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*Review*

# Medicalized Aesthetic Uses of Exosomes and Cell Culture-Conditioned Media: Opening an Advanced Care Era for Biologically Inspired Cutaneous Prejuvenation and Rejuvenation

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**Abstract:** Recent advancements in aesthetic medicine offer innovative cosmetic solutions to enhance patient skin quality and appearance. Advanced treatment options enable practitioners to effectively address skin aging signs, pigmentation imbalance, and loss of elasticity in ambulatory and home-based care regimens. Exosomes (nanoscale cell-derived vesicles) transport a variety of biomolecules and are pivotal in physiological intercellular communication. Importantly, exosomes have recently emerged as key endogenous players in tissular regeneration. More broadly (from an active ingredient purity standpoint), exosomes, stem cell secretomes, and cell culture-conditioned media have been clinically proven to exert multifaceted beneficial topical effects (anti-inflammatory, antioxidant, anti-aging, skin rejuvenation). Therefore, human, animal, and plant-derived exosomes or other refined sub-cellular biological fractions are gaining substantial interest within the aesthetic and cosmetic industries. Notably, such approaches are thought to be among the most promising novel contenders for advanced, biologically inspired skin prejuvenation and rejuvenation care. The present narrative review summarizes the latest clinically oriented research on exosomes and cell culture-conditioned media, highlighting their mechanisms of action in various topical applications. Furthermore, it explores the innovation landscape and currently commercially available products on the global cosmetic market and discusses the potential future applications of advanced, biologically inspired ingredients in the medical aesthetic industry.

**Keywords:** anti-aging treatments; cell culture-conditioned media; cosmetic products; dermatology; exosomes; extracellular vesicles; mesenchymal stem cells; secretome; skin rejuvenation; stem cell biology



**Citation:** Rodriguez, C.; Porcello, A.; Chemali, M.; Raffoul, W.; Marques, C.; Scaletta, C.; Lourenço, K.; Abdel-Sayed, P.; Applegate, L.A.; Pelissier Vatter, F.; et al. Medicalized Aesthetic Uses of Exosomes and Cell Culture-Conditioned Media: Opening an Advanced Care Era for Biologically Inspired Cutaneous Prejuvenation and Rejuvenation. *Cosmetics* **2024**, *11*, 154. https://doi.org/10.3390/cosmetics11050154

Academic Editor: Enzo Berardesca

Received: 12 August 2024
Revised: 2 September 2024
Accepted: 5 September 2024
Published: 7 September 2024

## 1. Introduction

Within a dense commercial market, many innovative cosmetic solutions are currently being developed and proposed for the enhancement of consumer/patient skin quality attributes. Notably, several modern aesthetic medicine approaches enable practitioners to effectively manage hallmark signs of cutaneous aging, such as wrinkling, elasticity loss, and pigmentation unbalance [1–4]. Therein, recent surging interest has brought exosome-based technologies to the forefront of scientific and industrial innovation [5–10]. Specifically, there has been a growing use of cell secretomes and exosomes as skin boosters, aimed at locally delivering growth factors, proteins, and other substances to support tissue repair and regeneration [11]. These compounds and advanced ingredients are often used after physical/chemical treatments such as fractional laser, microneedling, radiofrequency, and microdermabrasion treatments to support the cutaneous healing processes [1,3,4].

Exosomes are sub-cellular vesicular bodies that are produced and derived from human, animal, and plant tissues/fluids or cell cultures. Their use as active ingredients is notably bioinspired, as the main physiological functions of exosomes comprise intercellular communication and small cargo transport [1,2,12]. In therapeutic topical indications, various cell-free ingredients (e.g., exosomes, stem cell secretomes, cell culture-conditioned media) have been reported to exert beneficial actions, such as anti-inflammation, antioxidant, or anti-aging effects [1–6]. From a physiological standpoint, exosomes have been mechanistically described as key endogenous effectors of tissular regeneration [2]. In medicalized aesthetic settings, exosome products are available as facials, which involve applying exosome-rich serums or creams to the skin. Such protocols have been reported to support the skin's natural healing abilities, improve texture, reduce inflammation, and promote a more youthful appearance [1,6,8].

While exosomes and cell secretome ingredients may be derived from an array of exogenous sources, they may also be obtained from autologous biological samples. However, due to important processing technical limitations, autologous exosomes are considered to be similar to platelet-rich plasma (PRP) preparations [13]. Indeed, existing point-of-care processes cannot guarantee that only exosomes are isolated specifically and selectively. This observation was widely noted at the last Aesthetic and Anti-Aging Medicine World Congress (AMWC2024, March 2024, Monte-Carlo, Monaco). As an example, the company Meta Cell Technology (Barcelona, Spain) is commercializing a CE-certified machine that is claimed to produce autologous exosomes from patients' cells and tissues [10]. However, no specific exosome isolation method is used in this technology, which leverages biological sample photo-biostimulation for exosome release [14]. The obtention and use of autologous exosomes were thus set outside of the scope of this review, based on technical and analytical limitations that currently characterize this specific sub-field.

The present narrative review aims to comprehensively analyze the latest preclinical and clinical scientific research on exosomes, secretomes, and cell culture-conditioned media for topical medicalized aesthetic uses. Therefore, following brief descriptions of the known mechanisms of action (MoAs) of exosomes in topical applications, the available data on therapeutic and aesthetic cutaneous indications are set forth and discussed. This review notably highlights the current and rapidly evolving innovation landscape of exosomes in the global cosmetic industry, providing in-depth descriptions of commercially available products. Parallelly, this work underscores key challenges (i.e., regulatory and technical) around the market implementation of exosomes and related ingredients for medical aesthetic uses. To conclude, several critical assessments and perspectives are provided on the potential of such biologically inspired ingredients in the medical aesthetic industry for enhanced skin prejuvenation and rejuvenation care.

## 2. General Biological Characteristics of Exosomes

Exosomes constitute a type of extracellular vesicle (EV) that also includes microvesicles and apoptotic bodies. They are released by almost all cell types, including prokaryotes and eukaryotes [15–17]. Structurally, exosomes are lipid bilayer-enveloped EVs with a size

ranging from 30 to 200 nm. They originate through the fusion of multivesicular endosomes with the plasma membrane within the endocytic pathway [15,16]. Therefore, the term "exosome" should only be used when the endosomal subcellular origin of the EVs can be demonstrated [18]. This distinction is highly important to note, as many research groups erroneously report scientific work on alternative sub-cellular fractions (e.g., cell secretome and its extracts) under the term "exosome".

Due to their secretion by diverse cell types, exosomes can be detected in biological tissues and fluids such as breast milk, blood, serum, urine, saliva, amniotic fluid, and synovial fluid [19]. As they circulate in the bloodstream, they undergo multiple cycles of absorption and release, reaching different tissue layers [20]. Of note, transcytosis is thought to be the most probable mechanism for transporting exosomes across the endothelium [21]. Originally considered as physiological byproducts or cellular waste, recent studies have revealed the highly organized structure, composition, and significant function of exosomes in facilitating intercellular communication between donor and recipient cells [22]. Notably, exosomes achieve horizontal communication by homing to specific targets through surface proteins known as "zip codes" and delivering their cargo [23–26]. Importantly, the preserved tropism in released exosomes serves as recognition signals for uptake by similar recipient cell types [27]. Furthermore, exosome-mediated signal transduction is crucial for coordinating various biological reactions, such as immune regulation, stem cell functions, neural signaling, metabolic control, heart health, and tissular regeneration [28,29].

Biologically, exosomes contain a diverse range of cellular components, such as functional proteins, metabolites, and nucleic acids, which can impact the function of recipient cells [15–17]. Notable lipid component examples include cholesterol, ceramides, sphingomyelin, phosphatidylinosol, phosphatidylserine, phosphatidylcholine, phosphatidylethanolamine, and gangliosides [21]. In their lumen, exosomes contain nucleic acids such as DNA, mRNA, miRNA, and noncoding RNA. These molecules can thus be transferred between cells and potentially influence the behavior of recipient cells while being shielded from RNAse-mediated degradation [30].

Exosomes also transport growth factors and cytokines, which regulate cellular signaling, stimulate cell proliferation, and support tissue regeneration. Moreover, they contain a variety of transmembrane proteins and antigen-presenting molecules (e.g., MHC class I and MHC class II), indicating their potential role in immune responses [31]. Importantly, tetraspanin transmembrane proteins such as CD9, CD37, CD53, CD63, CD81, and CD82 play a crucial part in exosome biogenesis and the targeting of their recipient cells. In particular, CD9, CD63, and CD81 are often used as exosome biomarkers, as they are widely distributed across exosomes from various tissues [32]. Adhesion proteins (e.g., integrin, p-selectin) facilitate the binding of exosomes to recipient cells, whereas glycoproteins such as the galectin-3-binding protein serve as recognition molecules contributing to their immune tolerance [25,33,34].

Exosomes also transport additional signaling receptors, such as Fas ligand and TNF receptors. They notably shuttle heat shock proteins, which play a major role in cellular stress responses and immune regulation, or enzymes involved in various metabolic functions. Furthermore, exosomes contain cytoskeletal proteins (e.g., actin, tubulin, and filamin), indicating their potential involvement in regulating both cellular structure and dynamics. As they originate from the endocytic pathway, exosomes contain the endosomal sorting complex required for transport (ESCRT) protein machinery for membrane transport and fusion proteins such as GTPases and Rabs (i.e., GTPases family) [35]. Overall, the specific composition of exosomes can drastically vary depending on the type and physiological state of the parent cells, which leads to a heterogeneous and large family of sub-cellular vesicles (Figure 1) [34,36,37].

Notably, exosomes, secretomes, and conditioned media are biologically distinct, but these terms are often used interchangeably in aesthetic medicine (Figure 1). Specifically, conditioned medium is a broad term for the liquid containing all substances secreted by cultured cells. Secretomes include a wide array of extracellular vesicles (i.e., including

exosomes) and proteins. Exosomes are a specific type of extracellular vesicle with a defined size and content. While the scientific distinctions are clear, the terminology in aesthetic treatments can be imprecise (Figure 1).

