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Review Article

The Role of Extracellular Vesicles in the Skin and Their Interactions with Nanoparticles

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Abstract

Extracellular vesicles (EVs) include exosomes and microvesicles. They are released from cells under both physiological and pathological conditions. EVs can be isolated from a host of biological mediums, such as blood plasma, saliva, and skin. The role of EVs and their contents, including RNA, proteins, and signaling molecules, depends on the specific cells and organs from which they are derived and the diseased state. EVs play a key role in cell-to-cell communication. Although the role of EVs in skin biology is a developing field, recent literature suggests they play an important role in skin homeostasis, disease, and transdermal drug delivery. EVs have been shown to modulate skin pigmentation, aid in the cutaneous wound healing process and in the secretion of nanoparticles. This paper reviews the basics of EV biogenesis, their isolation and their role in the skin. We also review what is currently known about how nanoparticles may impact the contents of EVs in the skin.

Extracellular Vesicles – Biogenesis and Cargo

Extracellular vesicles (EVs) fall into two main categories, exosomes and microvesicles (MVs). They differ in their size, their formation mechanism, and consequently the cargo they may carry [1]. Exosomes are intraluminal vesicles formed inside multivesicular Bodies (MVBs), a subset of late endosomes that commonly range from 30-150 nm in size. They are released into the extracellular environment by MVB fusion with the plasma cell membrane [2-5] as depicted in Figure 1**.** Microvesicles (MVs) differ from exosomes in two principal ways: size and biogenesis. Literature suggests that MVs are larger than exosomes, with measurements ranging from 100 nm up to 1 μm [6,7]. Unlike the intracellular development of exosomes, MVs are released directly from the cell as a result of outward plasma membrane blebbing. Due to their larger size, MVs are easier to isolate than exosomes. Since the biogenesis of exosomes and MVs differ it is thought that they may carry membrane specific putative markers such as endosomal associated proteins (CD61, CD63) for exosomes and common plasma membrane anchor proteins (CD73, ARRCD1) for MVs [8,9]. However, discovery of distinct differential markers remains a challenge [10,11]. While little is currently understood about the mechanisms of how cargo is packaged into EVs, it is widely appreciated that exosomes and microvesicles carry functional mRNA, miRNA, lipids, proteins, and other signaling molecules that can alter cell function when they are taken up [4,12,13]. It is thought that there are three main mechanisms by which EVs facilitate intercellular communication [14]. The first is by direct EV-cell contact, similar to a ligand-receptor binding interaction that may trigger a signaling event and/or endosomal uptake. However, little is known about which proteins drive this type of interaction and if endocytosis occurs, how the EV cargo is delivered; directly into the cytosol or via endo-lysosomal escape [9,15]. Studies suggest that exosomes can efficiently enter cells as intact vesicles by surfing on filopodia that pull them into an endocytic vesicle. They are then transported to the endoplasmic reticulum membrane [16]. The second mechanism EVs use to facilitate intercellular communication is through phagocytosis. The third is by direct fusion of the EV with the cell membrane [4]. Similar to apoptotic cells, phosphatidyl serines are highly expressed on the lipid bilayers of EVs providing an "eat me" signal to phagocytic cells [17]. While nonspecific cell fusion may occur due to the similarity in the composition of EV and cell phospholipid bilayers, the predominant theory is that fusion requires the expression of specific proteins

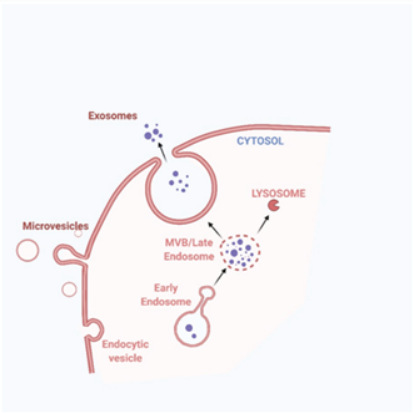

Figure 1: **Formation of Microvesicles and Exosomes.**
Exosomes are formed when an early endosome is transformed into a late endosome, or MVB. The MVB can then turn into a lysosome or release the exosomes into the extracellular environment. Microvesicles are formed from the plasma membrane blebbing outward.
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(e.g. syncytins) on the EVs [10]. As the EV and the cell interact, EV trans-membrane bound proteins bind cell receptors and structurally rearrange to insert hydrophobic residues into the plasma membrane. This induces reorganization in the lipid bi-layer forming a pore through which the EV cargo is released directly into the cytoplasm. While the mechanisms of exosome cell uptake are yet to be fully elucidated, they largely depend on exosome-cell-specific interactions to provide an efficient means for cell-to-cell communication [9].

