Extracellular Vesicles as Biomarkers and Therapeutics in Dermatology: A Focus on Exosomes

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Extracellular vesicles (exosomes, microvesicles, and apoptotic bodies) are ubiquitous in human tissues, circulation, and body fluids. Of these vesicles, exosomes are of growing interest among investigators across multiple fields, including dermatology. The characteristics of exosomes, their associated cargo (nucleic acids, proteins, and lipids), and downstream functions are vastly different, depending on the cell origin. Here, we review concepts in extracellular vesicle biology, with a focus on exosomes, highlighting recent studies in the field of dermatology. Furthermore, we highlight emerging technical issues associated with isolating and measuring exosomes. Extracellular vesicles, including exosomes, have immediate potential for serving as biomarkers and therapeutics in dermatology over the next decade.

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INTRODUCTION

Cell-to-cell communication is vital for human development and survival. Intercellular communication is regulated by extracellular hormones, metabolites, and cytokines or direct cell-to-cell contact. Still, there is an increasingly recognized role of extracellular vesicles (EVs) as "masters of intercellular communication" (Pitt et al., 2016b). EVs ("exosomes," "microvesicles," and "apoptotic bodies") carry complex cargo, including messenger RNAs, microRNAs, long noncoding RNAs, mitochondrial DNAs, single-stranded DNA, double-stranded DNAs, protein ligands, receptors, and transcription factors (Gross et al., 2012; Kalra et al., 2012; Keerthikumar et al., 2016; Li et al., 2014; Pitt et al., 2016b; Théry et al., 2002; Valadi et al., 2007). In this review, we highlight extracellular vesicle biogenesis, focusing on exosomes, in applications to dermatology. We discuss technical challenges in studying exosomes and future avenues for the development of EVs as biomarkers and therapeutics.

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Abbreviations: AB, apoptotic body; ESCRT, endosomal sorting complex required for transport; EV, extracellular vesicle; ILV, intraluminal vesicle; MCC, Merkel cell carcinoma; MSC, mesenchymal stem cell; MVB, multivesicular body; PBMC, peripheral blood mononuclear cell

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CURRENT DEFINITIONS, BIOSYNTHESIS, AND COMPOSITION OF EVs

EVs are similar to miniature versions of the parent cell, made of a lipid bilayer containing proteins and nucleic acids (Pitt et al., 2016b; Théry et al., 2001). EVs have been classically divided into broad categories: (i) exosomes, (ii) microvesicles, and (iii) apoptotic bodies (Figure 1).

Exosomes

Exosomes, mostly sized between 30 and 150 nm, are generated within multivesicular bodies (MVBs), which are endosome compartments in which "intraluminal vesicles" (ILVs) develop; these ILVs later become secreted into the extracellular space as "exosomes" (Pitt et al., 2016b). Exosomes are as durable as viruses, with the ability to deform elastically while maintaining vesicle integrity (Calo et al., 2014; Riazifar et al., 2017). Members of the Rab GTPase family modulate exosome secretion and act on different MVBs along "endosomal sorting complex required for transport" (ESCRT)-dependent and -independent pathways (Xu et al., 2016). ILVs within MVBs form when RAB5-positive early endosomes interact with the ESCRT complexes (which include ESCRT-0, -I, -II, and -III, each controlling different steps in cargo selection and exosome formation) (Henne et al., 2011; Hurley, 2008; McGough and Vincent, 2016). These specialized proteins recognize cargo marked for sorting (Piper, 2007). MVBs with ILVs can be targeted to lysosomes or transferred to the plasma membrane (involving RAB 11, RAB27, and RAB35) where SNARE proteins can promote exosome release (Cai et al., 2007; McGough and Vincent, 2016; Piper, 2007). Exosomes are highly enriched in certain tetraspanins, such as CD63, CD81, and CD9. Endosome-related protein TSG101 is a recognized marker (Raposo and Stoorvogel, 2013; van Niel et al., 2006). The protein ALIX also marks exosomes, as an adapter protein in cooperation with ESCRT complexes, contributing to ubiquitylation-independent cargo sorting into MVBs.

