aesthetic medicine

Review

Clinical potential of Extracellular Vesicles in Regenerative and Aesthetic Medicine

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Running Head: EVs in aesthetic medicine

Abstract

Extracellular vesicles (EVs) represent a heterogeneous class of spherical particles released by cells that are known for the essential role they play in cell to cell communication in various processes, both physiological and pathological. Over the years, they have been considered an useful tool for the diagnosis and treatment of various diseases, mainly cancer. Lately, however, their use has also extended to other fields of medicine, since they could be administered through minimally or non-invasive approaches in clinical treatments. Among those novel application fields, we find the aesthetic medicine, where the use of EVs aims to support skin rejuvenation and to improve and correct skin-related cosmetic defects, including wrinkles, hair loss, and acne scars.

This review provides a general perspective and the latest findings supporting the efficacy of EVs application in Aesthetic Medicine.

Keywords

Extracellular vesicles, rejuvenation, acne vulgaris, hair loss, scars, aging

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Introduction

Aesthetic Medicine is a practice that deals with developing and using minimally or non-invasive approaches in order to improve the aesthetic appearance, health and well-being of patients. It was born from the union of the multiple types of knowledge related to different fields of both medicine and surgery[1].
The most popular treatments in aesthetic medicine are specifically aimed to improve the appearance of the skin and eliminate aesthetic defects due to age (wrinkles, hair loss), or skin trauma, or surgical operations (scars). Although the most commonly used techniques for skin therapy include hyaluronic acid fillers, autologous fat transplant, botulinum toxin injection, and lasers, over the past decade other approaches based on biological elements had spread to skin treatments, included administration of Platelet Rich Plasma (PRP)[2-5] and Extracellular Vesicles (EVs)[6-8].
This review focuses on EVs and gives an overview of the latest evidence and benefits of their administration for aesthetic improvement.

Main skin defects treated by Aesthetic and Regenerative Medicine

Biological aging is a multifactorial and irreversible process, which involves a wide range of mechanisms that compromise normal cell functions, resulting in structural and functional alterations of organs and tissues[9,10]; on an aesthetic level, it manifests itself with skin morphological changes resulting in wrinkles, due to bone, muscle and fat alterations[11] (*Fig. 1A*). Aging is also manifested with hair thinning or loss, caused by the dysregulation of the hair follicles-stem cells and miniaturization of the hair follicles [12,13] (*Fig.1B*).
Skin lesions are a further process we are subjected to during life: they can occur as a result of physical trauma, post-surgical operations, acne, burns, etc. and can result in integral tissue repair, or in the formation of scars, the generation of which is mediated through the production of a granulation tissue and the differentiation of myofibroblasts, responsible for the deposition of collagen in wound sites and the consequent formation of scars[14]. Depending on the severity of the damage, wound healing can result in the formation of different types of scars: the simplest have a small, almost invisible line; others are abnormal tissue alterations, such as atrophic scars, scar contractures, hypertrophic scars, and keloid scars[15,16].
Among these skin lesions, the most common seem to be those induced by Acne vulgaris , a chronic inflammatory skin disease that usually occurs during puberty and lasts throughout adolescence. Face, back and chest are most affected and its onset is given by a series of multiple factors, which contribute to i) keratinization, sebum accumulation, and bacterial colonization of the skin pores; ii) formation of the whitehead which expands more and more due to the increasing accumulation of sebum and keratinization, resulting in the iii) formation of the blackhead; iv) follicular rupture which triggers an inflammatory state; v) stimulation of the wound healing process that often results in skin lesions, the most common of which are atrophic or hypertrophic scars[17] (*Fig.1C*).