Extracellular Vesicles – Isolation

EVs can be isolated from blood, cell culture supernatants, and from tissues [18,19]. The isolation methods vary in complexity depending upon the sample type as do the number of EVs that can be obtained. EVs are more readily obtained from liquid samples, but methods exist to extract them from solid tissues, such as the skin [19-21]. Current methods for isolating EVs routinely rely on differential ultracentrifugation, sucrose gradient ultracentrifugation, and commercial affinity capture columns [22-25]. However, many novel methods are under development using, for example, nanomembranes, nanowires, and microfluidic technologies [26-29]. Since microvesicles and exosomes differ considerably in size, the protocols used vary depending on whether isolation of microvesicles or exosomes is desired. Mass-dependent differential centrifugation protocols are commonly used to isolate extracellular vesicles. Typically, cell debris is removed at 500 g - 2,000 g, followed by isolation of MVs at 10,000 g, and then collection of exosomes by ultracentrifugation for many hours at 100,000 g. Isolation of larger MVs is possible at slower speeds, which also helps to prevent their fragmentation. After exosome isolation by ultracentrifugation, the supernatant is discarded to remove proteins but often protein aggregates pellet with the exosomes [23]. Hence, post-isolation steps may be performed using affinity columns or flow cytometry to purity exosomes based on expression of surface markers such as CD63 [4]. While CD63 is a common EV marker, other exosome makers include Alix, flotillin, Tsg101, and tetraspanins [30]. Markers for microvesicles include integrins, selectins, and CD40 [30]. Sucrose gradient ultracentrifugation is a preferred method for isolating exosomes because they have a density range of 1.13 to 1.22 g/mL. This allows them to float on a sucrose gradient, making it easier to separate them from extracellular debris and protein aggregates [1]. This process not only increases sample purity, but also reduces the stress of centripetal force on the sample. However, the downside of using sucrose gradient ultracentrifugation is that it can result in significant loss of EVs [23]. While these methods are currently the most widely preferred in the field, there is no accepted standard and isolation results depend on the type of equipment used [25]. Hence, further experimentation is required to establish the ideal isolation technique for standardization.

Extracellular Vesicles – In Skin Homeostasis and Disease

While there is still much to be learned about the basics of EV biogenesis and the mechanisms of cell uptake, their role in cell-to-cell communication in homeostasis and diseases is well established in many organ systems including the skin. EVs are released and exchanged between skin cells including keratinocytes, melanocytes, fibroblasts, and mast cells. For example, keratinocytes utilize exosomes to transfer miRNA to modulate pigmentation in the skin[3]. When exposed to UVB radiation, keratinocytes secrete exosomes containing miRNAs that when taken up by melanocytes can modulate tyrosinase activity to induce pigmentation [31]. Cicero observed that more than 30 different miRNAs were differentially expressed between Caucasian and Black keratinocyte secreted exosomes [31]. Keratinocyte-derived exosomes play an important role in cutaneous wound healing [21]. Exosomes isolated from a skin wound edge were compositionally different than those isolated from normal skin. They contained more surface N-glycans that promoted selective uptake by macrophages, converting them to a proresolving phenotype via the specific miRNA cargo. Studies demonstrate that the number of microvesicles produced by human keratinocytes varies in UVB dose-dependent fashion. Treatment with a UVB blocking agents decreased the release of microvesicles [32,33]. Lipid mediators such as Platelet-Activating Factor (PAF) and other PAF-receptor (PAF-R) agonists play a pivotal role in systemic immunosuppression induced by skin exposure to UVB or cigarette smoke [34,35]. Other studies showed keratinocytes release exosomes containing cytoplasmic 14-3-3 proteins that stimulate dermal fibroblasts [36]. These studies found that the concentration of the 14-3-3 protein isoforms depended on the differentiation state of the keratinocytes with more isoforms being present in exosomes secreted from cells cultured in high calcium differentiating media [37]. Exosomes released from keratinocytes can stimulate cutaneous and subcutaneous immune activity. PAF-R agonists are packaged in microvesicles secreted from keratinocytes that have undergone stress [32,33]. Microvesicles that contain PAF-R agonists can act locally in the skin by communication with mast cells or systemically to affect Treg generation to induce immunosuppressive responses [34,38]. Studies conducted with murine keratinocyte exosomes suggest that they were able to increase the expression of CD40 and the secretion of proinflammatory cytokines, leading to the enhanced maturation of dendritic cells [39]. Furthermore, neutrophils can endocytose keratinocyte derived exosomes. When neutrophils endocytose exosomes secreted from keratinocytes treated with cytokines (IL-17A, IL-22, IFN-γ, TNF-α) they induced neutrophil NETosis and expression of IL-6, IL-8, and TNF-α [40]. This observation was confirmed in vivo using an imiquimod-induced psoriasis mouse model that found keratinocyte derived exosomes expressed proinflammatory factors that activated neutrophils in the skin [40]. Exosomes play a key role in driving skin cancers. In the tumor microenvironment, exosomes are plentiful, and have an impact on the pathogenesis of the disease by optimizing the microenvironment for tumor progression and metastasis in melanoma [41]. Godert et al. [42] showed drug resistance in melanoma can be aided by exosome release. Detecting exosomes in early stages is one way to predict resistance. EVs are also associated with carcinoma pathogenesis. Studies focused on cutaneous Squamous Cell Carcinoma (SCC) found EVs can modulate the tumor microenvironment to produce deleterious effects [43,44]. Results found that Dsg2, a desmosomal cadherin often overexpressed in SCC, upregulated EV secretion enriched with the C-terminal fragment of Dsg2 and pro-mitogenic cargo [43]. Palmitoylation of Dsg2 was required for EV biogenesis. and pharmacologic inhibition of Dsg2 mediated EVs abrogated tumorigenesis in a mouse model due to suppression of miR-146a cargo and IL-8 signaling [44].