An international web-based collection of exosome cargo from various cell types is listed at EXOCARTA (http://www.exocarta.org). Although much of the cargo is interior, some proteins may exist exteriorly. Lipophilic morphogens, such as Wnts and hedgehogs, may exist on or in exosomes; however, this is not agreed on (Gross et al., 2012; Mittelbrunn et al., 2015; Riazifar et al., 2017). The profile of lipids on different types of exosomes depends on the cell producer, and the activity of membrane lipid scramblases, flippases, and floppases (Riazifar et al., 2017). How different

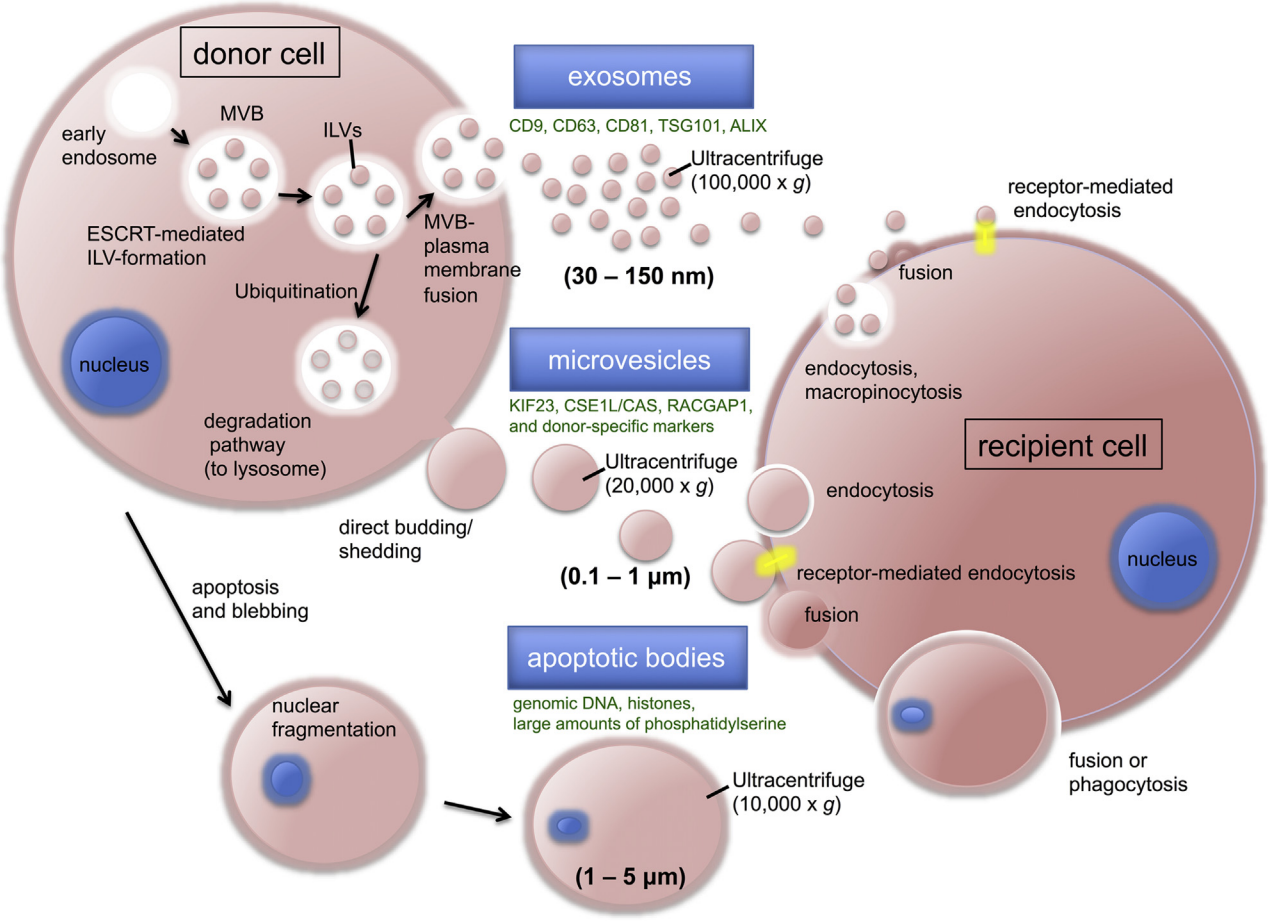

Figure 1. Three types of extracellular vesicles (EVs). Exosomes form in structures called multivesicular bodies (MVBs) derived from endosomes, and are typically marked by CD63, CD9, CD81, TSG101, and ALIX. Exosomes form inside of MVBs as intraluminal vesicles (ILVs) via ESCRT proteins sorted via RAB-mediated processes to be released at the plasma membrane, a process regulated by SNAREs. Microvesicles are released directly from the plasma membrane (direct shedding), which might be regulated, at least in part, by ESCRT-I, ADP-ribosylation factor 6 (ARF6), and/or acid sphingomyelinase (aSMase). Microvesicles may be marked by kinesin-like protein (KIF23), exportin-2/chromosome segregation like-1 protein (CSE1L/CAS), and Rac GTPase-activating protein 1 (RACGAP1). Apoptotic bodies are the result of apoptosis-driven cell fragmentation, containing genomic DNA and histones, and can contain the types of inner vesicle cargo that exosomes and microvesicles contain. Once released into the extracellular space, EVs can carry nucleic acids, proteins, lipids, and other associated molecules to cause broad effects in the recipient cells, including activation of cell signaling at the surface or regulation of molecules inside the recipient cell. Recipient cells uptake vesicles via fusion, endocytosis of all types, including macropinocytosis and receptor-mediated endocytosis. The biological functions of EVs are numerous, and largely depend on the cargo delivered to the recipient cell. Not shown: the "recipient cell" can become "donor cell," establishing a crosstalk in the other direction, consisting of vesicle exchanges that mediate complex biological processes (see Supplementary Table S1 online). ESCRT, endosomal sorting complex required for transport.