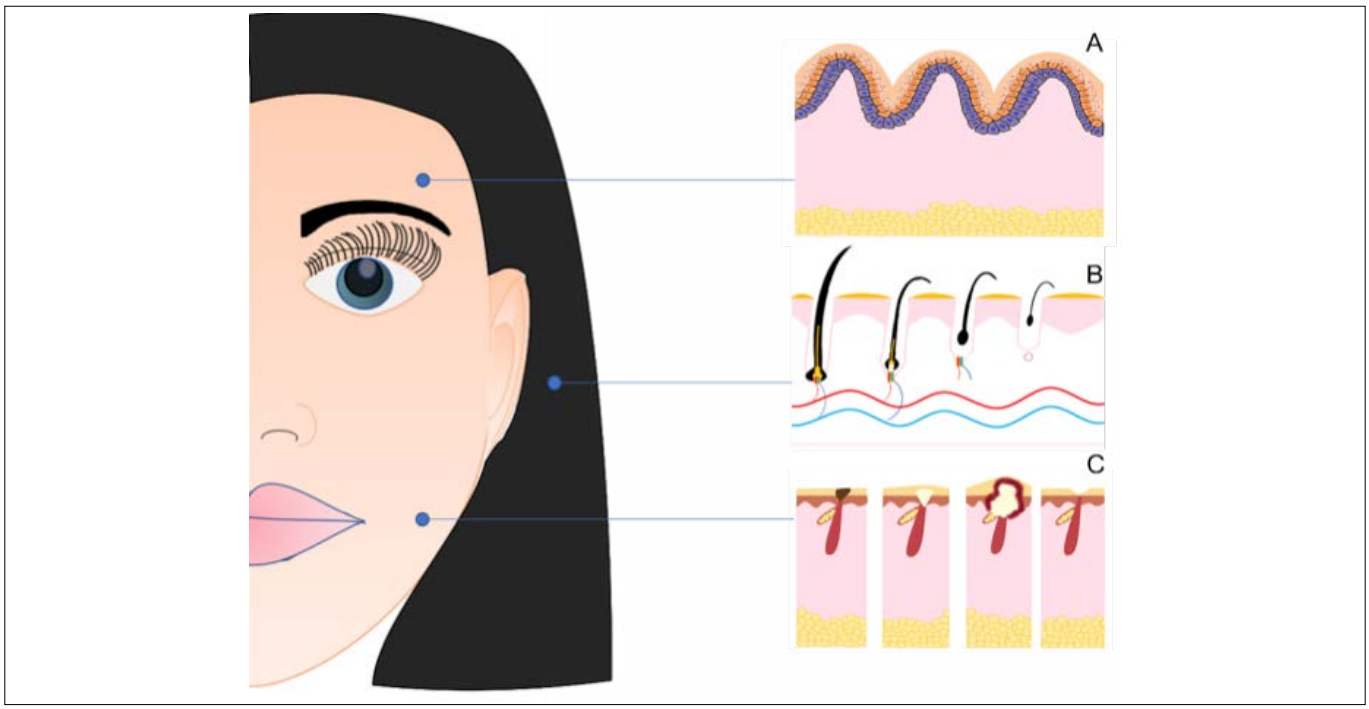

Figure 1 *- Examples of some of the most common aesthetic skin defects. A) represents how aged skin looks like: even if the segmentation of the skin layers does not change, the aged skin shows a thinning of the dermis, epidermis and elastic fibers; there is also degradation of collagen fibers, decrease of the junctions between dermis and epidermis, reduction in the vascularization of tissues and subcutaneous fat, causing a reduction in the elasticity and tone of the skin and leading to wrinkles appearing. In B) it's schematically reported how hair loss occurs: hair growth cycle is divided into 3 phases that are repeated cyclically: anagen (or growth phase), catagen (or regression phase) and telogen (or resting phase). Hair loss occurs when the period of the anagen phase reduces and the telogen phase increases, resulting in a miniaturization of the hair follicle and thinning of the hair. C) portrays how skin appears following the genesis of an acne scar. Pustule that forms gradually from a microcomedo leads to an inflammatory phase, which causes the rupture of the pustule and the attraction to the wound site of the various factors (both growth factors and cells) involved in tissue repair. The formation of the different types of scars occurs because of an overproduction of collagen at the wound site.*

