration of 2.0 × 109 particles/mL) to the entire face using a TEDS for 10 minutes. Patient 1: Six ASCE sessions, 1 each week. Patient 2: Six ASCE sessions, twice a week.	Improvement of the erythematous facial lesions	Patient 1: After treatment, had marked improvement of the erythematous facial lesions, was very satisfied, and the treatment was well tolerated. Patient 2: Showed excellent improvement.

Table 2 continued

Author year	Title	Application	Age Sex Patients recruited	Exosomes-Samples	Procedure	Measurements	Results
Ye, C [21]	hMSC exosomes as a novel treatment for female sensitive skin: An in vivo study. Prospective	Female sensitive skin	18–55 Female 22	Exosomes extracted from primary hMSC via UCF method. (Echo Biotech Co., Ltd., Beijing, China).	Prospective study. Two-week washout period prior. Visits at baseline (0 D), 7, 14, and 28 days after the use of the product. During the study, all volunteers applied 1 ml to the face twice a day (morning and evening) and stopped using their own facial moisturizers.	Clinical photographs with a VISIA^{TM}, TEWL, SSHI, SSB, SS-pH value, LAST, and skin a* value, at 0D, 7, 14, and 28 days. Satisfaction questionnaires at 7, 14, and 28 days. Objective symptoms including roughness, erythema. Subjective symptoms, including tension, burning, or itching, and dryness.	Objective symptoms and subjective symptoms, improved after 7, 14, and 28 day using hMSC exosomes ($p < 0.05$). TEWL and SSHI no significant results, SSB ($p = 0.026$), SS-pH, and a* ($p < 0.05$); all values were tended to return to the level of healthy skin. LAST decreased ($p < 0.05$). Subjects very satisfied were 75% on 7 days, 75% on 14 days, and 80% on 28 days. No adverse events reported.
Jang B [22]	Extracellular Vesicles from Korean Codium fragile and Sargassum fusiforme Negatively Regulate Melanin Synthesis.	Melanin Synthesis	Irradiation test: 35 (healthy) 29 and 59 years For the skin whitening efficacy test: 21 women 20 and 50 years	Prototype cream containing Codium fragile EVs (final concentration, 5 μg/ml)	Irritation test: A skin patch containing the test sample was applied to the test site for 24 hours, and then removed. Skin whitening efficacy test: The test products were each applied to half of the participant's face (on the right for placebo cream, on the left for test cream) once a day for 4 weeks	Irritation test: site graded at 30 min, 24 hours, and 48 hours post-removal using the IGS and the SOP of Korea Institute of Dermatological Sciences. Whitening efficacy test: The skin was imaged by VISIA-CA and the skin whitening was evaluated with a spectrophotometer.	The mean CII did not differ between sites treated with C. fragile EVs and those treated with control cream. The prototype cream containing C. fragile EV exhibited 0.94% improvement in skin whitening after 2 weeks and 1.31% improvement in skin brightness after 4 weeks.

Table 2 continued

Author year	Title	Application	Age Sex Patients recruited	Exosomes-Samples	Procedure	Measurements	Results
Cho BS [23]	Skin Brightening Efficacy of Exosomes Derived from Human Adipose Tissue-Derived Stem/ Stromal Cells: A Prospective, Split-Face, Randomized Placebo-Controlled Study.	Skin brightening efficacy in vivo of exosomes derived from human adipose tissue-derived mesenchymal stem/stromal cells	39–55 Female 21	Exosomes isolated using the TFF method from a cryopreserved bank of ASCs from the adipose tissue of a healthy donor, collected from ASCs cultured with serum-free and Xeno-free CEFOgroTM XF-MSC media. The final solution of ASC-exosomes was stored at −80 °C in small aliquots for further use.	Prospective, split-face, double-blind, randomized placebo-controlled study Group 1: 0.2 g of the placebo (without ASC-exosomes) Group 2: test with ASC-exosomes. Formulation was applied twice a day for 8 weeks. All volunteers rested for 30 min in a controlled environment at 20–24 °C and 40–60% relative humidity.	Skin brightening effect of ASCE BT and 2-, 4-, and 8-week PT. Melanin levels measured using a Mexameter. The average of five measurements was used to determine the improvement rate (%), following the formula = (value BT)−(value PT)/ (value BT) × 100. A) Reduction of melanin over time; Reduction of melanin in two age groups (40s and 50s), and representative images of two volunteers.	ASCE hyperpigmentation reduction was > in women aged <50 years (at 4 weeks) and diminished along with time (at week 8). The differences in the reduction of melanin levels between groups, were SS at 8-week PT. ASCE worked in vitro but there were not clinically relevant brightening effects in volunteers, probably due to inefficient delivery of exosomes into the deep dermis. No adverse effects were observed in any volunteer during or after the study.