exosome-lipid compositions might affect the affinity for different lipophilic proteins is unknown. Cholesterol and sphingolipids enable tight lipid packaging and structural rigidity of exosomes (Yanez-Mo et al., 2015). It will be important to study how lipid compositions differ in various exosome populations and what, if any, effects these differences may have on functional outcomes in dermatologic studies.

Microvesicles

Microvesicles are formed by direct shedding from the plasma membrane via outward invaginations. Microvesicles contain plasma membrane proteins, cytosolic proteins, nucleic acids, and other small molecules (Riazifar et al., 2017). Their sizes average approximately 200 nm, but range from 50 to 1500 nm (Pitt et al., 2016b; Raposo and Stoorvogel, 2013; Xu et al., 2016). Much is still not known regarding microvesicle biogenesis, although ARF6, acid sphingomyelinase activity, and ESCRT proteins are thought to be involved (Xu et al., 2016). Because microvesicles originate from plasma membrane pinching, microvesicles "pick up" cytosolic cargo on the periphery of the cell before secretion, whereas exosomes contain cytosolic contents accumulated near the formation of ILVs in MVBs (Riazifar et al., 2017).

Apoptotic bodies

Apoptotic bodies (ABs) are released during cell apoptosis, resulting in vesicles composed of organelle and plasma membranes with nuclear and cytoplasmic contents (Riazifar et al., 2017). ABs are larger than exosomes and microvesicles, typically ranging from 1,000 to 5,000 nm. Phagocytes usually take up ABs after being shed and eliminated rapidly from the human circulation, but can interact with many recipient cells before phagocytosis (Pitt et al., 2016b; Raposo and Stoorvogel, 2013; Xu et al., 2016). Uptake of

ABs can occur by macrophages but also by parenchymal cells, such as fibroblasts (Riazifar et al., 2017; Tso et al., 2010). ABs are more likely to contain genomic DNA and histones and can play a role in apoptosis-induced proliferation and regulation of immune responses (Ryoo and Bergmann, 2012). They carry large amounts of phosphatidylserine and annexin to recruit and activate phagocytosis (Poon et al., 2014). Uptake of ABs by recipient cells might affect their differentiation via transfer of nucleic acids and transcription factors. Because of their large size distribution and wide-ranging cargos, ABs have great potential for numerous functional outcomes to recipient cells.