Treatment	Application	Effects	Ref
Dermabrasion	- Facial Rejuvenation - Acne Scars - Scar revision - Facial Rhytids	Re-epithelialization within 7-10 days since dermabrasion. Post-treatment erythema, that usually resolves over time.	18-20
Laser Resurfacing	- Keloids - Hypertrophic Scars - Facial Rhytids - Facial Rejuvenation	IIncreasing in collagen production, decreasing in irregularities and increasing in skin firming; prolonged redness due to dispersed thermal lesions.	20,21
Chemical Peels	- Photoaging - Wrinkles - Acne	Production of collagen type 1 and 4 and elastin fibers, which contribute to the formation of new layers of epidermis; post-treatment is often characterized by inflammation that resolves over time. In the case of acne, reduction of sebum, comedones, pustules or papules.	22-24
Facial Fillers	- Acne Scars - Rhytids - Facial Sculpting / Contouring / Augmentation - Facial Rejuvenation	Depending on the fillers used, there is a different mechanism of action, which however lead to collagen synthesis as a final result, ensuring an improvement in the contours of aged or scarred skin. Side effects including erythema, edema, redness, and bruising can occur in the days post-treatment.	25-28
Micrograft	- Androgenetic alopecia	Micrografts have been shown to improve hair regrowth and density, and hair follicle development. However, further studies are needed to establish it as a safe and side-effect-free therapy.	29-31

***Table 1** - Most popular treatments in aesthetic medicine for skin rejuvenation, scars, wrinkle and androgenetic alopecia treatments; they ensure a smoother and more sculpted skin, improving its texture and contouring.*

Physical signs related to aging or skin lesions can affect a person's self-esteem, having an impact on personal relationships and social dynamics, as they cause discomfort, insecurity, and sometimes psychological problems; thus, people become more and more inclined to appeal to aesthetic treatments to improve or even correct aesthetic defects.
The most common treatments routinely used in aesthetic medicine are summarized in *Table 1*.
More recent approaches are based on regenerative medicine, a branch of medicine given by the set of biomedical approaches and clinical therapies based on the use of stem cells, scaffolds and biological molecules, capable of regenerating, repairing, or replacing parts of tissues or organs that have undergone structural and/or functional damage[32]. Among the different techniques of regenerative medicine, the attention of aesthetic medicine has turned to platelet derivatives, focusing more attentively on Platelet Rich Plasma (PRP), whose therapeutic efficacy is to be ascribed to the in loco administration of a wide range of growth factors and proteins, stored in platelets, able to support tissue repair and the regeneration processes. The administration of autologous PRP improves the skin elasticity and texture, and protects from aging and photoaging, due to the increase in elastin and collagen I expression, and in the proliferation of dermal fibroblasts, supporting the effectiveness of PRP in skin rejuvenation[2,33,34]. PRP has also shown significant effects in the treatment of hair loss since it can stimulate follicular and perifollicular angiogenesis, which is crucial for hair growth; PRP-based therapy has shown promising results in both women and men[35-37], with positive results becoming more evident 3 months after treatment, compared to the following 6-12 months[35].

Finally, the combined therapies based on PRP and Microneedling, or PRP and Fractional CO_2 laser, have been found to be useful for the treatment of acne scars: Microneedling and Fractional CO_2 laser are tecniques that remodel scars, improving their appearance and texture; the combination with PRP shows an improvement in wound healing and post-inflammatory hyperpigmentation, due to the high amount of growth factors it contains[38,39].
The administration of Extracellular vesicles is a further strategy lately taken into consideration to be used in aesthetic and regenerative medicine.

Extracellular Vesicles in Aesthetic and Regenerative Medicine

Extracellular Vesicles (EVs) are spherical particles secreted by all cells and enclosed in a phospholipid bilayer, ranging in size between 40 and 2000 nm; they mediate the intercellular communication in physiological and pathological processes and are generally classified according to their biogenesis and

