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REVIEW ARTICLE

Protein Corona on Extracellular Vesicles: Formation and Biological Function

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Abstract

Extracellular Vesicles (EVs) secreted by cells have become important agents of communication between cells. EVs promote tissue repair by transferring their molecular contents to target cells, influencing signaling pathways, metabolic functions, and gene expression. Recent studies indicate that numerous extracellular proteins interact with the surface of EVs dynamically, forming a layer referred to as the protein corona. The protein corona interacts with cell-surface receptors and enhances the specific absorption of EVs, thus affecting their therapeutic efficacy. The wide range of biomolecules can interact with the EVs' surface, and the thickness of these coronal proteins is significantly different within biological fluids, impacting EVs' kinetics, docking, uptake, biodistribution, and finally cell signaling. The elimination of the coronavirus protein from EVs remains a primary challenge and requires further study. Understanding the properties of the corona protein and eliminating it will be vital for optimizing cell-free therapies, opening new opportunities for progressing regenerative medicine. This review discusses the biogenesis of EVs and the formation of the protein corona. In addition, this review sheds light on the protein corona of EVs as a key factor influencing the function of EVs.

Keywords Extracellular vesicle, Exosomes, Protein corona

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1 Introduction

Cells release different types of extracellular vesicles (EVs) to communicate with each other.[1] EVs and secreted proteins, including growth factors and cytokines, have attracted interest as extracellular agents in cellular processes that support the functional restoration of damaged tissues.[1] EVs are nanoscale particles enclosed by a lipid bilayer that transport various molecular cargoes (proteins, RNAs, DNAs, signaling molecules) within their lumen.[2] EVs released by cells deliver their contents into the cytoplasm of target cells, thereby influencing intracellular activities such as signal transduction, metabolism, transcription, and translation.[3] EVs are found in many biological fluids.

In addition, EVs possess surface proteins that can either be integrated through transmembrane domains within the lipid bilayer or attached to the EV surface from biological fluids through non-covalent interactions.[4] The peripheral proteins present on EVs interact with receptors on the surfaces of recipient cells, which is crucial for EV targeting and internalization.[4] Many secretory proteins are known to accumulate around the EV surface through sequential protein–protein interactions, generating a stable arrangement referred to as the "protein corona."[4,5] Recent findings indicate that the protein corona is vital in mediating the positive impacts of EVs on tissue repair mechanisms.[4,5]

Close contact with diverse proteins and factors results in the creation of a protein-rich layer on the nanoparticle surface in biological fluids. This layer, referred to as the protein corona, is formed through various mechanisms.[6] Because of the similarities in size, structure, and active surface properties between synthetic nanoparticles and EVs, it is reasonable to suggest that protein corona formation may partially, but not entirely, influence the dynamic behavior of EVs.[7] Recent data indicate that the development of protein corona around nanoscale biomaterials can alter the fate of signaling cargoes in in vivo environments.[8]

Overall, it is likely that the formation of protein corona around EVs can alter their physiological properties and potentially their targeting ability. Nonetheless, numerous questions concerning the molecular basis of EV-mediated tissue regeneration remain unresolved and require further investigation. In this review, we describe EV terminology and biogenesis. In addition, we discuss protein corona formation and its impact on EV biology.