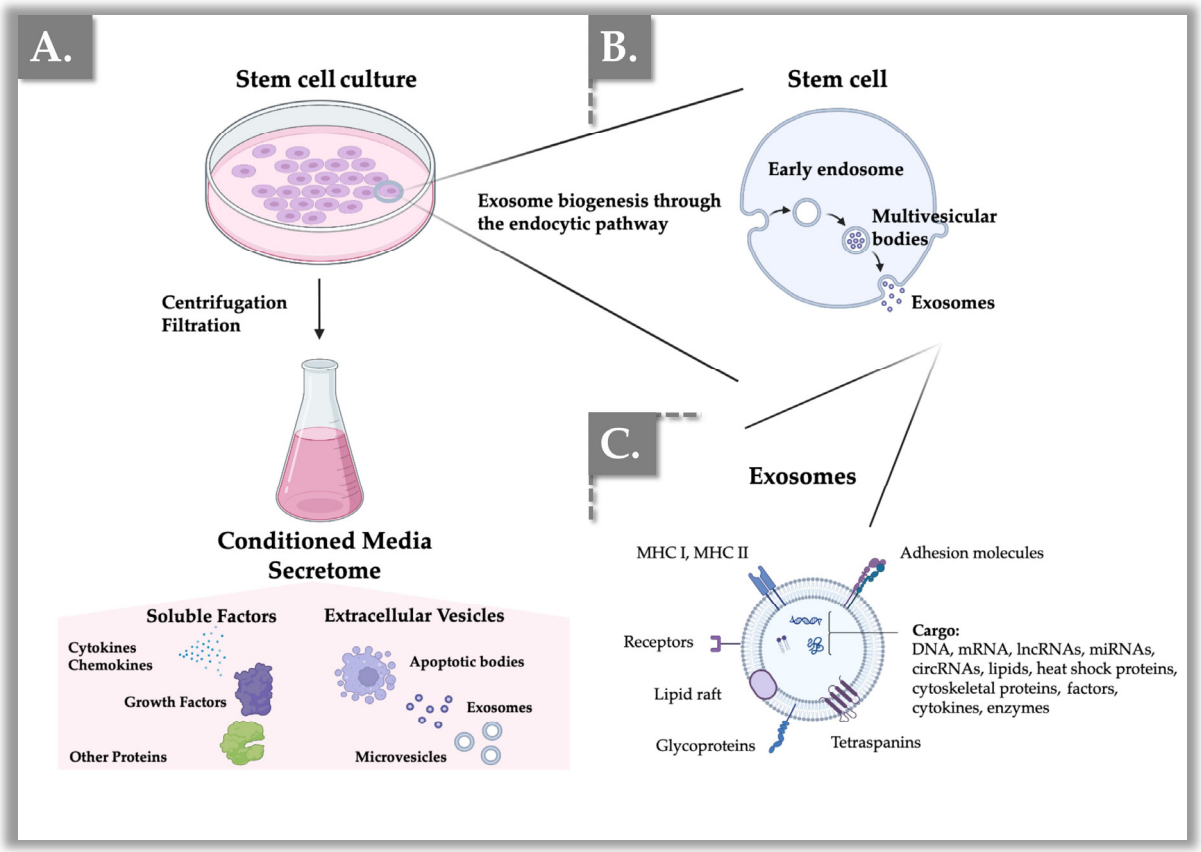


**Figure 1.** Exosome sourcing and characteristics. (**A**) Exosomes are obtained from stem cell cultures by centrifugation and filtration methods for primary isolation. (**B**) Exosomes are lipid bilayer-enveloped extracellular vesicles originating from the endocytic pathway. (**C**) Exosomes contain a diverse range of cellular components on their surface, such as adhesion proteins, antigen-presenting molecules, tetraspanins, receptors, glycoproteins, and lipids. They also transport cargo in their lumen, which may be shuttled to recipient cells. DNA, deoxyribonucleic acid; MHC, major histocompatibility complex; RNA, ribonucleic acid.

Of note, exosomes cannot be simply and specifically isolated from cell secretomes or conditioned media. Secretomes include all the molecules and biological factors released by cells into the extracellular space. The secretome mainly contains soluble factors (e.g., cytokines, chemokines, growth factors) and other soluble proteins and extracellular vesicles (e.g., apoptotic bodies, microvesicles, exosomes; Figure 1A). Therefore, the use of the term "exosome" requires that advanced separation processing and specific analytical methods are applied to the investigated biological extracts. Notwithstanding, few technical processes enable the obtention of exosome fractions with high degrees of purity, as soluble factors and alternative EVs are consistently present in trace amounts (Figure 1).

Importantly, exosomes are highly biocompatible and have enhanced stability, maintaining their integrity in circulation and even surviving gastrointestinal digestion [38–40]. Thus, exosomes offer several advantages in medical aesthetics, including low immunogenicity, good biocompatibility, targeting specificity, and strong tissue permeability. These properties also make them highly suitable as drug delivery vehicles. Their ability to transport bioactive molecules efficiently to targeted cells and tissues enhances the effectiveness of therapeutic applications in medical aesthetics.

## 3. Modes of Action of Exosomes in Cutaneous Applications

In the field of cosmetic and regenerative dermatology, several in vivo studies using EVs (i.e., sometimes incorrectly, inaccurately, or incompletely labeled as secretome or conditioned media) principally derived from stem cells have demonstrated promising potential in addressing various issues. Therein, the main indications of use include those for wound healing, scars, skin pigmentation, dermatoses, skin rejuvenation, and hair loss (Figure 2).

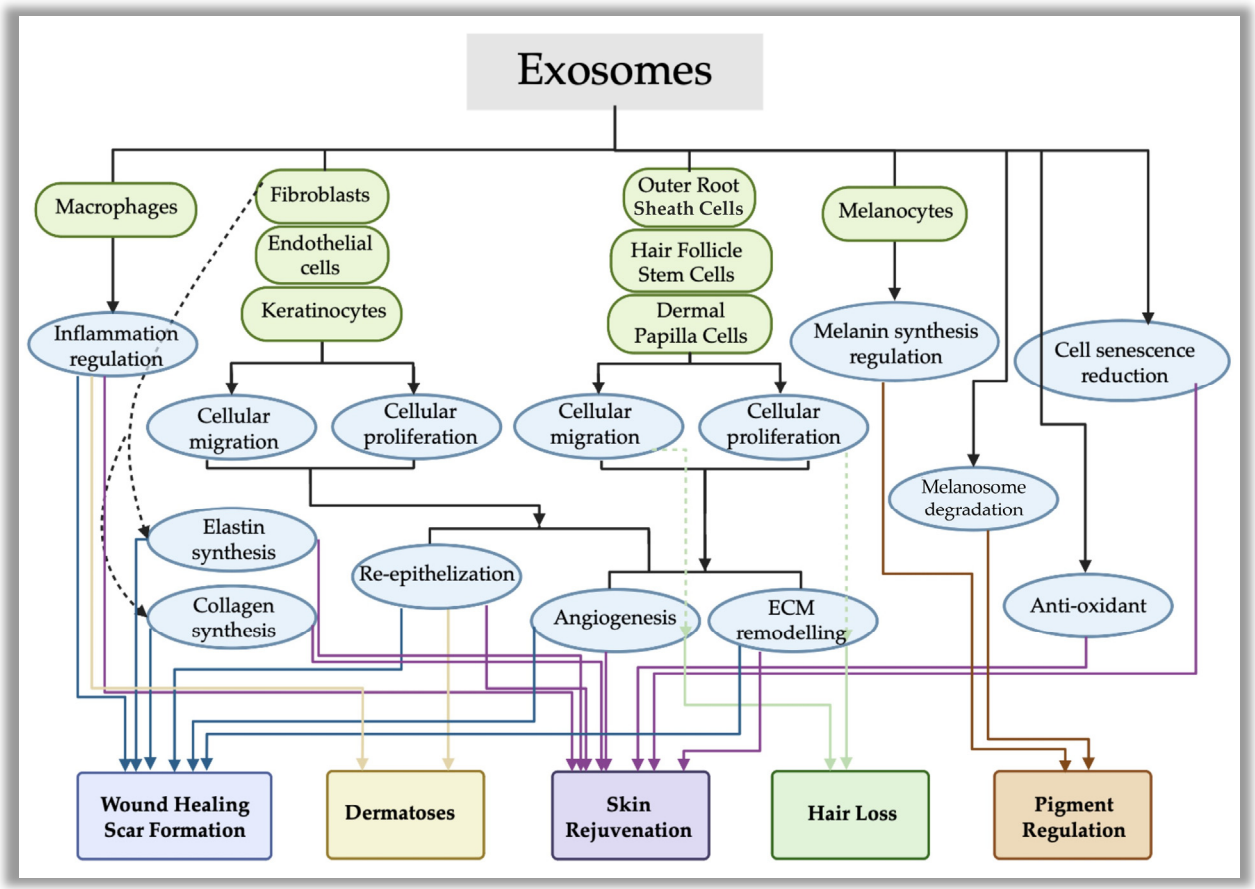


**Figure 2.** Mechanistic breakdown of the main documented effects of exosomes in skin-related applications. The listed biological functions were compiled from the available preclinical and clinical studies. ECM, extracellular matrix.

Importantly, exosomes' composition significantly influences their effects and in vivo applications. The presence of specific proteins and factors within exosomes dictates their regenerative properties. For example, adipose-derived exosomes, fibroblast exosomes, and those from umbilical cord cells are particularly noted for their regenerative potential [7–9]. To harness regenerative effects, exosomes must contain relevant proteins and factors associated with tissue repair and regeneration (Figure 1). Generally, the therapeutic potential and applications of exosomes in medicine are significantly influenced by the variability in their composition, which is determined by the cell type of origin and the physiological state of these cells. Exosomes reflect the molecular content of their parent cells, including proteins, lipids, RNAs, and other bioactive molecules, which play crucial roles in their therapeutic effects (Figures 1 and 2).