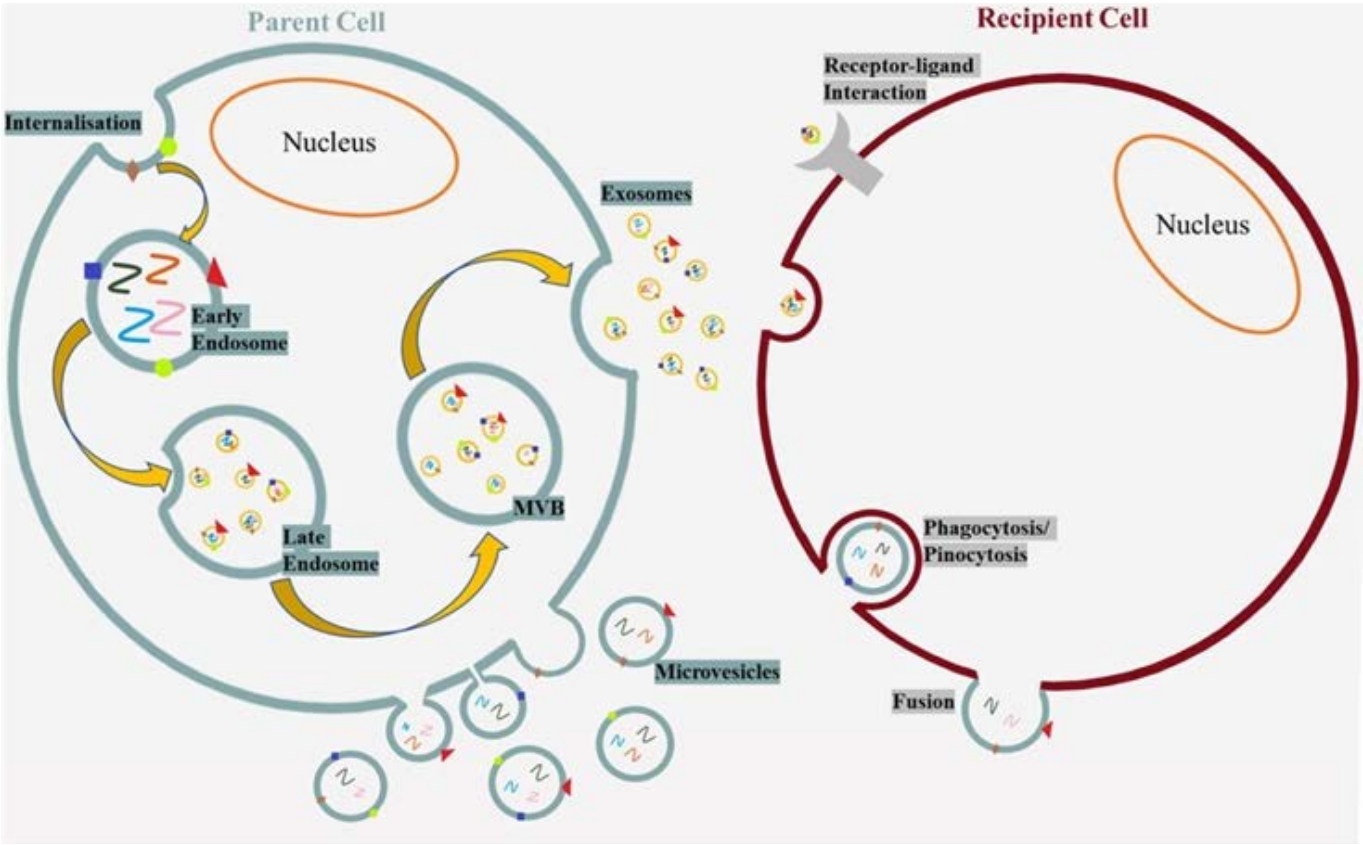

Figure 2 *- Biogenesis of Exosomes and Microvesicles, and EVs-mediated communication. Exosomes derive from the endolysosomal pathways, which involves the formation of "early endosomes" by inward budding of the cell plasma membrane; a further invagination of the endosomal membrane results in the formation of a "late endosome" leading to the generation and the release of intraluminal vesicles (ILVs) within the endosome and the formation of the so-called multivesicular bodies (MVB); the latter will fuse with lysosomes (in this case the contents in MVBs undergo degradation, not shown) or will merge with the cell membrane, releasing the ILVs, that make up the exosomes once outside the cells. The microvesicles, on the other hand, are released into the extracellular space by outward budding of the cell plasma membrane towards the extracellular space. Both exosomes and microvesicles, to carry out their role in communication, once released from the donor cell, interact with the recipient cells through various processes: phagocytosis/pinocytosis, fusion, and ligand/receptor or clathrin-mediated interaction. When this phase is finished, they can release their cargo inside the host cell, going on to perform their functions.*