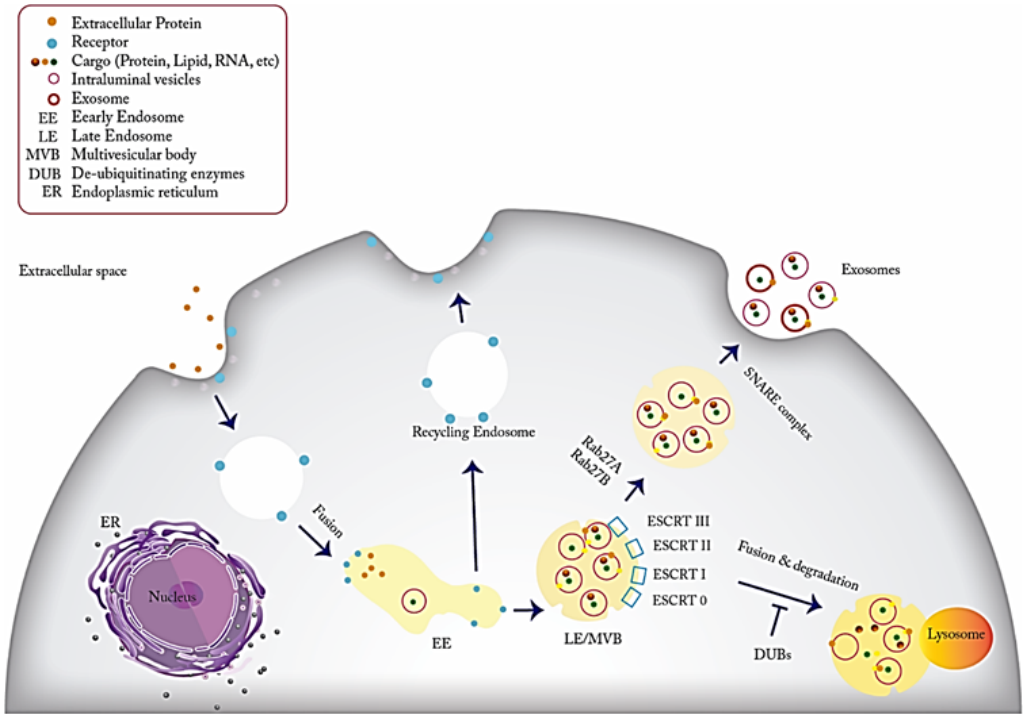

Figure 1 Biogenesis and release of exosomes from a cell. Exosomes are formed from multivesiclular body (MVB) within the cytoplasm, where different complexes such as ESCRT0, I, II, and III participate in loading and sorting cargo into exosomes. MVB may fuse with the plasma membrane and release exosomes into the extracellular space. Different Rab proteins (Rab27) and SNARE proteins contribute to the trafficking and fusion of MVB within the cytoplasm. Exosomes contain many molecules both in their lumen and surface. This figure was used from an article according to the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium.[15]

2 Extracellular Vesicles

Extracellular Vesicles (EVs) consist of various vesicles originating from different cell types, produced via several mechanisms.[2] These vesicles are essential for intercellular communication, as they enable the transfer of biological signals such as non-coding RNAs, coding RNAs, DNA fragments, proteins, and lipids to target cells.[1,9,10] Exosomes are nanosized extracellular vesicles (<200 nm) with a lipid bilayer, acting as mediators of intercellular communication and playing an important role in cellular physiology.[1,9,10] (Figure 1). They are found in various bodily fluids.[11,12]

According to the International Society for Extracellular Vesicles (ISEV), exosomes are typically categorized as small EVs that originate from multivesicular bodies (MVBs).[13] However, the term "exosomes" must be applied carefully and requires specific characterization techniques.[13] The most recent update of the Minimal Information for Studies of Extracellular Vesicles (MISEV) guidelines was published in 2023 as MISEV2023.[13] The latest updates are summarized in Figure 2.

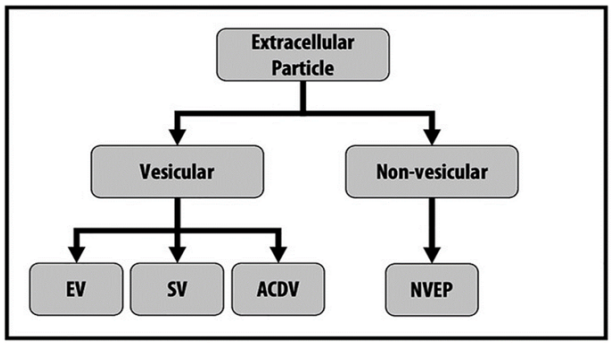

Figure 2 Nomenclature hierarchy of EP. Extracellular particles consist of both vesicular and non-vesicular types. This illustration shows differences that can be identified among categories of EPs, along with instances of potential naming conventions. EP: extracellular particle; EV: extracellular vesicle; SV: synthetic vesicle; ACDV: vesicle derived from artificial cells; NVEP: extracellular particle without vesicles. This figure was used from a previously published article.[2] This is an open-access article distributed under the terms of the Creative Commons CC BY license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

In practice, commonly utilized isolation protocols—such as differential ultracentrifugation, density gradient centrifugation, size-exclusion chromatography, and polymer-based precipitation—primarily separate EVs according to their physical characteristics, resulting in heterogeneous preparations rather than complete isolation of specific subtypes.[14] Moreover, surface markers commonly used to characterize EV subtypes, such as tetraspanins (CD9, CD63, CD81), are not restricted to a single vesicle class and exhibit considerable overlap.[14] Therefore, it is advisable to use the broader term "extracellular vesicles" and provide detailed information regarding operational features (e.g., size, surface markers) and isolation techniques employed, instead of defining strict subtype identities.[2] This approach enhances transparency and reproducibility, underscoring the importance of cautious interpretation of EV subtypes and the need for ongoing methodological refinement.

3 The protein corona of EVs

The EV surface continuously interacts with surrounding biological fluids, primarily interstitial fluid and blood plasma.[16] Various chemical and physical interactions occur between the proteins in these fluids and molecules on the EV surface, leading to the attachment of different proteins.[16] The attached proteins accumulate and form a corona-like layer surrounding the EV surface, known as the "protein corona," which appears as a 10–20 nm shadow in negative-staining transmission electron microscopy.[17,18]

Each EV subtype possesses specific mechanisms of biogenesis and release, resulting in unique patterns of protein corona development. Ectosomes emerge directly from the plasma membrane, primarily attracting secretory proteins from the extracellular space to their surface.[19] In contrast, the surface of exosomes initially interacts with soluble proteins within multivesicular bodies (MVBs), forming a layer called the "innate protein corona."[20] Upon release, exosomes continue to interact with secretory proteins in the extracellular environment, incorporating some into the existing innate protein corona and developing a new "acquired protein corona."[20] Consequently, the corona layer increases EV dimensions,[7,18] making its formation detectable using techniques that assess nanoparticle size, such as nanoparticle tracking analysis.[7,21]

The protein corona also alters the electrical potential between EVs and surrounding fluids, referred to as the "zeta potential."[7,22] EVs with an intact corona show zeta potential values between -5 mV and -50 mV;[7,23] however, the absolute value declines as corona thickness decreases.[24] Thus, variations in zeta potential may serve as a marker for changes in protein corona size.

