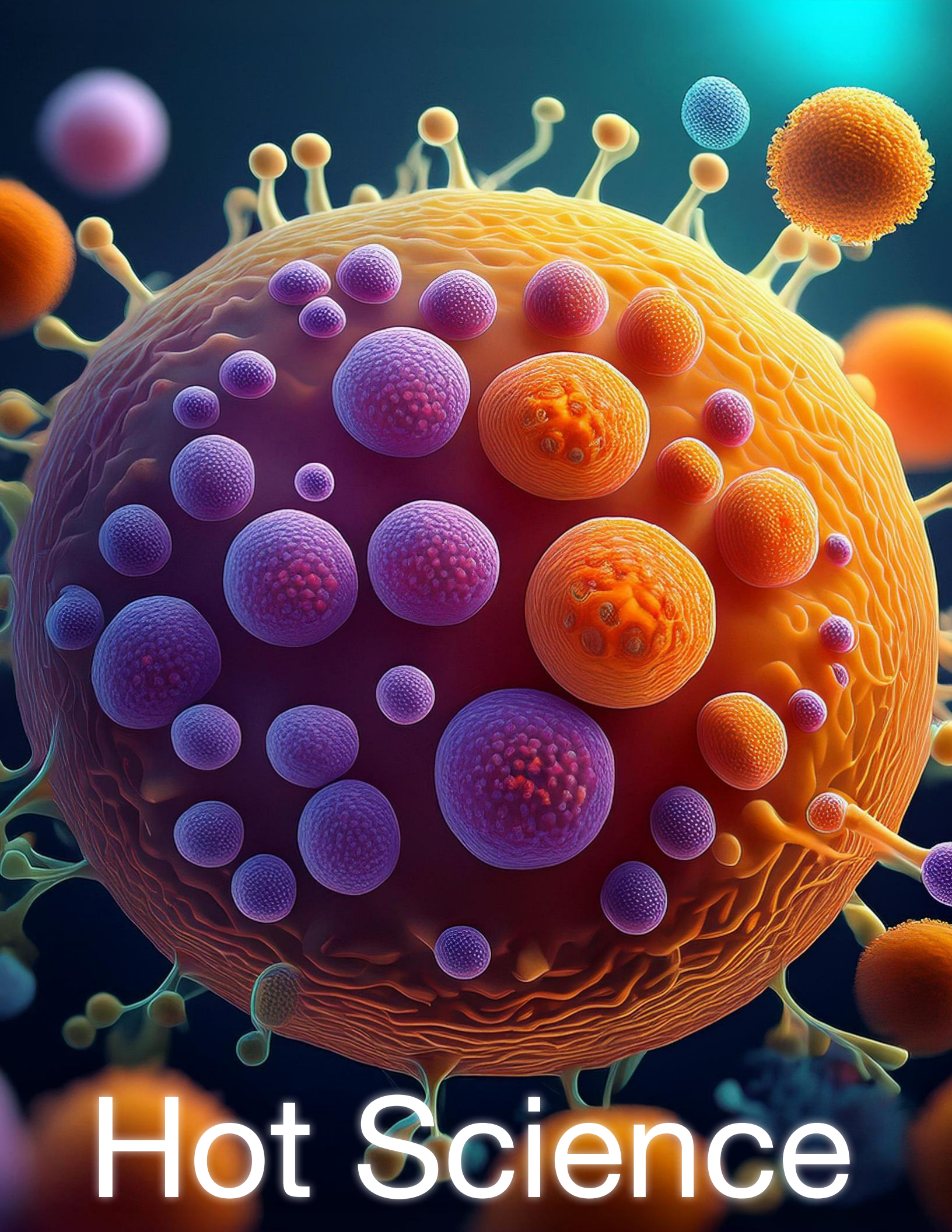
Hot Science

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La promesa de las vesículas extracelulares derivadas de macrófagos

The promise of macrophage-derived extracellular vesicles

Resumen

En los últimos años, el desarrollo de terapias ha sido impresionante. Desafortunadamente, estas terapias han presentado limitaciones, principalmente in vivo. En consecuencia, continúan los esfuerzos para diseñar mejores sistemas de administración de fármacos, entre los que destacan las vesículas extracelulares derivadas de macrófagos (MEVs). Debido a sus características naturales, principalmente sus marcadores de superficie, y sus métodos de producción accesibles. Estas características facilitan su uso en terapias contra el cáncer y enfermedades inflamatorias, con resultados prometedores. Por lo tanto, nuestro objetivo es resaltar por qué las MEVs están emergiendo como una estrategia prometedora para la administración de fármacos.

Palabras clave: Macrófagos, vesículas extracelulares, entrega de fármacos.