Table 2 continued

Author year	Title	Application	Age Sex Patients recruited	Exosomes-Samples	Procedure	Measurements	Results
Kwon, HH [24]	Combination Treatment with Human Adipose Tissue Stem Cell-derived Exosomes and Fractional CO2 Laser for Acne Scars: A 12-week Prospective, Double-blind, Randomized, Split-face Study.	Clinical efficacy and safety of adipose tissue stem cell-derived exosomes as an adjuvant therapy after application of fractional CO2 laser for acne scars.	19–54 years 18 men and 7 women 25	Exosomes from human adipose-derived stem cell-conditioned medium, using ExoSCRT™ technology (ExoCoBio Inc., Seoul, Republic of Korea)	A 12-week prospective, double-blind, randomized, split-face study. Patients received three consecutive treatment sessions of FCL (10,600-nm) to the whole face, at an interval of 3 weeks, with a follow-up evaluation 6 weeks after their final treatment session. Post-laser, one side of the face was treated with ASCE (1 mL) and the other side with control gel. For the next two consecutive days after each treatment, patients applied each solution twice a day to the designated half of the face.	Efficacy of scar improvement was assessed by the ECCA score at each visit, and IGA at the FFU visit. Secondary outcomes: % change in total ECCA at weeks 3 and 6, and IGA at week 12. Subjective symptoms: erythema, edema, dryness, and pain, daily for 7 days after each session. Hyperpigmentation, and secondary scarring, at the FFU visit. The MDD. Depressed volume of atrophic scars, mean volume of skin pores, and skin surface roughness, at each visit with 3D camera.	ES was milder, and PT downtime was shorter on the ASCE-treated side. Reduction in ECCA between both sides was SS at the SPT visit ($p < 0.01$). After 3 sessions, ASCE-treated side had > improvement on IGA ($p = 0.02$). At the FFU visit, all ASV, mean PV, and SSR decreased (only SS on the ASCE side). At the FPT week ES was SS, lower on the ASCE side ($p = 0.03$). Subjective symptoms tended to be milder on the ASCE side, (not SS). The MDD was < on the ASCE side. Side-effects, including PT pain, erythema, edema, and dryness on both sides, were resolved within 5 days, without scar formation or other permanent events.

EBI, exosome biostimulatory infusion; CaHA, calcium hydroxylapatite, P-BoNT-A, purified botulinum neurotoxin type A; CaHa, calcium hydroxyapatite; HA, hyaluronic acid; TNO-G, topical nitric oxide-generating; CU, cavitating ultrasound; IGA, investigator global assessment; CEAS, clinical erythema assessment scale; SSS, subjective satisfaction score; EI, erythema index; SSHI, skin surface hydration index; TEWL, trans-epidermal water loss; CTP clinical trial period; SS, statistically significant; hUCMSC-Exos, umbilical cord mesenchymal stem cell-derived exosomes; NAFL, nonablative fractional laser; PBASMP, Peninsula Blue Aurora Shumin Master plasma; PGAS, physician global assessment; HPE, human platelet extract; hASCEs, human adipose-derived stem cell exosomes; TEDS, transdermal electroporation delivery system; UCF, ultracentrifugation; mHMSC, human mesenchymal stem cells; SSB, skin sebum; SS-pH, skin surface pH; LAST, lactic acid stinging test; EV, exosome vesicles; IGS, irritation grading scale; SOP, standard operating procedures; CII, clinical irritation index; TFF, tangential flow filtration; BT, before treatment; PT, posttreatment; IM, intracellular melanin; hASC-CM, human adipose-derived mesenchymal stem cells-conditioned medium; FCL, fractional CO2 laser; ASCE, adipose tissue stem cell- derived exosomes; ES, erythema severity; ASV, atrophic scar volume; PV, pore volume; SSR, skin surface roughness; FFU, final follow-up, FPT, first posttreatment; SPT, second posttreatment; MDD, mean duration of downtime