Of note, cells with regenerative properties, such as stem cells, are particularly valuable for exosome-based therapies. Among these, adipose-derived mesenchymal stem cells (AD-MSCs) are the most extensively studied. AD-MSCs possess robust regenerative and immunomodulatory capabilities, and their exosomes are rich in factors that promote tissue

repair, reduce inflammation, and modulate immune responses [5,9]. These properties make exosomes derived from AD-MSCs highly effective in therapeutic applications, particularly in regenerative medicine.

In contrast, exosomes derived from cells in pathological states or from less regenerative cell types may contain different cargo, which could limit their therapeutic efficacy or even pose risks. Thus, selecting the appropriate cell type, particularly those with regenerative capacities like mesenchymal stem cells, is critical for optimizing the therapeutic potential of exosomes in clinical applications. Due to the implication of exosomes in many biological pathways and their multimodal effects, their known MoAs are presented hereafter in Section 3.1, Section 3.2, Section 3.3, Section 3.4, Section 3.5 by therapeutic or cosmetic indications (Figure 2).

### 3.1. Roles of Exosomes in Cutaneous Wound Healing

Wound healing is facilitated and accelerated by a range of EV bioactive components, which influence inflammation regulation, increase skin re-epithelization and tissue regeneration through cellular proliferation and migration, or promote angiogenesis and extracellular matrix (ECM) remodeling through collagen synthesis [41–44]. The main effector cells implicated in wound healing are macrophages, endothelial cells, fibroblasts, and keratinocytes (Figure 2) [42,45,46]. In detail, the most frequently observed signaling molecules are miRNA, lncRNA, and circRNA, followed by growth factors and other specific proteins [42,45,47].

Interestingly, various sources of exosomes have been found to impact wound healing, comprising adipose stem cells (ADSCs), bone marrow stem cells (BMSCs), human umbilical cord mesenchymal stem cells (hUC-MSCs), induced pluripotent stem cells (iPSCs), expanded potential stem cells (EPSCs), fetal dermal mesenchymal stem cells (FD-MSCs), urine-derived stem cells (USCs), oral mucosal lamina propria-progenitor cells (OMLP-PCs), menstrual blood-derived stem cells (MenSCs), Wharton-jelly mesenchymal stem cells (WJ-MSCs), human amniotic mesenchymal stem cells (hAMSCs), human amniotic fluid stem cells (hAFSCs), and human embryonic stem cells (hESCs) [42,45].

Specifically, EVs derived from umbilical cord mesenchymal stromal cells (UCMSCs) were shown to reduce inflammation through the action of cytokines and nucleic acids, promote angiogenesis, and stimulate the proliferation and migration of fibroblasts through TGF-β pathway activation [48–50]. EVs from human cord mesenchymal stem cells (hCM-SCs) were shown to influence the Wnt4 pathway, which is crucial for wound healing processes [51]. Wharton's jelly MSC EVs exhibited potential in activating endogenous VEGF-A to enhance angiogenesis through miRNAs [52]. Adipose stem cell (ADSC) EVs also exhibited therapeutic benefits for wound healing by activating the PI3K/Akt signaling pathway [53,54].

Generally, MSC EVs inhibit inflammatory cells and promote the polarization of M1 macrophages [48]. Moreover, they enhance angiogenesis by stimulating the proliferation and migration of endothelial cells. They also support the remodeling of the skin ECM and inhibit scar formation by increasing the proliferation and migration of fibroblasts. In some cases, EVs containing MALAT1 contribute to improved wound healing through miR-124 activation of the Wnt/β-catenin pathway [55]. Furthermore, exosomes containing miR-29a reduced scar formation by inhibiting the TGF-β2/Smad3 pathway [56].

Of note, blood MSC EVs have been shown to facilitate wound healing by regulating the polarization of macrophages and inhibiting inflammation [48]. Additionally, they increase angiogenesis and the proliferation and migration of fibroblasts. Overall, two meta-analyses including a total of 47 in vivo studies (i.e., involving diabetic and non-diabetic animals) showed a significant enhancement of wound closure rates for wounds treated with EVs compared to the control groups [57,58]. Additionally, the treatment with EVs was associated with superior neovascular density, re-epithelization rates, collagen deposition, scar width reduction, and the downregulation of inflammatory factors.

### 3.2. Roles of Exosomes in Scar Formation and Cutaneous Pigment Regulation

Adipose MSC (ADMSC) and umbilical cord MSC (UCMSC) EVs have been found to reduce scar formation and fibrosis by regulating ECM reconstruction, inhibiting myofibroblast differentiation, and reducing excessive fibrotic tissue formation [59,60]. Most often, such EVs are observed to limit myofibroblast formation and modulate collagen deposition towards physiological balance [61]. Human ADMSC EVs are also involved in cutaneous pigment regulation, as they modulate melanocyte melanin synthesis through miRNA- and MITF-dependent pathways, as well as independent signaling pathways [46,62,63]. Exosomes are also involved in pigment regulation via enhanced melanosome-based melanin degradation [44,61].

### 3.3. Use of Exosomes for Managing Dermatoses

Potential therapeutic options for the treatment of dermatoses include topical exosomes, which regulate cutaneous inflammation and re-epithelization (Figure 2). Specifically, the use of EVs has been thoroughly investigated for various dermatological conditions such as psoriasis and atopic dermatitis [64–72]. Notable improvements were clinically recorded and supported the further investigation of EV-based treatment approaches for alternative inflammatory skin diseases.

### 3.4. Use of Exosomes for Skin Rejuvenation

From a medical cosmetic standpoint, EVs were shown to play a significant role in skin rejuvenation and photoaging by increasing fibroblast and keratinocyte proliferation, resulting in increased levels of collagen, elastin, hyaluronic acid, and localized dermal fat production [44,46,63,73–76]. Therein, the regulation of inflammation and the promotion of cellular migration contributed to enhanced re-epithelization and angiogenesis. As concerns photoaging, ADSCs, iPSCs, and HUCMSCs demonstrated preclinical proofs of their ability to reduce cutaneous damages [73]. In an in vitro model of UVB-irradiated human dermal fibroblast photoaging, it was demonstrated that UVBs inhibited miR-29b-3p levels compared with controls, but bone marrow mesenchymal stem cell-derived exosomes restored miR-29b-3p levels and reversed the inhibition of cell migration, the oxidative stress caused by UVB irradiation, and the production of MMPs [74]. Moreover, in vivo experiments on photoaged mice exposed to UV radiation showed that ADSC-EV treatment decreased wrinkles, increased collagen expression and production, and decreased UV-induced MMP expression [75–78].

Moreover, it has been demonstrated that exosomes deploy significant antioxidant and cell senescence reduction effects, both positively supporting skin rejuvenation [44]. Optimized ECM remodeling, enhanced autophagy, and reduced oxidative stress in EV-treated skin have also been set forth as key drivers of skin repair and regeneration [44,61]. Mechanistically, exosomes are known to play a role in various regenerative pathways in the skin, such as NFκb, AP-1, MAPK, P-AKT, NRF2, and SIRT-1 [79].

### 3.5. Use of Exosomes for Managing Hair Loss

Interestingly, EVs from various cell sources have been considered and investigated for managing hair loss concerns. These EVs, which carry essential proteins and miRNA, play a role in promoting the proliferation and migration of dermal papilla cells, outer root sheath cells, and hair follicle stem cells by activating or inhibiting different pathways (e.g., Wnt/β-catenin or LEF1 signaling) [46,80–85]. Generally, the topical use of exosome-based products for hair loss management is considered a promising option for mild to moderate cases, or as an adjuvant approach in post-graft care.

## 4. Clinical Studies on the Topical Cutaneous Use of Exosomes

Several clinical studies have investigated the use of exosomes as a topical treatment option for various cutaneous applications [86–98]. Some studies employed microneedling or electroporation to enhance product absorption, while other studies combined topical

exosome use with additional treatments such as laser conditioning. The reported outcomes included the recorded effects on pigmentation, scars, post-treatment recovery, erythema, hair growth, female sensitive skin, wrinkles, biostimulation, skin hydration, and skin elasticity (Table 1).

**Table 1.** Listing of notable clinical studies for the assessment of the cutaneous effects of topical applications of exosome-based products. ADSCs, adipose stem cells; CaHA, calcium hydroxylapatite; CM, conditioned medium; EVs, extracellular vesicles; HA, hyaluronic acid; hUCMSCs, human umbilical cord mesenchymal stem cells; MSCs, mesenchymal stem cells; SCs, stem cells.

| Study Reference | Clinical Indication | Active Ingredients/ Concomitant Treatment | Clinical Results | Product/Protocol Details |
|---|---|---|---|---|
| Cho et al. [86] | Skin brightening | Exosomes from human adipose tissue-derived SC CM | Significant reduction in melanin levels; improvement in skin brightness | Topical formulation [1] with glycerin, 1,2-hexanediol, L-arginine, xanthan gum, carbopol, water for injection |
| Jang et al. [93] | Skin brightening | EVs from *Codium fragile* and *Sargassum fusiforme* | Improvement in skin brightness | Cream containing *Codium fragile* EVs, 5 µg/mL |
| Wang et al. [97] | Skin rejuvenation | Protein extracts from ADSCs/Microneedles | Improvement in melanin index, luminosity, brightness, elasticity, and wrinkles | Protein extracts from ADSCs |
| Proffer et al. [90] | Skin rejuvenation | Topical platelet exosomes for skin rejuvenation | Improvement in skin health; reduction in redness, wrinkles, and melanin production; improvement in luminosity and color evenness | Intensive Repair Serum from Rion containing human leukocyte-reduced apheresed platelet extracts |
| Lee et al. [94] | Skin rejuvenation | Human embryonic SC CM/0.25 mm microneedle roller | Significant improvement in pigmentation and wrinkles | CM secretory factors of endothelial precursor cells from human embryonic SCs |
| Chernoff et al. [88] | Tissue biostimulation | Exosomes from placental MSCs/Injected with CaHA/Cavitating ultrasound/LED therapy | Enhanced tissue biostimulation | Exosomes from Kimera Labs, 1 mL containing $10^6$ exosomes, botulinum toxin, HA, and CaHA |
| Prakoeswa et al. [98] | Photoaging | Amniotic membrane SC CM/Microneedling | Significant improvement in photoaging [2] | Amniotic membrane SC CM |
| Lueangarun et al. [85] | Androgenetic alopecia, hair repigmentation | Exosomes from human adipose-derived MSCs/Fractional picosecond laser | Hair regrowth; repigmentation of gray hair and poliosis circumscripta | ASCE + HRLV-S [1]: 20 mg of lyophilized exosomes with $10^{10}$ exosome particles |
| Tak et al. [95] | Androgenetic alopecia | Adipose-derived SC extract for androgenetic alopecia | Increase in hair count and hair diameter | T-Stem product: 1% ADSCE-CE in distilled water/Gentle massage |
| Han et al. [71] | Dupilumab-related facial redness in patients with atopic dermatitis | Exosomes from human adipose-derived MSCs | Decreased erythema; reduced expression of inflammatory molecules; increased expression of angiogenesis proteins | ASCE SRLV-S [1]: 20 mg of lyophilized exosomes applied with prism sonophoresis |