size into exosomes (40-120 nm), microvesicles (50-1000 nm) and apoptotic bodies (500-2000 nm)[40] (*Fig. 2*).
While the generation of exosomes involves the endolysosomal pathways, microvesicles are formed by the outward budding of the cell membrane. Lastly, apoptotic bodies are formed from the cell surface by cytoskeletal rearrangement, through the extroflection of the membrane of apoptotic cells[40,41]; they are less frequently involved in intercellular communications, which is why, generally, only exosomes and microvesicles are referred to[42,43].
The discovery of EVs can be traced back to the first studies on blood coagulation: initially, they were observed in 1946 by Chargaff and West in plasma, as procoagulant particles of platelet origin. Later, in 1967, Wolf isolated and characterized this material from blood samples defining it as "platelet dust"[44,45]. These studies were followed by others, which led to the isolation of EVs from a variety of cell types and biological fluids and the understanding that they are not, as was initially thought, the cells' waste material, but they can rather be considered as structures used by the cell as a communication mechanism (*Figure 2*), in physiological and pathological processes[45]. No less important is their involvement in the maintenance of homeostasis, angiogenesis, coagulation, inflammation and related response, and in the management of cellular waste[44,46-49]. Therefore, given their importance in cell biology, they are important targets for the development of new non-invasive and low-toxicity therapies.
EVs-based therapies are gaining more and more attention in different fields of medicine EVs, such as regenerative medicine. From several years now, stem cell-derived EVs (SC-EVs) have been studied for their potential application in the treatment of several tissue damages, such as organ-specific injuries, brain disorders, diabetes. Mesenchymal Stem Cells (MSCs) and Adipose Stem Cells (ASCs) are the most used sources of EVs; several studies have shown how EVs derived from the latter are involved, alone or in conjunction with other bioactive molecules or biomaterials, in a variety of processes related to tissue regeneration[50-52]. In this context, among the most novel studies, Yang et al. showed how the administration of human umbilical cord-derived exosomes (hUCMSC) encapsulated in a hydrogel to an induced skin wound in a diabetic rat leads to an upregulation of growth factors – vascular endothelial growth factor (VEGF) and transforming growth factor beta-1 (TGFβ-1) -, and therefore to an acceleration in wound healing[53]. Zhang et al. instead revealed that uMSC-derived exosomes combined with a hydrogel and applied to the site of a femoral fracture in a mouse model are able to improve bone healing by stimulating angiogenesis. The same group demonstrated

in vitro that these exosomes are able to increase the expression of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor-1α (HIF-1α), and contribute to the proliferation, migration and formation of tubes in endothelial cells[54]. More recently the same author has shown in his studies how the pre-treatment with ASCs-derived EVs are effective against hepatic damage from ischemia-reperfusion, as the EVs are able to induce an increase in superoxide dismutase and a reduction in inflammatory mediators such as IL-1β and TNF-α, protecting against mitochondrial damage, apoptosis and hepatocytes from injury[55]. No less important are the studies of Chen et al. on lesions of peripheral nerves, with which they demonstrated *in vitro* that ASCs- derived EVs, internalized by Schwann cells, can promote the proliferation, migration and myelination of axons and the secretion of trophic factors, accelerating the regeneration of peripheral nerves[56].
More recently, moreover, the EVs use has also extended to the novel field of aesthetic medicine. In the skin, indeed, EVs-mediated communication helps restoring normal cellular homeostasis, playing a key role in the four stages that make up the wound healing process[57,58]; in addition, several studies have shown that EVs help restoring damages due to skin degeneration, caused by processes such as aging, skin diseases, hair loss and the generation of scars[6,7,59]. The most used sources of EVs to this purpose are represented by stem cells (particularly mesenchymal stem cells (MSCs)), plants, and platelet derivatives[6,8,60-62].