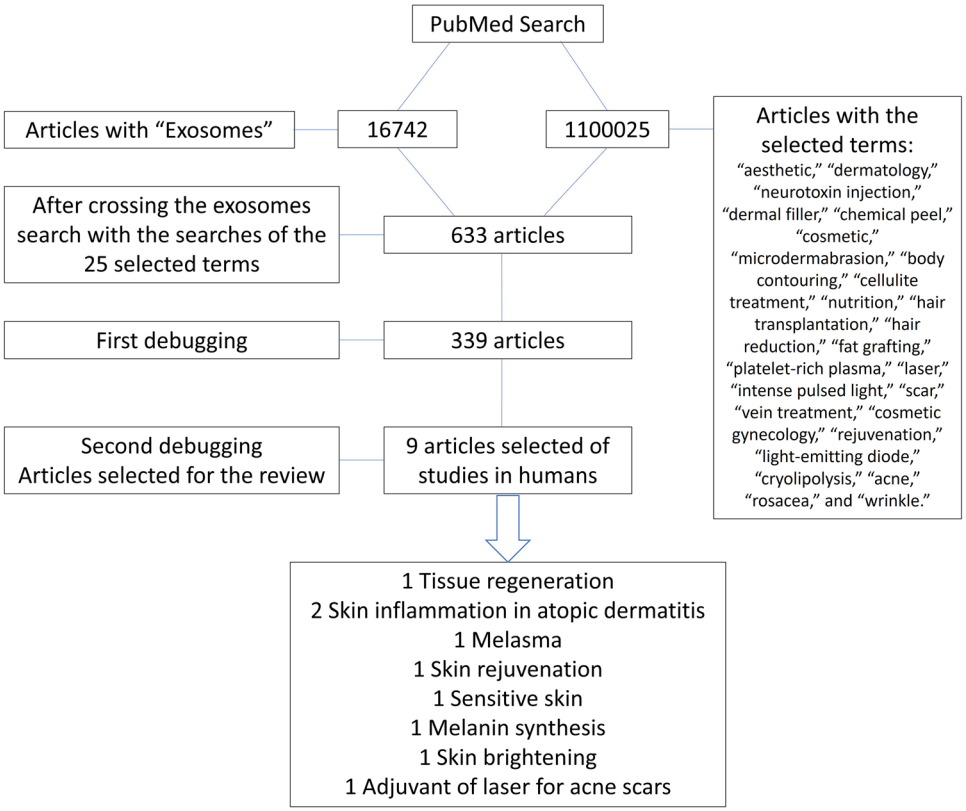

Fig. 3 PubMed search flowchart

term storage at −80 °C has also shown some concerns regarding changes in morphology and bioactivity have been reported [37]. Different cryoprotectants, such as penetrating and nonpenetrating cryoprotectants, are used to protect EV effectiveness. Cryopreservation using liquid nitrogen and cryoprotective agents may circumvent these issues, thereby achieving superior preservation of exosome morphology and function [19]. Freeze-drying is an emerging two-step technique to preserve EVs that includes sublimation and desorption, with 4 °C being the optimal storage temperature to freeze-dry EVs [56]. Spray-drying is a one-step method that is easier than freeze-drying. It can be used with various EVs, and the size of the products can be adjusted. In an automated manner, the EV solution is first atomized, and the heated gas sprays these droplets. Several factors can affect the stability of EV charging, such as EV solution feeding rate, atomization pressure, and outlet temperature. Furthermore, residual moisture can increase chemical instability by reducing the glass transition temperature of the solid particle state. Therefore, more research is required to consolidate this technique to produce EV-based therapies [57].