Exosome RNA cargo

Functional messenger RNA and miRNAs can be transferred via exosomes (Valadi et al., 2007). All RNA species are thought to be inside of exosomes, including messenger, ribosomal and transfer RNA, long noncoding RNA, microRNA, picoRNA, vaultRNA, and Y-RNA (Riazifar et al., 2017). Exosome RNA cargo can mediate recipient cell functions via transcriptional, posttranscriptional, and epigenetic modalities. Functional RNA in cells was first described in 2006 in murine stem cell-derived exosomes (Ratajczak et al., 2006; Yanez-Mo et al., 2015). Most RNAs within exosomes are less than 700 nt (nucleotide) (Batagov and Kurochkin, 2013; Chen et al., 2010; Yanez-Mo et al., 2015). Exosomes have been shown to contain intact mRNA, but there is an enrichment of 3′ untranslated region mRNA fragments; it is unclear if this represents degraded mature mRNA, or if these fragments serve a purpose of regulating stability and translation in recipient cells. There is evidence suggesting that the 3′ untranslated region of mRNAs acts as a 25-nt "zipcode" (with a short CTGCC domain) sequence to target mRNAs into exosomes (Bolukbasi et al., 2012). Functional transfer of RNA has been suggested when exosomes enriched in Oct4 mRNA resulted in an increase in Oct4 protein expression in bone marrow cells (Ratajczak et al., 2006; Yanez-Mo et al., 2015).

MicroRNAs are approximately 20–21 nt regulatory molecules that are transcribed as pri-miRNAs, cleaved by Dicer into pre-miRNAs, bound by Ago proteins, and loaded into the miRNA-induced silencing complex for regulation of target mRNAs (Yanez-Mo et al., 2015). miRNA-induced silencing complexes have been shown to accumulate at sites of MVBs, suggesting that miRNA can be easily incorporated into ILVs (Gibbings et al., 2009; Yanez-Mo et al., 2015). The loading of miRNAs into exosomes may be regulated by heterogeneous nuclear ribonucleoprotein A2B1 (Gibbings et al., 2009; Yanez-Mo et al., 2015). It is currently unclear how exosomal miRNA cargo varies from different skin cell types; however, recent reports reveal that miRNAs play significant roles in dermatology (reviewed in this article).

Biodistribution and uptake of exosomes

Cells can internalize exosomes through (i) direct membrane fusion, (ii) clathrin-dependent endocytosis, (iii) macropinocytosis, and (iv) receptor-mediated endocytosis (Riazifar et al., 2017). The balance between exosomes generated and cleared by the body determines the level of exosomes in circulation. Some studies suggest that exosomes are cleared from the circulation within 30 minutes in normal physiologic conditions (Takahashi et al., 2013; Willekens et al., 2005). The interaction and uptake of exosomes may be facilitated in part by intercellular adhesion molecules and integrins (Nolte-'t Hoen et al., 2009; Yanez-Mo et al., 2015). The uptake of exosomes appears to be largely dependent on exosome-cell-specific interactions, highlighting the need for more studies on the uptake of exosomes derived from different cell types.

EXOSOMES IN CUTANEOUS HOMEOSTASIS, PATHOLOGY, AND REPAIR

Exosomes are important regulators of skin homeostasis and pathology. Below, we highlight some important examples of how exosomes participate in skin homeostasis, disease, and repair.

Cutaneous immune responses

Immunologists and neuroscientists have long been interested in exosomes as mediators of immune cell and neural cell crosstalk. The skin relies on both the nervous system and the immune system for essential functions, such as thermoregulation and infection control. During infection, exosomes can convey pathogen-derived proteins, nucleic acids, lipids, and carbohydrates, serving as antigen presenters, turning on innate immune receptors to induce host defense and immunity. These qualities make exosomes potential components of immune-based therapies and vaccine preparations (Schorey and Harding, 2016).