**Table 1.** *Cont.*

| Study Reference | Clinical Indication | Active Ingredients/ Concomitant Treatment | Clinical Results | Product/Protocol Details |
|---|---|---|---|---|
| Park et al. [91] | Dupilumab-related facial redness in patients with atopic dermatitis | Human adipose-derived MSC exosomes for dupilumab-related facial redness | Improvement in erythematous facial lesions | ExoCoBio technology [1], $2 \times 10^9$ particles/mL/Electroporation |
| Ye et al. [92] | Female sensitive skin | Human MSC exosomes for female sensitive skin | Improved roughness, scaling, erythema, tension, burning, and itching symptoms | Exosomes from human MSCs |
| Kwon et al. [87] | Acne scars | Exosomes from human adipose tissue-derived SC CM/Fractional $CO_2$ laser | Significant improvement in acne scars; less erythema; reduced post-treatment downtime | ASCE gel [1], 1,2-hexanediol, glycerin, ammonium acryloyldimethyltaurate/VP copolymer, L-arginine, water for injection |
| Zhou et al. [96] | Atrophic acne scars and skin rejuvenation | ADSC-CM/Fractional $CO_2$ laser resurfacing | Improvement in skin hydration, elasticity, collagen, and elastin density | ADSC-CM |
| Wang et al. [89] | Melasma | hUCMSC-exosomes/Non-ablative fractional laser | Improvement in melasma symptoms | hUCMSC-exosomes with various non-ablative treatments |

[1] Exosomes isolated through the ExoSCRT technology from ExoCoBio (Seoul, South Korea). [2] A clinical study demonstrated skin parameter improvements related to photoaging, such as reduced pore appearance, wrinkles, and UV spots in the group treated with AMSC-CM [98].

Exosome-based treatments have shown promising results in clinical studies for conditions like acne scars and melasma, often demonstrating faster healing and improved skin texture compared to traditional treatments like laser therapy or chemical peels (Table 1). However, the outcomes can vary due to the lack of standardization in exosome preparation, which can lead to inconsistent results. While exosomes offer the potential for more targeted and less invasive treatments, their efficacy relative to traditional therapies requires further investigation through controlled trials.

Interestingly, clinical studies have demonstrated that combining exosome-based treatments with microneedling or laser therapy is more effective than using microneedling or laser therapy alone (Table 1). The synergistic effects of exosomes with traditional methods lead to improved outcomes in photoaging treatment, with improved melanin indexes, skin brightness, skin roughness, wrinkles, skin elasticity, and subject satisfaction [96–98]. This combination approach leverages the strengths of both exosomes and established techniques, offering a superior therapeutic option compared to traditional methods used on their own [1–3].

Of note, among the 15 clinical studies listed in Table 1, two studies clearly mention the ASCE SRLV-S and ASCE HRLV-S products from ExoCoBio (Seoul, South Korea) [71,85]. Additionally, other clinical studies, albeit not directly mentioning the ExoCoBio products, were conducted by ExoCoBio and utilized the same exosome isolation technique that the company has patented (i.e., ExoSCRT technology) [86,87,91]. One clinical study mentioned the Kimera Labs product, and it should be noted that Kimera Labs (Miramar Beach, FL, USA) received a US Food and Drug Administration (FDA) warning and should not be currently commercializing their products for aesthetic indications [88]. Finally, one clinical study mentioned the use of the Rion cream product (Rion Aesthetics, Rochester, MN, USA) [90].

Importantly, no FDA or EMA EV-based products are approved at the moment. However, clinical trials related to wound healing (i.e., NCT05078385, NCT02565264, NCT04134676,

NCT06253975, NCT05475419, NCT04235296, NCT03686449, NCT04664738, NCT05475418), keloid formation (i.e., NCT04326959), androgenetic alopecia and hair loss (i.e., NCT06066827, NCT06239207, NCT05658094), skin rejuvenation (i.e., NCT05508191, NCT06217627, NCT05813379), psoriasis (i.e., NCT05523011, NCT05523011), atopic dermatitis (i.e., NCT05969717), and melasma (i.e., NCT06221787) are registered according to www.ClinicalTrials.gov (accessed on 25 June 2024).

## 5. Commercialized Exosome Products

### *5.1. Commercial Exosome Product Formula Analyses*

For commercialized exosome products, the ingredients list is sometimes provided, but not consistently. The components may not be comprehensively listed, and the information may not be easily accessible or clearly presented. The ingredients list was reported for some products of ExoCoBio/Benev, Medipost, Rion Aesthetics, Elevai, hMSC Skincare, and DP Derm, which were retained for further analyses (Table 2).

**Table 2.** Listing of market-leading commercial exosome/secretome product information. Data were compiled from publicly accessible repositories [1] or product packaging and labeling materials. Detailed product ingredient listings are provided in Table S1. CM, conditioned medium; HA, hyaluronic acid; MSCs, mesenchymal stem cells; SCs, stem cells.

| Company (Headquarters) | Product | Formulation Presentation | Active Ingredient Source | Uses/Applications | Storage Information [2] |
|---|---|---|---|---|---|
| ExoCoBio/Benev (Seoul, South Korea) | ASCEplus SRLV/HRLV/IRLV | 20 mg lyophilizate + 5.0 mL solution | Plant/*Rosa damascena* | Topical/Skin rejuvenation | 2–8 °C |
| ExoCoBio/Benev (Seoul, South Korea) | ExoBalm | 20 mg lyophilizate capsule + 20 mL cream | Plant/*Rosa damascena* | Topical/Skin rejuvenation | 4–8 °C during 28 days after reconstitution |
| ExoCoBio/Benev (Seoul, South Korea) | Soothing Gel Mask | Gel mask | Plant/*Rosa damascena* | Topical/Calming, cooling, recovery, hydration | Ambient |
| Croma Pharma (Leobendorf, Austria) | EXO/E Serum | Solution | Plant/*Ustilago cynodontis*, *Piper nigrum* L SC, *Withania somnifera* root SCs | Topical/Skin revitalizing complex | Ambient |
| Stemica Labs (Beirut, Lebanon) | Secretome from UCMSCs | Solution | UCMSCs | Mesotherapy/Skin rejuvenation and hair restoration | Ambient |
| Medipost (Seongnam City, South Korea) | NGF-574H Hair Serum | Solution | CM of hUCBMSCs | Topical/Hair growth in androgenic alopecia | Ambient |
| Medipost (Seongnam City, South Korea) | NGF-574H Solution | Solution (mesotherapy; 3 to 6 sessions every 2 weeks) | CM of hUCBMSCs | Mesotherapy | Ambient |
| Rion Aesthetics (Rochester, MN, USA) | Intense Serum | Serum | Human platelet extract | Topical/Anti-aging skin care, skin rejuvenation | Ambient |
| Exocel Bio (San Diego, CA, USA) | Exovex Revive | 5.0 mL cryopreserved solution | Placental MSCs | Topical, microinfusion after microneedling | −80 °C to −20 °C |

**Table 2.** *Cont.*

| Company (Headquarters) | Product | Formulation Presentation | Active Ingredient Source | Uses/Applications | Storage Information [2] |
|---|---|---|---|---|---|
| Exoqure, Resilielle (Los Angeles, CA, USA) | Resilielle Age Zero Exosomes | 5.0 mL cryopreserved solution | WJ-MSC | Topical with microneedling, laser | −80 °C, 15-month shelf life; −20 °C, 6-month shelf life; refrigerator, 3-month shelf life |
| JuveXo (Miami, FL, USA) | JuveXO Skin | 5.0 mL solution | Umbilical MSCs | Topical with microneedling, dermabrasion, laser therapy | Ambient |
| PrimaCure (Incheon, South Korea) | E-50 Skin: Dry Ampoule | Lyophilizate | Salmon-tested cells cultivated in salmon embryonic SC media | Topical/Skin rejuvenation, skin inflammation, hair loss, hair growth | Ambient |
| AnteAge MDX (Irvine, CA, USA) | Exosome Solution | Lyophilizate + 6 mL HA solution | hBMSCs and hUCSCs | Topical with microneedling, radiofrequency, laser, and other ablative treatments | 2–8 °C |
| DP Derm (North Miami Beach, FL, USA) | MG-Exo-skin Serum | 5 mL solution (5×) | MSCs | Topical with microneedling | Ambient |

[1] Reviews of accessible information (i.e., in English, French, or Spanish) were performed for products with a clear mention of exosomes, secretomes, and/or conditioned media. [2] When the storage temperature was not specified, it was typically assumed that the products are stored at ambient temperature.

It should be noted that only a fraction of the listed ingredients in the analyzed products are considered active, with the other components serving as excipients (Table S1). Specifically, as concerns the Benev ASCE plus SRLV product from ExoCoBio, leucine and isoleucine are considered the active ingredients, while all other ingredients (i.e., including exosomes) are considered inactive ingredients, according to the National Institute of Health (Tables 1 and S1) [99]. As concerns the Benev ASCEplus HRLV from ExoCoBio, the only active ingredient is biotin, according to an FDA report [100]. For the NGF-574H injectable solution, the distributor website cites panthenol as the only active ingredient [101]. In the Medipost shampoo, zinc pyrithione, panthenol, niacinamide, and biotin are considered active ingredients [102].