Different lipids, proteins, and carbohydrates on the EV surface selectively interact with soluble proteins, resulting in unique corona layers with distinct molecular profiles.[25] The outer EV membrane generally includes phosphatidylserine, an anionic lipid that attracts fibroblast growth factors and Raf-1 via electrostatic interactions.[25,26] Integrins are abundant surface proteins that contain domains such as the RGD-binding domain, which interacts with extracellular matrix (ECM) proteins like vitronectin and laminin, and metal ion-dependent

adhesion sites that associate with the GFOGER motif of collagen.[27] Tetraspanins, prevalent on EV surfaces,[2] contain conserved cysteine residues in extracellular loops and harbor thiol groups that can react with thiols of other proteins, such as albumin, depending on their redox state.[25,28] Heparan sulfate, a common polysaccharide on EV surfaces as proteoglycans,[2] interacts with growth factors and ECM components possessing heparin-binding domains, including fibroblast growth factors and fibronectin.[29,30]

Interactions between the EV surface and external proteins initially generate a thin layer, which becomes denser by sequential recruitment of soluble proteins via protein–protein interactions. Phosphatidylserine recruits milk fat globule-epidermal growth factor 8 (MFGE-8) through electrostatic and hydrophobic interactions,[31] while its serine head-group binds annexins.[26] Extracellular nucleic acids are attracted by nucleic acid-binding proteins, such as AIFM1, CDH5, and albumin,[32,33] forming the "biocorona," which contains DNA, RNA, and proteins.

EVs can be characterized by size, sedimentation rate, density, and molecular weight thresholds (kDa).[2,19] Due to its dynamic and non-covalent nature, the protein corona is highly sensitive to processing methods.[18] Intensive concentration or separation techniques may affect protein aggregation levels and corona composition, influencing EV bioactivity and therapeutic efficacy.[18] For example, using molecular weight cut-off filters during EV concentration selectively captures proteins, leading to variability in corona composition.[7,18] Similarly, size-exclusion chromatography can partially remove densely packed coronas, reducing corona-mediated biofunctions.[17,18] Ultracentrifugation, which relies on high centrifugal forces, may also cause substantial loss or alteration of corona constituents, affecting subsequent cellular interactions and biological responses.[17,18]

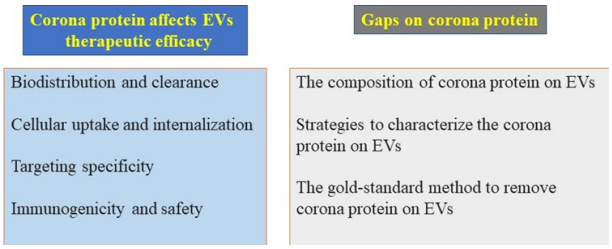

Figure 3 Corona protein on EVs affects therapeutic efficacy and gaps in the corona protein

The impact of the protein corona on EV therapeutic efficacy and remaining knowledge gaps are illustrated in Figure 3. These findings underscore the necessity of customized concentration and purification methods that preserve or deliberately modify the protein corona to achieve intended functional outcomes. In some cases, reconstituting the corona post-isolation by incubating EVs with selected soluble proteins or recovered fractions may restore or fine-tune its composition,[7,18] improving reproducibility and enabling more precise therapeutic applications.

4 Conclusion

Different proteins in the corona layer influence the extracellular roles of EVs and play a crucial role in the uptake of cargoes by recipient cells, with their composition and function being significantly impacted by the chosen isolation methods. The exact functions and processes of the protein corona remain largely unclear and require more clarification. Nonetheless, this variability, dependent on context, also poses considerable challenges, such as EVs diversity and the requirement for accurate control and standardization of corona profiles for clinical application. Generally, our understanding of coronaviruses and their elimination is still in its early stages, leaving some challenges in this area that require additional study. There is a need for standardization of coronavirus characterization and engineering methods. Tackling these challenges necessitates a thorough understanding of the mechanisms behind the formation and regulation of the protein corona, the intricate interactions between the constituent proteins, and the consequent changes in EVs behavior. Future endeavors should concentrate on creating sophisticated methods to deliberately design the EVs surface and regulate protein corona formation, allowing for accurate management of EVs bioactivity and safety characteristics.

Declarations

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Authors' Contributions
All authors contributed to the initial idea generation, study design, data collection, and manuscript drafting. All authors have read and approved the final version of the manuscript and declare no disagreement over its contents.

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