Summary

In recent years the development of therapies has been impressive. Unfortunately, many of these therapies have presented some limitations, mainly in vivo. Consequently, efforts continue to design drug delivery systems that address these limitations, among which are macrophage-derived extracellular vesicles (MEVs). This is due to their natural characteristics, mainly their surface markers, which enable their function as messengers and their production methods accessible. These features have facilitated their use in developing therapies against cancer and inflammatory diseases, yielding promising results. Therefore, our objective is to highlight why MEVs are emerging as a promising drug delivery strategy.

Keywords: Macrophages, extracellular vesicles, drug delivery.

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Introduction

Over the past decade, humanity has experienced an unprecedented discovery rate of novel therapies. Each day, we witness the relentless innovation, research, and testing of an expanding array of molecules. Furthermore, in contrast to previous decades, today's treatment landscape holds remarkable diversity, encompassing traditional small molecules, peptides, nucleic acids, antibodies, and even intricate protein systems. However, despite these strides, many of these therapies encounter formidable obstacles, which limits their *in vivo* potency. Among these obstacles are their short half-lives, variable blood concentration levels, poor elimination kinetics, imprecise targeting, and propensity for adverse reactions [1, 2]. Consequently, there exists a pressing need for novel approaches for drug delivery.

Initially, nanoformulations emerged as a frontrunner for this task. However, they often exhibit a markedly tendency to accumulate in tissues with heightened vascular permeability, such as zones with inflammation or cancerous sites. Due to the phenomenon known as the enhanced permeability and retention effect

(EPR) [1, 2]. Furthermore, drug-loaded nanoparticles frequently face a rapid clearance from the body [1, 2]. As a result, the search for alternative vehicles led to the exploration of extracellular vesicles (EVs) as promising substitutes. This mainly due to their intrinsic involvement in cellular communication [1, 2]. However, despite some promising results, efficiently loading therapeutic agents into EVs remains a formidable obstacle.

With this in mind, macrophages have emerged as promising mediators to obtain drug loaded EVs. Macrophages are located throughout the body, undertaking a wide range of crucial tasks. Among their arsenal of functionalities, the capacity to generate EVs stands out. Macrophages use MEVs as capable messengers to transport a diverse set of bioactive molecules to neighboring cells, orchestrating different biological responses [3]. Within the context of MEVs, they can be classified as apoptotic bodies (ApB), microvesicles and exosomes [4]. Notably, MEVs are distinguished by the expression of CD47, a surface molecule pivotal for evading immune system surveillance [3]. This and other surface molecules give MEVs significant advantages, including diminished immunogenicity and prolonged stability [2, 3]. Furthermore, their capacity to cross tissue barriers, such as the blood-brain barrier (BBB), amplifies their therapeutic potential [2, 3]. This is due to the presence of integrin lymphocyte function-associated antigen 1 (LFA-1), which interacts with its ligand, intercellular adhesion molecule 1 (ICAM-1), within the membrane of brain cells [5]. In this way, MEVs possess unique characteristics that makes it a promising system for drug delivery. Therefore, in this paper, we aim to demonstrate why MEVs appear to be the drug delivery system of the future by highlighting their mechanisms, production methods, and current applications.

How does macrophage-mediated drug delivery work?

Naturally, MEVs possess a remarkable ability to encapsulate a diverse array of bioactive molecules, encompassing proteins, RNA, DNA, and lipids [3]. Upon secretion, MEVs are internalized by recipient cells, where they exert regulatory control over gene expression or signaling pathways within these cells [6]. For example, in the context of colorectal cancer, it has been found that MEVs contains proteins that promotes T cell proliferation and activation, and enzymes related to prostaglandins (PGs) and thromboxanes (TXs) production [6]. Which modulates a pro-inflammatory signaling within this context [6].

MEVs are mainly uptaken through endocytosis or direct fusion. Endocytosis is mediated through different mechanisms such as clathrin-mediated endocytosis, macropinocytosis, phagocytosis, among others [3]. While direct fusion, in this context, is mediated through proteins like Rab and SNAREs, or in response to an acidic stimulus [3]. Moreover, transmembrane proteins play a fundamental role in facilitating MEVs uptake [4]. For example, MEVs (specifically microvesicles) derived from M1 macrophages have shown to improve doxorubicin delivery into tumor sites [4]. This is attributed to a high presence of the C-C chemokine receptor type 2 (CCR2), due to the interaction between CCR2 and C-C Motif Chemokine Ligand 2 (CCL2) (Figure 1) [4]. After MEVs bind to target cells, SNARE proteins found within the membrane of MEVs motivate membrane fusion, thereby improving the efficiency of drug delivery (Figure 1) [4].