Generally, the available evidence suggests that exosome/secretome-based products contain a wide range of additional inactive constituents (Tables 2 and S1). Moreover, some companies claim to have growth factors, collagen, peptides, and other molecules in their products, but they do not specify whether these molecules are included in the exosomes, which, by definition, contain such cargo, or if they are molecules that were added during the product formulation process. Furthermore, one reasonable technical assumption is that all lyophilized products contain cryoprotectants and lyoprotectants such as sugars, amino acids, or glycerol to ensure the stability of the delicate ingredients throughout the lyophilization process.

Overall, it may be extremely difficult to precisely determine the exact composition of a given commercial product [103]. Specifically, a 2023 review by Asadpour et al. reported that there are 114 companies and private clinics in 28 different countries providing allogenic SC secretome-based treatments to consumers for skincare and hair loss. Therein, almost 50% of the companies were identified as marketing secretome-based therapies, and the authors noted that the cell sources for such treatments are frequently undisclosed [103].

However, due to the complex nature of finished product analysis from a quantitative standpoint, manufacturer-provided information currently represents the only tangible source for determining exact product compositions and contents.

From a general safety standpoint, it should be noted that long-term safety and efficacy data for exosome-based treatments in dermatology are currently limited. Nevertheless, in topical uses, and to the best of our knowledge, all the available studies highlighted an absence of adverse effects (Table 1).

### *5.2. Exosome Ingredient and Product Sourcing Considerations*

As previously mentioned, exosomes can be derived from multiple biological sources, such as human donors, animal cells, or plant cells. Therein, critical importance is set on the selection of the starting donor material, which needs to be tested for communicable diseases (e.g., viruses, prions). Such basic qualification steps are necessary to ensure the safety and quality levels of the sub-cellular products that are derived from the retained starting materials. Moreover, age, sex, health status, and weight of donors are factors that have a potential impact on the regenerative potential or the proliferative capacity of the established cell source [104]. In detail, some companies (e.g., ExoCoBio) leverage the donor characteristics to support the quality attributes of their products.

In addition to the starting material sourcing and qualification phase, the specific in vitro manufacturing processes applied to the cells are directly related to the quality and functionality attributes of the isolated exosomes/secretomes. Indeed, the methods used to isolate and expand the cell source influence the basic characteristics and functionalities of the derived exosomes/secretomes [104,105].

### *5.3. Secretome Products for Potential Technical Rationalization*

Generally, the isolation of exosomes from cultured cells presents significant biological and technical obstacles, making it a challenging task. Importantly, the act of proving the process of endocytic biogenesis for exosomes is crucial to ensure the unique presence of exosomes. Furthermore, methods including ultracentrifugation and ultrafiltration, commonly employed by companies commercializing exosomes, present technical challenges in isolating only exosomes without also co-isolating other sub-cellular and soluble particles.

As concerns most products that exist on the commercial aesthetic market, it is more appropriate to use the term "secretome" instead of "exosome" (Figure 1) [106,107]. The contents of the cell secretome are constantly dynamic and influenced by the specific type of cell that secretes it, as well as microenvironmental factors, such as cell culture conditions [108,109]. Another term for secretome is conditioned medium, which contains bioactive components responsible for tissue repair and regeneration through autocrine and paracrine effects in intercellular signaling [110–113]. As an example, an in vitro study demonstrated that human dermal fibroblasts pre-treated with ADSC-conditioned medium (CM) exhibited significantly increased proliferative activity and reduced cellular senescence compared to normal human dermal fibroblasts [114].

Topical applications of CM also demonstrated important effects related to skin conditions. A clinical study showed that the topical application of SC CM can significantly reduce wrinkles, pigmentation, and skin pores [111,112]. A topical gel containing hypoxically cultured human neonatal cell CM was assessed in a split-face clinical trial and showed significant improvement in wound healing following laser treatment, exhibiting a dose-dependent response. Additionally, it demonstrated a significant reduction in transepidermal water loss and decreased levels of inflammation by reducing macrophage and neutrophil infiltration, increasing regulatory T cell infiltration, and downregulating the expression of inflammation-related genes (i.e., interleukin-1β, interleukin-6, chemokine ligand 1, and chemokine ligand 2) [112,113]. Another topical gel, which contained hypoxically cultured amnion MSC CM, was demonstrated to enhance angiogenesis and re-epithelization as well as decrease inflammation in an in vivo model of wound healing [113].

One of the major challenges in successful exosome-based therapeutics is the inefficient productivity of exosomes. The yield of exosomes is typically less than 1 µg of exosomal protein (i.e., representing 1 billion EVs per 1 mL of culture medium), whereas the effective dose of exosomes is approximately 10–100 µg of exosomal protein per mouse in most studies [115,116]. Commercial EV products differ in their dosage, which typically range from 5 to 25 billion EVs/particles per application. Therefore, effective, large-scale exosome production methods are required to meet the therapeutic demands. The use of bioreactors offers a promising solution for the large-scale production of EVs. Depending on the cell types, bioreactor compatibility needs to be assessed to ensure the integrity and preservation of the biological activity of the exosomes [117].

### *5.4. Current Best Practices in Commercial Exosome Isolation*

As previously mentioned, the standardization and consistency of exosome isolation are complex and unresolved issues in exosome-based research and applications. Currently, there is no accepted standard for ensuring the purity and consistency of exosomes, which poses a significant challenge across various fields, including aesthetic medicine and therapeutic applications.

In aesthetic medicine, manufacturers commonly rely on a combination of centrifugation, ultracentrifugation, and filtration techniques to isolate exosomes. These methods are widely used due to their relative simplicity and ability to yield exosomes in sufficient quantities for commercial use. However, these techniques often result in preparations that may contain a heterogeneous mix of vesicles and other contaminants, leading to variability in the final product [18,19].

In therapeutic applications, more precise and sophisticated methods, such as size-exclusion chromatography, immunoaffinity capture, and microfluidic techniques, are being explored to enhance the purity and consistency of exosome preparations. These approaches aim to isolate exosomes with greater specificity, but they also introduce additional complexity and are not yet universally adopted or standardized [18,19].

The lack of standardized protocols across different applications remains a critical issue, as it hampers the reproducibility and reliability of exosome-based therapies. Establishing rigorous standards for exosome isolation, characterization, and quality control is essential for advancing their clinical and commercial potential [18].

## 6. Analysis of EU and Global Regulatory Frameworks for Exosome-Based Products

### *6.1. Technical Hurdles in the Registration and Market Implementation of Exosome Products*

Multiparametric factors contribute to the slow registration of exosome-based products. Notably, there are currently no injectable FDA-approved products, and available topical preparations are highly heterogeneous [118]. Furthermore, there is an absence of widespread consensus on standardized protocols for isolating, storing, and identifying exosomes [46]. It is important to underscore that treatments involving exosomes, secretomes, or EVs have not yet been approved (i.e., FDA or EMA) for application in aesthetic medicine. This fact is attributed to several factors, including the lack of standardization in isolation, purification, and characterization methodologies for exosomes [46,119,120]. The lack of standardization currently poses challenges for regulatory approval and consistency in clinical outcomes. Variability in exosome isolation methods, such as ultracentrifugation, size-exclusion chromatography, and precipitation, can lead to differences in the purity and functionality of the final product. Standardization would involve developing universally accepted guidelines for exosome purity, potency, and characterization, including the specific markers that define exosomes versus other extracellular vesicles. As an example, studies of toxicity profiles, biodistribution, and pharmacokinetics may be required. These standards would not only facilitate regulatory approvals but also ensure that exosome-based products are safe, effective, and of consistent quality across different clinical settings.

Of note, the FDA currently classifies exosomes as biological products. However, exosomes may be classified as cosmeceutical ingredients and therefore must respect the fact

that their effects must be restricted to the skin [120]. Overall, from a regulatory standpoint, developing a standardized quality control workflow for exosome products would be necessary [105]. Importantly, regulatory pathways for exosome-based products are likely to differ between therapeutic and cosmetic applications. In the United States, the FDA categorizes products based on their intended use. Therapeutic exosomes might be classified as biologics, requiring approval under the Biologics License Application pathway, which involves rigorous clinical trials and manufacturing oversight. Cosmetic exosome products, if not intended to treat, cure, or prevent disease, might be regulated as cosmetics or devices, but they still must meet safety and labeling requirements. In the European Union, the EMA may also classify exosome therapies as advanced therapy medicinal products (ATMPs) for therapeutic uses, necessitating a centralized marketing authorization process. For cosmetic applications, exosome products need to comply with the EU Cosmetics Regulation, which demands evidence of safety and efficacy but has less stringent requirements than ATMPs.

To potentially harmonize the classification and approval processes for exosome-based products, regulatory bodies could establish clear guidelines that differentiate between their use as cosmetics and therapeutic agents. This might involve creating specific regulatory pathways that consider the unique properties of exosomes, such as their cellular origin and intended use. Collaborative efforts between international regulatory agencies could also lead to unified standards, reducing the complexity and cost of obtaining approvals across different regions.

Overall, obtaining regulatory approval for exosome-based products is challenging due to the lack of clear guidelines and standardized manufacturing processes. The FDA and EMA have yet to approve any exosome-based products, largely because of concerns about product consistency, safety, and efficacy. These challenges could slow the adoption of exosome therapies in clinical practice, as companies may need to invest heavily in research and development to meet regulatory requirements. This delay could also affect the availability and affordability of these treatments.

### *6.2. Off-Label Uses and Illicit Commercialization of Exosome Products*

Notwithstanding the described limitations of exosome product registration, several companies have taken the risk of marketing their products for aesthetic medicine indications. Most notably, in September 2023, Kimera Labs (Miramar Beach, FL, USA) received a warning letter from the FDA for commercializing MSC exosome products for various uses, including those concerning skin applications [121]. The warning letter explained that exosome products fall under the classification of biological products in the Public Health Service Act and are categorized as drugs. Moreover, Kimera Labs also demonstrated a negative response to the sterility test and exhibited notable deviations from the requirements of current good manufacturing practices (cGMPs). It is important to note that in May 2023, the company obtained an investigational new drug (IND) application for the intravenous product XoGloPro to address COVID-19 symptoms [122]. However, they have also gone beyond this framework and commercialized the product for skin repair, regrowth, and rejuvenation purposes.