Temperature is not the only factor that can affect the preservation of EVs. Some studies have confirmed that pH can affect the characteristics and preservation of EVs. For example, a low pH cell culture (pH 5) was observed to affect EV production by increasing their protein content and zeta potential. On the other hand, low pH increased the uptake of EVs in recipient cells [58]. Storage of exosomes under acidic (pH 4) or alkaline (pH10) conditions can increase exosome aggregation as well as exosome uptake by cells compared to storage at pH 7 [59].

Conclusions

There is no doubt about the scientific basis and growing interest in exosomes in aesthetic medicine. The developments are at an early stage, and there is an urgent need for companies to spend more money on comprehensive

Table 3 List of interventional clinical trials involving exosomes for aesthetic medicine treatments identified in the ClinicalTrials.gov database (Search date: October 19, 2023).

Study title	Type Phases	Sex Age	Design Purpose	Conditions Interventions	Status Enrolled* Results
Exosome effect on prevention of hair loss NCT05658094	Int NA	All A, OA	Single group (no masking) Prevention	Hair Loss/Alopecia Device: Exosome	Recruiting 20 No
Effect of plasma-derived exosomes on cutaneous wound healing NCT02565264	Int Early Phase 1	All Ch, A. OA	Single group (no masking) Treatment	Ulcer Other: plasma-derived exosomes	Unknown 5 No
Mesenchymal stem cells derived exosomes in skin rejuvenation NCT05813379	Int Phase 1-2	Female A, OA	Single group (no masking) Prevention	Anti-Aging Combination product: Exosome injection	Recruiting 20 No
Safety and tolerability study of msc exosome ointment NCT05523011	Int Phase 1	All A, OA	Single group (no masking) Treatment	Psoriasis Drug: Exosome ointment	Completed 10 No
Pilot study of human adipose tissue-derived exosomes promoting wound healing NCT05475418	Int NA	All A	Single group (no masking) Treatment	Wounds and Injuries Procedure: Adipose tissue-derived exosomes	Not yet recruiting 5 No
Therapeutic potential of stem cell-conditioned medium on chronic ulcer wounds NCT04134676	Int Phase 1	All A, OA	Single group (no masking) Treatment	Chronic Ulcer Drug: Conditioned media	Recruiting 38 No
Effect of time-restricted feeding on fat loss and cardiometabolic risk factors in overweight adults NCT03459703	Int NA	All A, OA	RCT Parallel (no masking) Treatment	Obesity Behavioral: Early Time-Restricted Feeding, control schedule, structured weight loss program	Recruiting 90 No
Development of a nutrigenetic test for personalized prescription of body weight loss diets (obekit) NCT02737267	Int NA	All A, OA	RCT Parallel (no masking) Treatment	Body Weight Changes Behavioral: Moderately high protein diet, low fat diet	Unknown 260 No
Induced pluripotent stem cell-derived exosomes for the treatment of atopic dermatitis NCT05969717	Int Early Phase 1	All A, OA	RCT Parallel (no masking) Treatment	Atopic Dermatitis Drug: GD-iExo-001	Recruiting 20 No

*Oct. 19, 2023

Intervent, interventional; Ch, child; A, adult; OA, older adult; RCT, randomized clinical trial

research on topics involving exosomes to develop standardized and reliable procedures for their approval and application in clinical practice. On the other hand, the uncertainty about the general content of exosome products, such as miRNA, proteins, and lipids, purity degree, and safety issues still need to be resolved for an appropriate clinical application.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest to disclose.

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Informed Consent For this type of study, informed consent is not required.

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