EVs and Aging

Several *in vitro* and animal model studies have demonstrated the great potential of EVs in the treatment of aging and photo-aging. The administration of MSCs-derived EVs is among the most studied applications. For example, in a study conducted on mouse models, Wang et al. have shown how engineered EVs derived from the human umbilical cord MSCs (hucMSCs) can promote the proliferation and migration of human dermal fibroblasts (HDFs) and increase the expression of ECM proteins, including collagen, elastin, and fibronectin[63]. On the other hand, Zhang showed that the combined administration to derma of EVs deriving from hucMSCs with Sponge Spicules increases the absorption of the EVs themselves in the mouse skin, improving the appearance of wrinkles, UV-induced photo-aging, and the expression of constituents of the ECM[64]. Zhao et al. investigated the effect of subcutaneous administration of the human placenta MSCs (hPMSCs)-derived EVs incorporated into a chitosan hydrogel on naturally aging mice; the chitosan hydrogel allowed a slow and prolonged release of the EVs, contributing a prolonged therapeutic effect over time. The authors demonstrated an improvement in the skin of aged mice, assuming that the effect is due to a reduction in the expression of MMPs and an increase in the expression of TIMP, as well as to a regeneration of the ECM in aged dermal fibroblasts[65].
In addition to MSCs, other sources of stem cells have been investigated as EV as a resource for the treatment of aged skin. For example, Oh et al. studied the *in vitro* effect of EVs derived from induced pluripotent human stem cells (iPSCs) on HDF naturally aged or UV-treated to induce photo-aging. Their results showed that iPSCs-derived EVs administrated to senescent HDFs increased the collagen production and, at the same time, decreased the production of senescence-associated β-Galactosidase and MMP1, MMP3 (MMPs, that have been found to be upregulated in aged fibroblasts, quicken the degradation of skin matrix, impairing the skin's innate regenerative ability)[66]. Similarly, Choi et al. demonstrated that EVs derived from the human adipose stem cells (HASC) ameliorates *in vitro* induced photo-aging on HDF by increasing levels of elastin, collagen I, II, III, and V, TIMP-1 (which inhibits MMPs), and Transforming Growth Factor-β (TGF-β) involved in the synthesis of the ECM[67].
Finally, Hu et al. demonstrated how exosomes derived from 3D HDF models provide an up-regulation of collagen I and TGF-β levels and a down-regulation of MMP-1 when administered in nude mice in which photo-aging had been induced with UV rays[68].

EVs and Hair growth

EVs have been evaluated for their involvement in the different phases of hair production, particularly since Wnt molecules, involved in hair follicle morphogenesis and growth, were found in the EVs cargo[69]. In this regard, Rajendran et al. have shown that MSCs-derived EVs can induce an *in vitro* increase in IGF-1 and VEGF levels in the cells of the dermal papilla (DP) and in the expression levels of the anti-apoptotic factor Bcl-2, which activates the pathway of MapK and Erk cell signaling; in vivo, on the other hand, the intradermal injection of MSCs-derived EVs into mice is able to induce the hair growth by increasing the levels of Wnt3 and Wnt5[70]. Similarly, they showed that also EVs derived from dermal fibroblasts can activate in vitro Wnt/β-catenin signaling pathways and induce proliferation of DP cells, demonstrating their pro-proliferative capacity in hair follicle cultures[71]. Moreover, the same group studied macrophages as an alternative source of EVs, capable of stimulating DP cells, confirming the results already obtained *in vitro* with the previously described studies, demonstrating that the Wnt contained in the EVs derived from macrophages can stimulate the Wnt/β-catenin signaling pathways and increase Bcl-2 levels, thus suggesting the therapeutic potential of these sources of EVs in the treatment of hair loss[72].
If, on the one hand, the effect on hair growth has been evaluated by the direct administration of the EVs, on the other hand studies have been conducted using microgels for the administration of EVs: Chen et al., for example, focused their attention on the administration of EVs derived from human DP cell cultures, encapsulated in oxidized sodium alginate (OSA) and its related effects. OSA ensures the prolonged release of EVs, which up-regulate the expression levels of Wnt3, b-catenin, and MMP-3, resulting in the promotion of hair growth[73].
Finally, Hu et al. isolated EVs from DP cells from 3D cultures and tested them *in vitro* and on mouse models to evaluate hair follicle production versus the only DP cell administration. They demonstrated that EVs isolated from 3D cultures induce greater hair growth, compared to control EVs isolated from 2D cultures, also because of the higher levels of β-catenin expression and the down-regulation of inhibitors of the Wnt factors,