As previously set forth, there are currently no exosome-based treatments or medical devices approved for clinical use by the FDA or the EMA, despite several ongoing clinical trials. However, exosomes may be defined as cosmetic ingredients. The FDA defines "cosmetics" as "articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance" [123]. Similarly, the European Commission defines "cosmetics" in EU Regulation 1223/2009, Article 2.1.a) as "any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours" [124]. Notwithstanding, it is important to

stress again that exosomes have not yet been officially categorized for cosmetic use by the FDA; thus, the specific regulatory requirements remain uncertain [125].

Practically, within the EU, exosomes are included in the CosIng database, which lists all cosmetic ingredients. They are listed under the names "human adipose derived mesenchymal cell exosomes", "human cord blood progenitor cell exosomes", "human umbilical mesenchymal stem cell exosomes", "milk exosomes", "human adipose stromal cell exosomes", "human amniotic fluid induced pluripotent cell exosomes", "human dermal fibroblast induced multipotent cell exosomes", "human adipose derived mesenchymal stem cell exosomes", "human umbilical endothelial cell conditioned media", "human tooth pulp stem cell conditioned media", "human adipose derived stem cell conditioned media", "human neonatal fibroblast/keratinocyte conditioned media", "plant conditioned media", etc. Such ingredient nomenclature may be found on most marketed cosmetic products in the exosome field under the INCI component listing.

### *6.3. Dangers of Off-Label Uses for Commercial Exosome Products*

Three main potential safety concerns are applicable to exosome-based products. Firstly, there is a risk of transmitting adventitious viruses or other pathogens from donor cells to recipients. Secondly, non-autologous exosomes may trigger immune responses, leading to inflammation, allergic reactions, or even rejection by the recipient's immune system. Thirdly, exosomes may carry oncogenic factors like specific microRNAs or proteins that could promote tumor growth or metastasis in recipients. Thus, the off-label use and illicit commercialization of exosome products carry significant risks, particularly when these products are not subjected to the rigorous testing (e.g., assays for viruses, mycoplasma, endotoxins, and sterility) and regulation required for safe medical use.

Furthermore, the long-term safety and efficacy of exosome-based products remain largely untested [126]. Moreover, toxicological evaluations have shown that some exosome products, such as those derived from human adipose tissue-derived mesenchymal stem cells (i.e., ASC-exosomes), are generally considered safe, with no adverse effects in various toxicity tests (e.g., skin sensitization, photosensitization, and acute oral toxicity) [126]. Lastly, the lack of standardized manufacturing processes and quality control measures in the commercialization of exosomes can result in products with varying potency and purity. This variability can compromise expected outcomes and safety. Therefore, a cautious and evidence-based approach is essential to ensure the safety and effectiveness of exosome treatments.

### *6.4. Harmonization of Regulations for Exosomes and EVs*

Notwithstanding current industrial practices, the available evidence points towards global regulatory harmonization, under which, exosomes derived from cultured cell sources will be classified as biological products and drugs. Therein, there could be separation into various drug categories, depending on the product doses and intended application sites (e.g., topical use), similar to existing practices for over-the-counter painkillers and anti-allergy medications. Due to the dosing scale that is considered in exosome and EV products, the quantitative aspects of the preparations may even be considered close to those of homeopathic preparations. Notwithstanding, the use of cell culture methods requires the application of cGMPs for product manufacturing, including all QA and QC measures for release. Additionally, the availability of biological assays for the assessment of product activity is of high importance for the validation of product quality, potency, and stability.

## 7. Exosome Use in Cosmetic Products and Aesthetic Medicine Protocols

Exosomes have a significant scientific background, supported by in vitro, in vivo, and clinical studies. Their therapeutic effects have been demonstrated in this context along with their MoAs [1–4,20,31]. However, the use of exosomes in cosmetics differs from their therapeutic use due to several factors. These include dosages, application methods, formulations (i.e., including excipients), and other ingredients involved in the

finished product. Furthermore, EV function may be affected by various storage conditions, presenting considerable difficulties in maintaining their integrity within a formulation. Importantly, exosomes used as cosmetic ingredients may not produce identical effects to those examined in the aforementioned clinical studies [1,2].

Indeed, a clinical study conducted to evaluate the efficacy of ASCE plus exosomes (i.e., obtained from ExoCoBio) in treating dupilumab-related facial redness in patients with atopic dermatitis revealed significant changes not only in clinical scores but also in the expression of molecules within the stratum corneum [71]. This indicated that the product's clinical effects are produced through specific MoAs that alter the production of inflammatory and angiogenesis proteins.

Furthermore, the regulation and study of exosome MoAs are continuously evolving due to the youthful nature of the field. This necessitates further regulatory consensus and scientific research work. Over the past year, many websites and companies have updated their marketing strategies for exosomes. In detail, companies that previously marketed exosomes for aesthetic purposes have shifted towards developing drug-containing exosomes to treat diseases. Furthermore, several companies continue to sell products containing exosomes, but they have stopped explicitly mentioning "exosomes" in their marketing messages. With numerous changes occurring in this field, some previously available websites are no longer accessible.

From a safety standpoint, while it may appear that topically applied exosomes are less effective, they may in fact decrease immune reactivity. Indeed, the stratum corneum is highly reactive to immune responses, and topically applied exosomes effectively reduce immune reactivity in this layer, leading to a global decrease in inflammatory signals [67,68]. Moreover, an in vivo experiment in a mouse model demonstrated that topically applied exosomes are mostly confined to the stratum corneum [67]. Following topical application, mesenchymal stem cell exosomes exhibited minimal penetration beyond this outermost layer of the skin. Specifically, less than 1% of the exosomal fluorescence was detected in the culture medium after 24 h, indicating that the exosomes were largely retained within the stratum corneum rather than being excreted or systemically absorbed [67]. Locally, exosomes were utilized by the cells within the stratum corneum to inhibit complement activation, particularly the formation of the terminal complement complex (C5b-9). This inhibition led to a reduction in the release of IL-17, a pro-inflammatory cytokine primarily produced by neutrophils through the process of NETosis [67]. Overall, the data suggested that the exosomes exerted their therapeutic effects locally within the skin's stratum corneum by modulating the immune response, rather than being excreted from the body or entering the systemic circulation. Interestingly, despite this limited penetration, topically applied exosomes significantly reduced psoriasis biomarkers [67].

One effective method for achieving this outcome is micro-channeling (e.g., microneedling), which explains its wide application in clinics. In detail, microneedling allows molecules to bypass the stratum corneum barrier by creating numerous micro-holes, enabling transdermal molecule delivery to reach the epidermal/dermal tissue [3,75].

As regards clinical protocols, the topical application of exosomes could be regulated under cosmetic jurisdictions. However, many clinics offer exosomes combined with micro-channeling to maximize the function of exosomes and enhance skin rejuvenation and regeneration effects. Therein, it remains unclear if substances applied with the aid of micro-channeling are still classified as cosmetics. Notwithstanding, it is crucial to improve the penetration of exosomes applied topically into deeper layers of the skin [73,75,76]. Indeed, topically applied exosomes are unable to penetrate the skin barrier and therefore mainly act within the stratum corneum.

## 8. Galenic Form and Storage of Exosome-Based Products

### *8.1. Topical Formulation Possibilities for Exosome Products*

Due to the biological nature of exosome/secretome ingredients, the finished product formulation and stability attributes are of critical importance for the quality of the provided

care. Specifically, the biological activity of secretome ingredients, including exosomes, is highly sensitive to storage conditions. Factors such as temperature, pH, and the use of cryoprotectants play critical roles in preserving the stability of these products. Improper storage, such as exposure to fluctuating temperatures or incorrect pH levels, can lead to the degradation of the exosomes' cargo and membrane integrity, reducing their effectiveness. Therefore, it is crucial to adhere to specific storage protocols, including the use of appropriate cryoprotectants (e.g., in lyophilized form), and maintain storage at ultra-low temperatures (e.g., if the product is in liquid form) to ensure the consistency and safety of exosome-based products. Additionally, no incompatibilities should exist between the components of the final formula (i.e., including primary packaging materials), and the finished product should maintain the integrity of the ingredient up until application. Importantly, due to the highly heat- and time-sensitive nature of exosome ingredients, specific aseptic formulation measures are necessary and terminal sterilization is excluded. Of note, most commercially available products are manufactured as semi-solid creams, serums, or lyophilizates (Figure 3).