Keratinocytes can regulate cutaneous immunity through exosomes. A study involving murine keratinocyte exosomes showed that they could stimulate dendritic cells to develop a mature phenotype, increasing CD40 expression and producing IL-6, IL-10, and IL-12 (Kotzerke et al., 2013). Circulating immune cells, such as monocytes, have been shown to release exosomes containing 14-3-3 proteins to increase matrix metalloproteinase-1 in dermal fibroblasts and facilitate cell migration (Medina and Ghahary, 2010). Exosomes from parasites can also regulate infection control. In Leishmania infection, when exosomes are released, they turn on HSP100/gp63 and activate dendritic cells and macrophages toward fighting off the infection (Schorey and Harding, 2016). Thus, from host to pathogen, exosomes regulate important crosstalk in the skin to control infection.

Immune cell-derived exosomes have been used as cancer therapeutics. Dendritic cell-derived exosomes in phase I and II clinical trials appear to stimulate T cells to fight tumor cells in metastatic melanoma, nonsquamous cell lung carcinoma, and advanced colorectal cancer with good safety profiles (Besse et al., 2016; Dai et al., 2008; Escudier et al., 2005; Pitt et al., 2016a). Future studies should focus on how to further develop immune cell exosomes as biomarkers and therapies for infection, autoimmunity, and cancer.

Cutaneous pigmentation

Human keratinocytes release exosomes that modulate pigmentation. Exosomes carry specific miRNAs to melanocytes and modulate pigmentation status by altering gene expression and tyrosinase activity (Kim et al., 2014; Lo Cicero et al., 2015). Alternatively, one study showed how keratinocytes could inhibit melanogenesis: keratinocyte exosome-derived miR-675 decreased microphthalmia-associated

transcription factor levels, the master transcriptional regulator of melanogenesis, in melanocytes (Kim et al., 2014). Another study revealed how keratinocyte exosomes turn on pigment production: keratinocyte exosomes stimulated with UVB increased tyrosinase activity in melanocytes (Lo Cicero et al., 2015). Furthermore, transfection of melanocytes with specific keratinocyte pre-miRNAs increased melanin production and gene expression of microphthalmia-associated transcription factor (Lo Cicero et al., 2015). Thus, keratinocyte exosomes have roles in delivering RNA cargo to alter melanocyte pigment production in microphthalmia-associated transcription factor-dependent and microphthalmia-associated transcription factor-independent pathways (Lo Cicero et al., 2015). More studies of this nature are required to gain insight into how exosomes might be used to manipulate pigmentation in hypo- and hyperpigmentation disorders (Lo Cicero et al., 2015).

Melanoma

Exosomes from malignant cells can influence benign cells to acquire metastatic characteristics (Pitt et al., 2016b; Zomer et al., 2015). Isola et al. provide a comprehensive review of exosomes in melanoma progression. In brief, exosomes are released by primary tumor melanoma cells and communicate with the microenvironment (Isola et al., 2016; Valadi et al., 2007). Melanoma-derived exosomes, once in circulation, can home toward sites of metastasis, resulting in vascular leakage and immune cell recruitment, which are processes in pre-metastatic niche formation (Peinado et al., 2012). A major search is on to establish melanoma-specific exosome markers. Detection of exosomal miRNAs in the plasma of metastatic melanoma patients provided a source of useful biomarkers to predict disease progression (Mirzaei et al., 2016; Pfeffer et al., 2015). Exosomes derived from patients with melanoma contained higher concentrations of melanoma inhibitory activity and S100B secreted by malignant melanoma cells (Alegre et al., 2016; Isola et al., 2016). Exosomal miR-17, -19a, -21, -126, and -149 were also increased in patients with melanoma (Pfeffer et al., 2015). MiR-222 within melanoma-derived exosomes was proportional to the metastatic ability of melanoma cell lines (Felicetti et al., 2016; Isola et al., 2016). Thus, these are examples of studies identifying candidate exosome biomarkers in melanoma, with potential for development as screening tools in dermatology.