resulting from their administration. These results are because EVs from 3D cultures contained higher levels of miR-218-5p, which is essential for the development of hair follicles and, consequently, for hair growth[74].

EVs and Scars

The role of EVs in wound healing processes is well known since they are important for communication in all healing phases; the main sources of EVs in this context are represented by neutrophils, macrophages, platelets, and MSCs. Therefore, EVs have been considered as potential non-toxic therapeutic agents for the treatment of skin wounds, and in particular for the treatment of scars[57,75].

Zhu et al. described how the administration of EVs derived from human adipose stem cells (hASCs) prevented the differentiation of myofibroblasts and the synthesis of collagen I, and thus the formation of scars in rabbits in which a wound had been produced[76].

Furthermore, in vivo, the application of a gel consisting of exosomes derived from hASC after laser treatment, showed a remarkable improvement in the healing of acne scars and accelerated healing, with a decrease of post-treatment side effects, such as edema, pain, dryness, or erythema, compared to treatments carried out with the administration of a gel without exosomes[77].

Despite some of the studies that demonstrate the therapeutic potential of EVs in tissue repair, it has also been shown that their efficacy is sometimes limited due to the reduced half-life and to the rapid clearance of these particles[78-81], forcing to multiple administrations for treatment. An alternative to this problem is given by the administration, in situ or subcutaneously, of hydrogels in which the EVs are encapsulated, ensuring a prolonged release over time and consequently greater efficacy[82].

Plant derived EVs

Even if most sources of EVs are human-derived, in recent decades the isolation and use of plant-derived EVs has become increasingly widespread, as they have different therapeutic properties. Despite their discovery dates back to 1967[83], only more recently plant-derived EVs are gaining medical relevance. In plants the EVs play two fundamental roles: i) defense against pathogens; ii) regulation of communication and nutrient exchange in plant-microbial symbiosis processes[84,85]. Studies conducted in animal models have shown that the EVs of grapes, strawberries, broccoli, grapefruit, ginger, carrot and orange, have mainly antioxidant and anti-inflammatory activities for different types of human pathologies[86,87]. Furthermore, studies conducted on citrus fruits have shown that the EVs derived from them have anti-neoplastic activities in different types of tumors, although the mechanisms of action are still uncertain[88-90].

In the dermatological field there is still a limited number of studies concerning plant-derived EVs for therapeutic purposes. Şahin et al. demonstrated *in vitro* how the exosomes derived from wheat contribute positively to the healing processes of skin wounds: the tests showed how exosomes of wheat increase the expression and production of collagen I, and the proliferation and migration of fibroblasts in wound healing. They also have a pro-angiogenic effect, due to the increase in the formation of tubular structures *in vitro*[91]. Moreover, Cho demonstrated *in vitro* how EVs derived from ginseng roots have positive effects on senescent fibroblasts of the human dermis, as they are capable of downregulating the activity of β-gal associated with senescence[92].

Conclusion

This review aimed to provide a general overview of the latest findings on EVs supporting the potential application as new potential therapeutic agents in the field of aesthetic medicine, and particularly in rejuvenation, photo-aging, scarring and hair loss. Although it has been established that EVs are promising, non-invasive tools for the treatment of aesthetic defects, as they show good therapeutic efficacy, further studies are needed to clarify their mechanisms of action and improve the degree of purification and isolation.

Above all, it is needed to clarify whether their administration can lead, in the long term, to the onset of any side effects or adverse events, which can affect the patient's health.

Conflict of interest disclosure

The Authors declare that they have no conflict of interest.

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