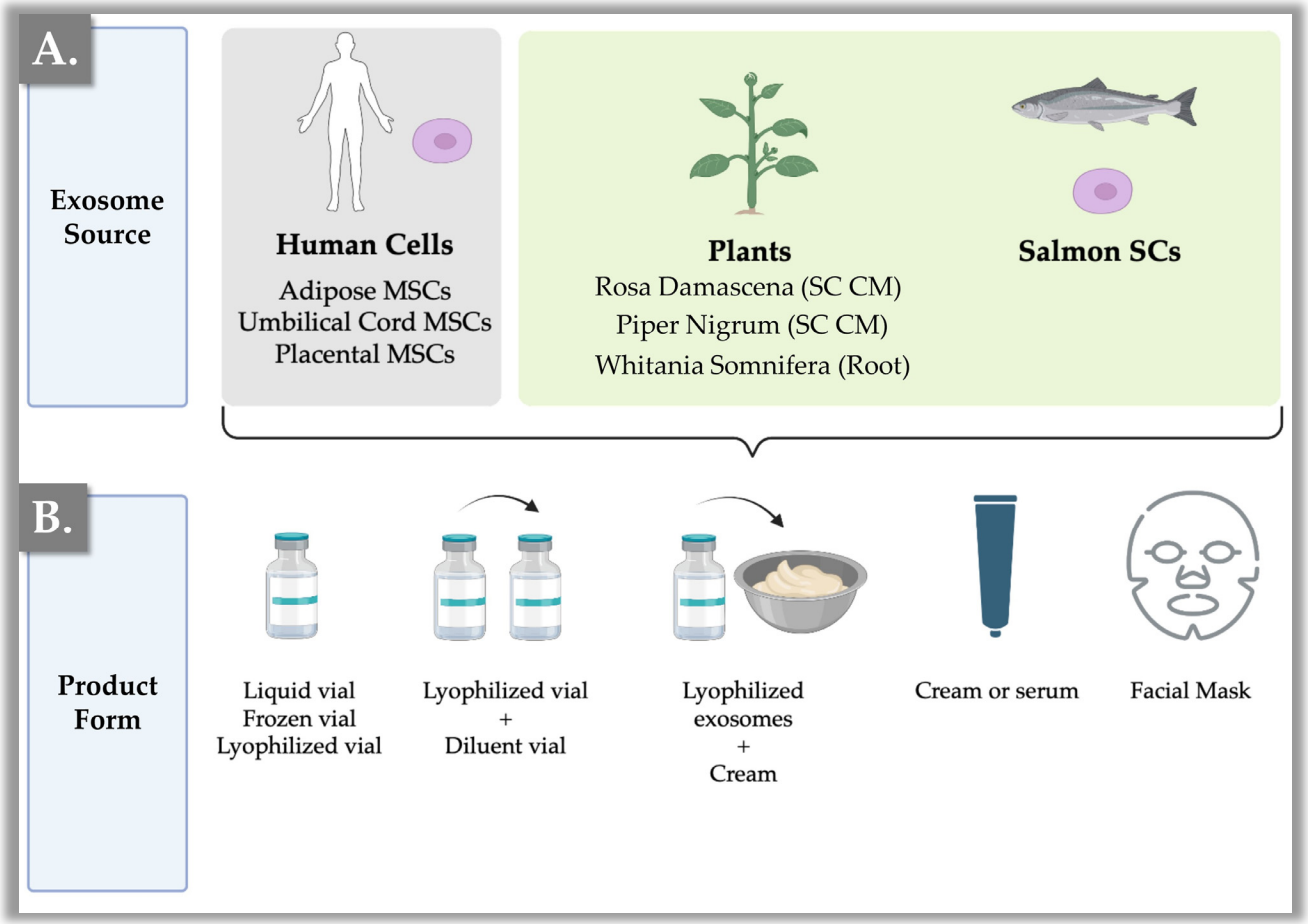


**Figure 3.** Current commercial landscape of exosome products. (**A**) In the commercial landscape, exosome products are primarily derived from human MSCs, but can also be derived from plant cells (e.g., *Rosa damascena*, *Piper nigrum*, *Whitania somnifera* root) and salmon stem cell CM. (**B**) The commercial products available on the market exhibit a diverse range of galenic forms. There are 3–5 mL vials that can be liquid (i.e., stored at room temperature), frozen (i.e., stored between −20 °C and −80 °C), or lyophilized. Lyophilized exosome vials usually need to be reconstituted with a diluent vial before application. Creams containing exosomes (i.e., ready to use or be reconstituted) can also be found. Exosome-containing facial masks and eye patches are also available. CM, conditioned medium; MSCs, mesenchymal stem cells; SCs, stem cells.

As concerns cream formulations, a preparation containing CM from 3D-cultured human adipose MSCs demonstrated a considerable skin whitening effect following a two-week application period [127]. The creme formulation involved an oil-in-water emulsion, with the CM being incorporated at 1% into the cream mixture [127]. Another formulation example by Dong Wha Pharmaceutical (Seoul, South Korea) offers a serum and cream that include lyophilized hUCBMSC CM and are combined with humectants and emollients before use [128]. In clinical studies, these products were shown to increase wound healing and decrease post-laser redness [128]. As concerns lyophilized commercial preparations, ExoCoBio commercialized an exosome-containing cream that consists of a 20 mL cream base formulation incorporating 20 mg of lyophilized plant exosomes, which the user needs to mix before application. The prepared cream should be stored at temperatures between 4 °C and 8 °C for up to 21 days. Additionally, ExoCoBio provides a calming gel mask that contains pre-solubilized plant exosomes (Table 2).

### *8.2. Storage Conditions and Stability of Exosome/Secretome Products*

Storage conditions, particularly temperature, have a significant impact on the biological activity of exosome-based products. For example, storing exosomes at −80 °C helps preserve their integrity and functionality over time, while storage at higher temperatures, such as 4 °C or −20 °C, can lead to the degradation of key components like miRNAs, reducing their therapeutic potential. Freeze–thaw cycles and pH shifts during storage can also affect exosome stability, leading to diminished efficacy or altered biological effects.

In order to preserve the biological activities of secretome ingredients, it is important to store them appropriately. The secretome is highly unstable and requires specific storage conditions. Indeed, the molecules present in the secretome are notably prone to enzymatic degradation, protein oxidation, or protein aggregation [129,130]. It is crucial to remember that each study of storage conditions applies specifically to the type of cell being studied. Thus, it remains uncertain whether these results can be applied to EVs from different cell sources that vary in surface lipids or proteins and cargo content [131,132]. However, exosomes offer greater stability compared to other soluble factors in the secretome. One thermodynamic study of liposome stability showed that the specific surface composition plays a crucial role in maintaining the liposome's stability [133]. Various factors, such as temperature, pH, and preservation techniques, can influence the physiological and biological characteristics of the considered EVs [134].

For ready-made cosmetic products, long-term storage and sustained effectiveness at ambient temperature are important factors to consider [44]. At the ingredient level, a study demonstrated that storing EVs in PBS over time leads to drastically reduced recovery, particularly for pure EV samples at all tested temperatures, starting within days [135]. Alternative buffers may facilitate EV preservation at −80 °C, maintain stability throughout several freeze–thaw cycles, and drastically improve EV recovery in downstream applications [135]. To date, there is still a lack of consensus concerning the overall impact of storage conditions on EVs, yet it appears that process, formulation, and temporal parameters play key roles in frozen ingredient stability [135,136]. As concerns the use of lyophilization processing for ingredient stabilization, it was reported that a preparation supplementation with sugars (e.g., sucrose, trehalose, mannitol) significantly contributed to augmenting the maintenance of the quality attributes/therapeutic potency of biomolecules [137,138]. Furthermore, lyophilization offers the possibility of concentrating samples, which is especially important for CM-based preparations [137].

#### 8.2.1. Frozen Storage for Exosome Products

In a patent describing EV formulation, it was demonstrated that the NTA-determined EV concentration and size remained stable after one week of storage at 4 °C, −20 °C, and −80 °C [132]. However, the levels of miRNA decreased to less than 50% after 30 days at temperatures of 4 °C and −20 °C. At a temperature of −80 °C, there was minimal alteration in the levels of miRNA [131]. Another study used flow cytometry analysis to investigate

the impact of the storage temperature on the physical and functional characteristics of antibacterial EVs derived from human neutrophilic granulocytes [139]. Storing the EVs at 4 °C for one day caused a reduction in EV quantity and antibacterial efficacy. Storing them at −20 °C for 28 days preserved the number of EVs but nearly eliminated their antibacterial effectiveness. Contrastingly, storage at −80 °C for 28 days maintained both the physical and functional characteristics of the EVs. While storage at −80 °C is optimal, it drastically increases costs and limits transportation and logistical options [139].

The clinical use of secretome products necessitates a minimum shelf-life extension of 6 months. According to MISEV2023 recommendations, storage should be at −80 °C in PBS [20]. However, it is underscored that freezing and thawing can cause alterations in the shape, activity, size, and concentration of EVs. Freezing may result in ice formation, exposure to ice–liquid interfaces, buffer salt precipitation causing pH shifts, and particle-rich phases due to the cryo-concentration of the vesicles. Furthermore, PBS use can cause an acidic shift during freezing, which, in turn, destabilizes the membrane proteins of the EVs [140]. Some authors reported that the concentration of EV fractions remained stable when preserved at either 4 °C or −80 °C for a period of one month [141].

#### 8.2.2. Lyophilized Storage for Exosome Products

The lyophilization of exosome-based products presents several challenges, including the potential for aggregation and loss of bioactivity during the freezing and drying processes. The choice of cryoprotectants, such as trehalose, is critical to maintaining the structural integrity of exosomes. However, lyophilization can also introduce stresses that may cause changes in exosome size or surface properties, impacting their effectiveness. These challenges complicate the development of stable, commercially viable exosome formulations and require careful optimization of the lyophilization process to ensure product consistency and efficacy.

Lyophilization is commonly used to condense and preserve active ingredients during storage and transportation. Additionally, it offers an optimal ingredient format for subsequent cosmetic product formulation [44]. A notable in vitro and in vivo experiment demonstrated that lyophilized secretome had similar effects on wound closure rates. Importantly, this suggested that the process of lyophilization did not diminish the potency of the secretome [142]. Furthermore, polyacrylamide gel electrophoresis (PAGE) analysis indicated that the proteins and RNA in the secretome were preserved after lyophilization [143]. Of note, lyophilization and subsequent rehydration did not result in a significant decrease in the bioactivity of EVs either in vitro or in vivo when compared to EVs that were not subjected to lyophilization [131,132]. Notwithstanding, it was set forth that lyophilization can induce stress during the freezing, dehydration, and rehydration stages, necessitating the addition of suitable stabilizers. Indeed, rehydration can notably cause the swelling of amphiphilic molecules present in the vesicle bilayer and potentially detrimental osmotic effects [140].

Laboratory studies on the lyophilization of EVs from different cell sources, such as MSCs and HUVECs, have shown that the total particle amount decreases with lyophilization compared to storage at 4 °C or −80 °C. This decrease may be attributed to the formation of aggregates. Notwithstanding, it was found that the qualitative properties of EVs were not significantly influenced by lyophilization. Furthermore, trehalose was shown to be a superior cryoprotective agent compared to mannitol and polyethylene glycol 400 [144]. Overall, exosomes offer greater stability compared to other soluble factors and ingredients, and lyophilization processing appears to be an optimal technical solution for the preparation and storage of active exosome-based ingredients.