Merkel cell carcinoma (MCC)

MCC is an aggressive skin cancer with a poor prognosis. Biomarkers for early detection of MCC and response to treatment are needed. A proteomics investigation examined exosomes from four different MCC cell lines and identified 164 common proteins, many of which were implicated in motility, metastasis, and tumor progression, including integrins, tetraspanins, intracellular signaling molecules, chaperones, proteasome components, and factors involved in translation (Konstantinell et al., 2016). This study illustrates the importance of gaining insights into exosome protein cargo, laying foundations for identifying exosome proteins that could be measured in "liquid biopsy" for prognostic and diagnostic biomarkers related to MCC progression.

Psoriasis

Psoriasis results, in part, from dysfunction of the immune system. Exosomes can modulate production of pro-psoriatic cytokines. Phospholipase A_2 is highly expressed in psoriatic lesions, and it was recently found that mast cells produced exosomes containing phospholipase A_2, generating neolipid antigens and recognition by CD1a-reactive T cells, resulting in the production of IL-22 and IL-17a (Cheung et al., 2016). Circulating and skin-derived T cells from patients with psoriasis revealed increased sensitivity to phospholipase A_2 versus healthy control T cells. These findings suggest mast cell exosomes are important mediators in the pathogenesis of psoriasis, and inhibiting exosomal phospholipase A_2 and/or CD1a may represent therapeutic strategies for psoriasis.

Stem cell exosomes in skin repair and regeneration

Many groups have reported that exosomes, particularly from mesenchymal stem cells (MSCs) and peripheral blood mononuclear cells (PBMCs), promote skin repair. One study found that exosomes were a main effector of circulating PBMCs (Beer et al., 2015). PBMC exosomes were the major biological components of the PBMC secretome (of both irradiated and nonirradiated cells) that enhanced cell mobility (Beer et al., 2015). This study also emphasized that the secretome of irradiated PBMCs contained differentially expressed genes involved in angiogenesis and pro-regeneration pathways (Beer et al., 2015). There is accumulating evidence that several signaling pathways are stimulated by human MSC exosome treatment (derived from various MSC types; Supplementary Table S2 online). Exosomes may aid wound repair in a cell density-dependent manner. For example, human umbilical cord-MSC-exosomes were able to stimulate dermis repair (in low cell density conditions) by stimulating Wnt4/β-catenin signaling, cutaneous repair, and angiogenesis in rat burn wounds. In high cell density conditions, near the later part of wound healing, human umbilical cord-MSC-exosomes delivered 14-3-3ζ, which phosphorylates yes-associated protein, promoting a "brake" of cell proliferation and stimulating wound remodeling. In these wound healing studies, different exosome-producer cell types, methods of isolation, methods of exosome application, and dosing were used. More studies should focus on these variables in functional in vitro and in vivo assays.

TECHNICAL ISSUES: A FOCUS ON EXOSOME PURIFICATION

There are numerous isolation methods for exosomes, microvesicles, and ABs, yet there is no gold standard method for every downstream application. Isolation methods for exosomes include differential centrifugation and density-gradient centrifugation, sucrose cushion centrifugation, gel-permeation chromatography, affinity capture methods, microfluidic devices, synthetic polymer-based precipitation, and membrane filtration (Xu et al., 2016).

Differential centrifugation involves removal of cell debris and larger vesicles at 500−2,000*g*, and then larger ABs at 10,000*g*; exosomes are isolated by ultracentrifugation at 100,000*g* (Théry et al., 2006; Xu et al., 2016). This method does not separate exosomes from small microvesicles and

high molecular weight protein complexes, protein-RNA complexes, and viruses (Tauro et al., 2012; Xu et al., 2016). To further purify exosomes, sucrose cushion ultracentrifugation can be used, resulting in less mechanical stress to the exosomes and high purity, but not without the risk of sample loss (Théry et al., 2006; Xu et al., 2016). A recent study found that the buoyant densities of exosomes in sucrose cushions were 1.10–1.11 g/ml and those of secreted microvesicles were 1.18–1.19 g/ml (Xu et al., 2015). The final isolations of exosomes are sensitive to *g* force, the rotor type (fixed angle or swinging bucket), the angle of rotor sedimentation, radius of the centrifugal force, pelleting efficiency (rotor and tube k-factors), and solution viscosity, which are difficult to make universal across studies (Taylor and Shah, 2015). Despite these challenges, differential ultracentrifugation remains the most established method for isolating exosomes for biochemical and functional studies.

