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Mini Review

Ligand-based Exosome Affinity Purification (LEAP) Column Chromatography: A Tool for Clinical Applications

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Abstract

Exosomes are nano-sized extracellular vesicles that play essential roles in intercellular communication, carrying biomolecules such as proteins, lipids, and RNAs that can influence physiological and pathological processes. The isolation of pure exosomes is critical for both basic research and clinical applications, including diagnostics and therapeutics. Traditional exosome isolation techniques, such as ultracentrifugation, lack specificity and may yield impure samples, making the need for advanced isolation techniques evident. Ligand-based exosome affinity purification (LEAP) column chromatography has emerged as a novel method that utilizes specific ligands targeting exosome surface markers, providing a highly specific, gentle, and scalable approach to exosome isolation. This mini review explores LEAP chromatography's mechanism, benefits, and potential for clinical applications, emphasizing its g.rowing importance in exosome-based diagnostics and therapies.

Keywords: exomes, affinity chromatography, biomarkers, drug delivery, systems regenerative medicine

1. Introduction

Exosomes, small extracellular vesicles (30–150 nm), are secreted by nearly all cell types and are essential for intercellular communication [1]. They carry a diverse array of bioactive molecules, including proteins, lipids, and nucleic acids, which reflect the physiological or pathological state of their parent cells [2]. This unique cargo has positioned exosomes as valuable candidates for diagnostic biomarkers, particularly in oncology, cardiovascular diseases, and neurodegenerative conditions [3]. Exosomes are also being explored as therapeutic agents and drug delivery systems due to their ability to cross biological barriers, target specific tissues, and be engineered to carry therapeutic payloads [4]. However, their use in clinical settings depends on reliable, high-purity isolation techniques that preserve exosome structure and functionality [5].

Isolating exosomes from complex biological fluids such as blood, urine, and saliva presents substantial challenges due to their small size and the presence of other extracellular particles, such as microvesicles and protein aggregates [6]. Traditional isolation techniques like differential ultracentrifugation, precipitation-based methods, and filtration lack the specificity required for high-purity exosome separation [7]. Ultracentrifugation, for instance, is labor-intensive and often co-isolates other vesicles and protein contaminants, while precipitation techniques can result in co-purification of undesired proteins and lipoproteins [8]. These limitations hinder the downstream use of exosomes for diagnostic or therapeutic purposes, where purity and integrity are paramount [6].

Ligand-based exosome affinity purification (LEAP) column chromatography has emerged as a promising solution to these limitations [9]. This technique utilizes affinity ligands, such as antibodies targeting exosome-specific surface markers (e.g., CD63, CD9, and CD81), which are immobilized on a resin matrix [10]. These ligands selectively bind exosomes based on their surface markers, enabling efficient separation from other vesicles and contaminants [11]. LEAP chromatography offers high specificity, gentle isolation conditions that preserve exosome integrity, and scalability for both research and clinical applications [12]. With its ability to provide high-purity exosome preparations, LEAP chromatography is well-suited for advancing exosome-based diagnostics and therapeutic applications, addressing the pressing need for standardized, clinically viable exosome isolation methods.

2. LEAP Column Chromatography: Mechanism and Composition

LEAP chromatography employs a resin embedded with specific ligands that target surface markers commonly found on exosomes, such as tetraspanins (e.g., CD63, CD9, and CD81) [13]. The resin typically consists of a porous matrix (such as agarose or polystyrene) functionalized with affinity ligands, which can include antibodies or antibody fragments that selectively bind exosome markers [14]. Spacer arms, often made of hydrophilic polymers like polyethylene glycol (PEG), may be incorporated to enhance ligand

accessibility and reduce steric hindrance [15]. Chemical linkers, such as N-hydroxysuccinimide (NHS) esters or maleimide groups, attach these ligands securely to the resin backbone, ensuring that they remain stable throughout the purification process [16]. This ligand-based specificity allows LEAP chromatography to capture exosomes while excluding other particles, offering high-purity exosome samples with preserved structure and function. The method's scalability and compatibility with various sample types make it suitable for both research and clinical production.

3. Advantages of LEAP Chromatography Over Traditional Methods

LEAP chromatography offers several advantages over traditional exosome isolation techniques [17]:

1. High specificity: The ligand-based approach selectively binds exosomes, reducing the contamination seen in methods like ultracentrifugation, which often co-isolates nonexosome vesicles and proteins.

2. Enhanced purity and structural integrity: By gently capturing exosomes through affinity interactions, LEAP preserves the structural and functional integrity of exosomes, which is crucial for downstream analyses and therapeutic applications.

3. Scalability and adaptability: LEAP chromatography can be scaled for different sample volumes, making it suitable for both small research studies and larger clinical production processes.