### *8.3. Stabilization Processing of Exosome Products*

#### 8.3.1. Formulation with Cryoprotectants/Lyoprotectants

Frozen storage as well as lyophilization require cryoprotectants. Cryoprotectants are commonly employed in preservation processes, with options such as penetrating

cryoprotectants (e.g., dimethyl sulfoxide [DMSO] or glycerol) and non-penetrating agents like trehalose and sucrose [134]. Frozen storage of the secretome at −80 °C with DMSO optimally maintains the stability of the sample, in contrast to frozen storage without cryoprotectants. This indicates that DMSO can be used in reasonable proportions (i.e., subtoxic amounts) as a cryoprotectant for the long-term storage of secretome ingredients, even though it may not preserve all vesicle morphologies in the sample [145]. Lyophilization also requires the use of cryoprotectants, such as trehalose, to prevent aggregation of the secretome [143]. It was demonstrated that EVs are more stable in the presence of sucrose or potassium phosphate buffer than in sodium phosphate buffer or phosphate-buffered saline during freeze–thaw cycles. A neutral pH also leads to less aggregation [140]. Generally, the use of sugars (e.g., mannitol, trehalose) as combined cryoprotectants and lyoprotectants is optimal for simple and effective biological ingredient stabilization.

Of note, the cryoprotectant used in the sourcing of cells for EV production is considered to be the most important. The overall cell survival and recovery should indeed be optimal (i.e., >80% cellular viability upon initiation). Specifically, if the retained cell preservation system does not allow high cell stability, it should be reconsidered before establishing the downstream processing of EVs from the cell source. Several studies have demonstrated that exosomes lyophilized with stabilizers such as trehalose maintain their bioactivity after rehydration, with minimal changes in size, structure, or functional properties [140–144]. However, the lyophilization process must be carefully controlled to avoid damage to the exosome membrane, which could occur during the freezing, dehydration, or rehydration stages. The use of appropriate cryoprotectants and careful optimization of the lyophilization cycle are crucial to ensuring that the exosome product remains potent and effective. We showed in a recent study the importance of assessing combinations of cryoprotectants with a grading table to compare the results after the process of lyophilization (i.e., including various quality controls, e.g., presence of a cake, residual water, uniformity, resuspension time, or meltback) [146].

#### 8.3.2. Quality Considerations: Ingredient Resuspension after Lyophilization

An important parameter linked to the lyophilizate form is the resuspension time and quality, which can be limiting factors for clinicians and consumers. A study demonstrated that human red blood cells that had been lyophilized and subsequently rehydrated exhibited analogous levels of molecular components and cellular characteristics than the control group [147,148]. The parallel between red blood cells or liposomes and exosomes is interesting, as they share similar biological formats/structures. A study found that the reconstitution of highly concentrated lyophilized proteins was complete within one minute when the protein-to-sugar ratio was less than 1. However, when the ratio exceeded 1, the reconstitution times increased in a nonlinear manner [149]. Another study revealed that the reconstitution time was primarily determined by the formulation viscosity, which was dependent on the protein-to-sugar ratio [150]. The study determined that disaccharides do not offer a protective effect on lyophilized cells when they are present only in the extracellular environment and that extensive vitrification may not provide significant benefits [150]. Overall, high importance is set on the careful design of lyophilization formulas and cycle recipes in order to obtain an appropriately stabilized product with acceptable resuspension characteristics [151].

## 9. Conclusions and Perspectives

The growing use of secretomes, exosomes, and all potential formulations of EVs in the field of cosmetic and aesthetic medicine highlights their promising role in supporting tissue repair and regeneration. Although exosome- and secretome-derived products have demonstrated beneficial effects on skin texture, inflammation reduction, and overall skin quality attribute enhancement, important challenges remain regarding their isolation, characterization, MoAs, widespread cosmetic application, and regulation. Despite these challenges, in vitro, in vivo, and clinical studies confirmed their potential to address various important

cutaneous issues. Moreover, clinical trials using exosomes or secretome ingredients have notably confirmed their tolerability and efficacy, providing tangible scientific support for their future use. Differences in cosmetic applications, such as sourcing, dosage, formulation, and product stability, necessitate prospective research to optimize the use of exosomes and alternative EVs and facilitate their regulation. In detail, there are limitations due to the lack of reliable manufacturing methods, limited understanding of the mechanisms of action of the product owing to its complexity and involvement in multiple pathways, regulatory challenges, and scalability issues. Establishing standardized guidelines for manufacturing, isolation, storage, stability, dosage, sourcing, and safety is necessary. Addressing these factors and obtaining more clinical data will enable the homogeneous utilization and more straightforward regulation of secretome products and exosomes. The molecular profiling (i.e., of proteins and lipids) of secretomes could be an option to better characterize the products and help in establishing more precise mechanisms of action. Importantly, the commercial market for these products is constantly evolving, with frequent manufacturer website changes and applications shifting between aesthetic and therapeutic uses. Indeed, the market is rapidly expanding, with significant growth expected. Additionally, a substantial number of clinics are offering this treatment directly to consumers. Since 2024, numerous articles in cosmetic and beauty magazines have been highlighting these emerging beauty treatments, making exosomes a trending topic. However, the increasing interest in exosome therapies suggests that regulatory agencies will eventually develop specific guidelines. The implications for future market entry are significant. As the regulatory landscape evolves, exosome-based products may face stringent requirements for demonstrating safety, efficacy, and consistency in manufacturing processes. Companies entering this market will need to navigate these regulations carefully, which may involve extensive clinical testing and post-market surveillance activities. Generally, the promise of exosome and secretome products in the cosmetic industry holds substantial potential, requiring a rigorous approach to fully exploit their capabilities in improving skin health and appearance.

**Supplementary Materials:** The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/cosmetics11050154/s1: Table S1: Listing of ingredients for the commercially available products described in Table 2.

**Author Contributions:** Conceptualization, C.R., A.P., M.C., C.M., L.A.A., F.P.V. and A.L.; methodology, C.R., A.P., C.M., C.S., L.A.A., F.P.V. and A.L.; software, C.R., A.P., C.M. and A.L.; validation, C.R., A.P., M.C., W.R., C.M., C.S., K.L., P.A.-S., L.A.A., F.P.V. and A.L.; formal analysis, C.R., A.P., M.C., C.M., K.L., L.A.A., F.P.V. and A.L.; investigation, C.R., A.P., C.M., K.L., F.P.V. and A.L.; resources, A.P., L.A.A. and A.L.; data curation, C.R., A.P., M.C., C.M., K.L., L.A.A. and A.L.; writing—original draft preparation, C.R., A.P. and A.L.; writing—review and editing, C.R., A.P., M.C., W.R., C.M., C.S., K.L., P.A.-S., L.A.A., F.P.V. and A.L.; visualization, C.R., A.P., C.M., L.A.A. and A.L.; supervision A.P., L.A.A. and A.L.; project administration, A.P. and A.L.; funding acquisition, A.P. and A.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study received no external funding and was not supported by any specific grants or institutional programs.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Acknowledgments:** Artwork templates were partly generated with www.biorender.com, accessed on 25 July 2024.

**Conflicts of Interest:** Authors C.R., A.P., C.M. and K.L. were employed by LOUNA REGENERATIVE SA (Geneva, Switzerland) during this study. Author F.P.V. was an invited scientist at LOUNA REGENERATIVE SA (Geneva, Switzerland) during this study. Author A.L. was employed by LAM Biotechnologies SA (Epalinges, Switzerland) and TEC-PHARMA SA (Bercher, Switzerland) during this study. The remaining authors declare no conflicts of interest for this study.

## Abbreviations

| | |
|---|---|
| ADSCs | adipose stem cells |
| ATMP | advanced therapy medicinal product |
| BMSCs | bone marrow stem cells |
| CD | cluster of differentiation |
| circRNA | circular RNA |
| CM | conditioned medium |
| CaHA | calcium hydroxylapatite |
| cGMPs | current good manufacturing practices |
| CHUV | Lausanne University Hospital |
| DMSO | dimethyl sulfoxide |
| DNA | deoxyribonucleic acid |
| ECM | extracellular matrix |
| EV | extracellular vesicle |
| EMA | European Medicines Agency |
| EPSCs | expanded potential stem cells |
| ESCRT | endosomal sorting complex required for transport |
| EU | European Union |
| FD-MSCs | fetal dermal mesenchymal stem cells |
| FDA | US Food and Drug Administration |
| HA | hyaluronic acid |
| hAFSCs | human amniotic fluid stem cells |
| hAMSCs | human amniotic mesenchymal stem cells |
| hCMSCs | human cord mesenchymal stem cells |
| hESCs | human embryonic stem cells |
| hUC-MSCs | human umbilical cord mesenchymal stem cells |
| HUVECs | human umbilical vein endothelial cells |
| INCI | international nomenclature of cosmetic ingredients |
| IND | investigational new drug |
| iPSCs | induced pluripotent stem cells |
| LED | light-emitting diode |
| lncRNA | long non-coding RNA |
| MenSCs | menstrual blood-derived stem cells |
| MHC | major histocompatibility complex |
| miRNA | microRNA |
| MoA | mechanism of action |
| mRNA | messenger ribonucleic acid |
| MSCs | mesenchymal stem cells |
| NLF | nuclear localization factor |
| NTA | nanoparticle tracking analysis |
| OMLP-PCs | oral mucosa lamina propria-progenitor cells |
| PRP | platelet-rich plasma |
| PBS | phosphate-buffered saline |
| PAGE | polyacrylamide gel electrophoresis |
| QA | quality assurance |
| QC | quality control |
| Rabs | GTPases families |
| RNA | ribonucleic acid |
| SCs | stem cells |
| TNF | tumor necrosis factor |
| WJ-MSCs | Wharton-jelly mesenchymal stem cells |
| UCMSCs | umbilical cord mesenchymal stromal cells |
| USA | United States of America |
| USCs | urine-derived stem cells |
| UV | ultraviolet |

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