Synthetic polymer-based precipitation, such as with polyethylene glycol, is a highly effective method for isolating crude mixtures of any EV type from fluids, depending on which EVs are present in the starting fluid. This method can coisolate nonvesicular proteins, ribonucleoprotein and lipoprotein complexes (Xu et al., 2016). In a comparison of ultracentrifugation/sucrose cushion versus polyethylene glycol 6000 with the subsequent washing step with phosphate buffered saline, both methods resulted in exosomes of high purity (Rider et al., 2016). This suggests that polyethylene glycol combined with washing during a final ultracentrifugation step can be exploited to develop pure preparations of exosomes for downstream analyses.

Filtration with low-protein binding membranes and nanomembrane ultracentrifugation spin devices have been used (Cheruvanky et al., 2007; Merchant et al., 2010); however, sample loss of exosomes can be high due to them sticking to membrane pores (Xu et al., 2016). High-performance liquid chromatography gel permeation has been used for high-throughput exosome isolation from human fluids (Muller et al., 2014; Welton et al., 2015), although it requires highly specialized equipment. Affinity capture methods involve the use of monoclonal antibodies targeting an antigen on exosomes (Atai et al., 2013; Balaj et al., 2015; Christianson et al., 2013; Clayton et al., 2001; Ghosh et al., 2014; Keryer-Bibens et al., 2006; Oksvold et al., 2014; Xu et al., 2016; Yoo et al., 2012); however, this method results in a lower yield, and antibody-antigen complexes may impair downstream functional analyses of the exosomes (Théry et al., 2006). Microfluidic devices trap exosomes via affinity capture (examples are Exochip, iMER, µNMR, nPLEX), by nanoporous membranes or by trapping exosomes on nanowires (Liga et al., 2015; Wang et al., 2013; Xu et al., 2016). Currently, there is no universal agreement which methods are best for isolating exosomes from human plasma, with reports suggesting that size exclusion chromatography should be employed before ultracentrifugation or precipitation methods (Muller et al., 2014; Xu et al., 2016). In summary, because there are no "gold standard" methods for purifying exosomes for further analysis, trial and error is likely required to identify the optimal purification method for chosen downstream analyses.

Methods to measure exosome quantity are numerous, including optical methods (e.g., nanoparticle tracking, flow cytometry), resistive pulse sensing, and transmission electron microscopy (Smith et al., 2015; Xu et al., 2016). Transmission electron microscopy involves direct visualization of exosome structure and morphology, but preparation for imaging likely alters structural appearance; cryo-transmission electron microscopy typically preserves structures of membranes in native states (Xu et al., 2016). Nanoparticle tracking analysis, such as NanoSight, can measure size distribution (10–2,000 nm) and concentration (up to billions of particles per milliliter) (Filipe et al., 2010; Xu et al., 2016). Flow cytometry is limited in the ability to detect all particles and frequently requires bead linking to exosomes on antigens, limiting the isolation to antibody-antigen interactions in the sample being measured (van der Pol et al., 2014; Xu et al., 2016). It is common to measure purified exosomes as an estimation of protein content in samples using the micro-bicinchoninic assay (Webber and Clayton, 2013; Xu et al., 2016). The measured protein content can be correlated to particle concentration and specific exosome antigens using enzyme-linked immunosorbent assays. Measuring whole protein content does not exclude measurements of contaminating protein aggregates or EV-adherent proteins. Lysing the vesicles before measurements of total protein allows for better assessment of total protein content on the interior and exterior of exosomes. To date, several diagnostic platforms have been developed, including ELISA-based microarray chip technologies (Costa-Silva et al., 2015), immunomagnetic exosome technologies (Shao et al., 2015), and methods based on nuclear magnetic resonance (Shao et al., 2012). None of these measures predict functional downstream activity of exosomes. The field of exosomes will likely benefit from continued investigations on how isolation methods, processing, and storage affect functionality of exosomes from various cell types in multiple downstream applications (Xu et al., 2016).