4. Reduced sample contamination: Unlike precipitation and filtration techniques, which may include contaminants, LEAP chromatography offers a cleaner separation, yielding exosomes with minimal protein or lipoprotein contamination.

4. Clinical Applications of LEAP Chromatography

Given the purity and functionality of exosomes isolated by LEAP chromatography, the technique holds promise for various clinical applications:

4.1. Diagnostics and biomarker discovery

Exosomes are increasingly recognized as carriers of disease-specific biomarkers [18]. By isolating exosomes from biological fluids like blood, urine, and saliva, LEAP chromatography enables the discovery and validation of biomarkers associated with cancer, cardiovascular diseases, neurodegenerative disorders, and infectious diseases [19]. High-purity exosomes allow for more reliable biomarker profiling, increasing diagnostic accuracy [20]. For example, cancer-related exosomes often carry oncogenic proteins or nucleic acids that can provide early indications of tumor presence and progression [21].

4.2. Therapeutic exosomes in regenerative medicine

Exosomes have demonstrated potential in regenerative medicine, particularly in wound healing, cardiovascular repair, and neurological regeneration [22]. The mild purification process of LEAP chromatography preserves the biological function of exosomes, allowing them to be used as therapeutic agents [23]. Clinical-grade exosomes isolated through LEAP chromatography could be used to deliver proteins, RNAs, or drugs to specific cells or tissues, enhancing therapeutic outcomes in regenerative therapies [10].

4.3. Drug delivery and targeted therapies

Exosomes are natural carriers that can deliver therapeutic molecules across biological barriers, making them attractive for drug delivery [24]. LEAP chromatography can isolate exosomes with high specificity, ensuring that only functional and intact vesicles are used in drug delivery systems [20]. Exosomes can be engineered to carry therapeutic cargo and target specific cells, such as tumor cells in cancer therapy [25]. High-purity exosomes minimize the risk of immune reactions and improve targeting efficacy, making LEAP chromatography essential for developing exosome-based delivery systems [26].

4.4. Liquid biopsy for noninvasive disease monitoring

LEAP chromatography facilitates the isolation of exosomes from readily accessible body fluids, making it a valuable tool in liquid biopsy [21]. Exosomes reflect the molecular profile of their cell of origin, providing real-time insights into disease states without the need for invasive tissue biopsies [27]. This is particularly useful in monitoring cancer progression and response to treatment, as exosome cargo can reveal changes in tumor biology over time [28].

5. Limitations and Challenges

While LEAP chromatography offers significant advantages, several limitations must be considered. The high cost of ligand-functionalized resins can be a barrier to widespread use, particularly in large-scale studies or routine clinical applications. Additionally, not all exosome populations express the same surface markers, which can limit the method's applicability to certain cell types. Further research is needed to expand the range of affinity ligands and optimize protocols for different exosome subtypes.

6. Future Perspectives

The growing interest in exosome-based diagnostics and therapeutics underscores the need for reliable and efficient isolation methods. LEAP chromatography has the potential to become a standard technique in clinical laboratories, offering a reproducible and scalable method for obtaining pure exosomes. Future developments could include multi-ligand resins capable of capturing a broader range of exosome populations and cost-effective manufacturing techniques to lower the expense of affinity-based resins. With these advancements, LEAP chromatography may further support the clinical translation of exosome-based therapies and diagnostics.

7. Conclusion

LEAP column chromatography represents a promising advancement in exosome isolation technology, offering high specificity, purity, and scalability. By enabling the reliable isolation of functional exosomes, LEAP chromatography addresses the limitations of traditional methods and enhances the feasibility of exosome-based clinical applications. The method's utility in diagnostics, regenerative medicine, drug delivery, and liquid biopsies highlights its potential to revolutionize personalized medicine and improve patient outcomes. Continued research and development of LEAP technology will likely expand its clinical applications, paving the way for novel diagnostic and therapeutic strategies based on exosomal biology.

Declarations

Compliance with ethical standards

Not applicable.

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Data availability statement

Data is available on request from the corresponding author.

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Not applicable.

Conflicts of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization: N.M.M.; Data curation: A.T.; A.B.; Formal analysis: K.R.Zh.; A.A.U.; Methodology: A.T.; A.B.; Project administration: N.M.M.; Resources: R.S.; S.M.M.; A.B.; Software: A.T.; A.A.U.; Supervision: N.M.M.; Validation: A.A.U.; Visualization: K.R.Zh.; Writing, original draft: A.T.; A.B.; Writing, review and editing: R.S.; S.M.M.; N.M.M.; A.T.; All authors have read and agreed to the published version of the manuscript.

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