Extracellular Vesicles – Role in Nanoparticle Transport and Drug Delivery

Nanoparticles are currently used in a wide variety of skincare products including sunscreens, anti-wrinkle creams, and moisturizers [45,46]. The ability of nanoparticles to penetrate the skin is size-dependent [47]. However, many studies have shown that nanoparticles can more readily penetrate through barrier-disrupted skin [48-50]. Nanoparticles are capable of modulating skin immune responses in the context of skin allergy [51-53]. Nanoparticle uptake into EVs

is currently an unexplored and important possible mechanism for modulation of skin immunity. There is evidence to indicate that EVs and nanoparticles can interact. Recent literature suggests that gold and iron oxide nanoparticles can be endocytosed through macrophages and released through exosomes [54,55]. Another recent study showed that mesenchymal stem cells could uptake gold nanoparticles and secrete them in exosomes [56]. EVs and nanoparticles are widely being exploited for drug delivery in cancer [57,58]. However, it has yet to be examined how nanoparticles interact with or alter EV formation in the skin. Given their size, physiochemical properties, and function in terms of transferring genetic material and other signaling molecules, exosomes may be optimal for use in Transdermal Drug Delivery (TDD). Currently, liposomes are highly exploited as TDD systems [59]. Solid lipid microparticles can also be used as a TDD system [60,61]. Liposomes and exosomes are similar in that they are both composed of (phospho) lipids [62,63]. However, the primary drawbacks of using liposomal delivery are its inability to target a specific skin layer and the transfer of the liposomes systemically. Exosomes exhibit cell tropism, which makes them ideal for targeting tissues [64]. In addition, unlike liposomes, the biogenesis of exosomes carries targeting information from the cell of origin, which can be exploited by targeting distinct tissues and potentially skin layers [56]. Exosomes are able to bypass barriers, making them excellent vehicles for targeted TDD. Studies have shown that exosomes have a homing selectivity [56,64], as observed in neuron [65] and cancer [66] studies, making them highly attractive for targeted drug delivery [67,68]. Furthermore, immunogenicity can be reduced significantly by using self-derived exosomes for drug delivery, which is a unique and distinct property of exosomes that delineates them from liposomes and makes them prime candidates for precision medicine [65,67]. However, it should be noted that exosome production and isolation via immortalized cell lines may present challenges in avoiding immune detection. Furthermore, it should also be noted that unlike liposomes and solid lipid microparticles, exosomes are generated from cells, providing them with the potential for genetic alteration of their original cells to change their contents. Exosomes have been studied as drug delivery systems because of their ability to carry proteins and RNA, which are difficult to deliver via liposomes. While liposomes must be internalized via specific pathways, exosomes have unique binding properties that allow them to be internalized into endosomes, presenting an alternate mechanism for delivery of RNA [62]. Exosomes have the potential to become a valuable tool for TDD, as they are less immunogenic and cytotoxic, and have high drug-carrying capacity [69]. Currently, there is a deficit of any clinical trials, development of drugs, or literature relating to the development of EVs for TDD. This is largely due to inadequate and nonstandardized isolation and characterization methods for EVs. Nonetheless, EVs have the potential to be loaded with drugs either before or after secretion [27]. If loaded before secretion, transfection and co-incubation can be used. If loaded after secretion, direct mixing or electroporation can be utilized. Future studies should focus on progress towards solving the aforementioned issues surrounding the application of EVs in TDD.

Conclusion

Current research has broadly focused on determining the role of EVs in disease states such as cancer. However, further emphasis should be placed on clarifying the biological pathways and mechanisms that lead to EV biogenesis in the skin, how EVs affect dermatological processes, and their interactions with nanoparticles. Previous studies have illuminated the types of information that can be contained in EVs, but application of this information requires further exploration. Identification of specific biomarkers for both microvesicles and exosomes will aid in isolation, purification, and characterization. Prior to EV characterization, a standard of post-isolation purity must be established. Currently, methods allow for the determination of the contents of EVs, and some have been found to contain signaling molecules. Yet systematic definitions of all the individual functions of EV cargo have not been elucidated. Once such a taxonomy has been constructed, further work can be done to examine the impact of nanoparticles on EV biogenesis and the composition of their contents.

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