NEW DIRECTIONS FOR THE STUDY OF EVs IN DERMATOLOGY

As a part of the efforts to garner collaboration and consolidate understanding of this newly evolving field, the International Society for Extracellular Vesicles was launched in 2011 (Raposo and Stoorvogel, 2013). There are many unanswered questions regarding the methods to optimize EVs for clinical use, how to enrich for certain populations of EVs and from which tissues or cell types such enrichment would be most useful. Furthermore, it is still unclear exactly how to use the vast information stored in and on EVs to design better biomarker testing to impact clinical decision making.

Although there have been advances in our understanding of the role of EVs in cutaneous biology, we summarize the following areas that need development in parallel with basic science investigations of EVs in dermatology.

(1) *Biomarker development:* Scientists should optimize how EVs, particularly exosomes released into the circulation, can be effectively and cleanly isolated. It should be determined whether specific cargo "signatures" are sensitive and specific for the presence, or onset, of

dermatologic disease (e.g., autoimmune disease, cancers, development of wounds). Once the isolation method has been selected, exosomes can be subjected to mass spectrometry and RNA sequencing. These can be referenced to the disease of interest, and sensitivity and specificity can be determined for the disease of interest.

(2) *Therapeutic development:* One can determine the cell types that produce vesicles with potential for therapeutic properties (e.g., treatment of autoimmune disease, cancers, wounds, etc.). Furthermore, vesicles can be engineered for specific cargo transfer (e.g., proteins, miRNAs) with potential to treat dermatologic diseases. Taking advantage of the natural production process of exosomes, loading exosomes with specific cargo can be achieved via (i) genetic modification of the producer cells and (ii) passive approaches, such as electroporation and chemical conjugation (Smyth et al., 2014; Wahlgren et al., 2012). Proteins can be loaded into exosomes by introducing a genetic construct that codes for a fusion protein of tetraspanin CD63 with the protein of interest. MicroRNAs can be enriched into exosomes by fusing the protein Ago with CD63 or transfecting modified oligonucleotides (e.g., XMIR/AXMIR oligos, System Biosciences, Palo Alto, CA) into the producer cells and harvesting the resulting exosomes. CD63 linked to poly A binding protein can recruit specific messenger RNAs into exosomes; long noncoding RNAs can be enriched in exosomes by fusing polycomb repressive complex 2 with CD63 (Riazifar et al., 2017). Thus, bioengineering of exosomes is a tool to investigate how exosomes may interact with dermatologic processes and pathology.

Ultimately, the future of exosomes as therapeutics relies on finding optimal producer cell(s), culture condition(s), and stimulation condition(s), while producing homogeneous (or purposely heterogeneous) exosomes on a large scale, compatible with good manufacturing practices and approved by the Food and Drug Administration for clinical trials. For each particular investigation/application, the starting point to answer questions will depend on the analysis of exosome profiles (protein content, particle size and distribution, and exosome-specific markers) present from different cell types over different time periods. Investigators should keep in mind that the chosen isolation procedure can likely affect downstream applications (see "technical issues" above). Finally, testing exosomes in functional assays is crucial to gaining an understanding of potency resulting from the aforementioned collection and isolation methods. Once an investigator has determined the above parameters, one can experiment with scaling up the number of producer cells and collection media. Once the exosomes are purified, they can be tested in functional assays to determine if scaling up affects exosome-dependent activities.

Within the next decade, EVs, especially exosomes, have potential to change the way dermatologic disease is screened, diagnosed, and treated. Exosomes in blood circulation may predict the onset of a dermatologic disease before it can be seen on the skin. The first step to realizing the potential of EVs in dermatology is by increasing awareness in the field. Basic investigations will continue to inform the practical design of biomarkers and therapies, all of which should occur in parallel.

CONFLICT OF INTEREST

The authors state no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2017.04.021.

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