Methods for the production of drug-loaded MEVs

Typically, the MEV manufacturing process comprises two distinct stages [5]. The initial stage consists of loading the desired molecules into the MEVs. Due to the increasing attention in this field, there is a continuous evolution and development of new techniques. However, despite this diversity, there are some notable trends. On one hand, the molecules can be cultured with macrophages, allowing them to be encapsulated in MEV [2, 7]. Some of the predominant techniques will be discussed below:

- **Incubation:** Incubation is one of the oldest

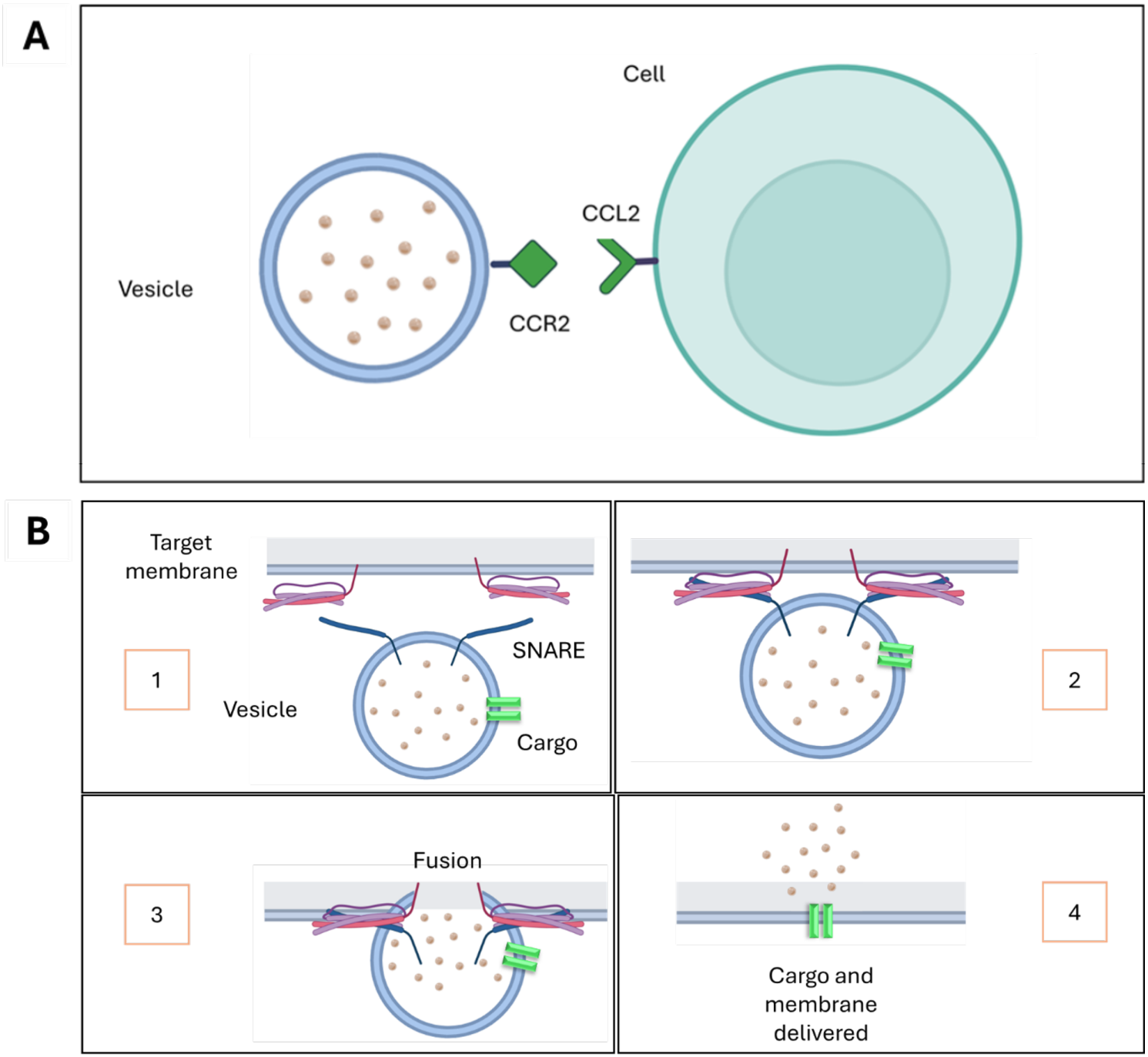

Figure 1. Illustration of a possible cellular uptake mechanism of MEVs. A) MEVs recognize cancerous cell though CCR2-CCL2 interactions. B) SNARE proteins within MEV enhance membrane fusion, releasing the cargo within the cancerous cells.

and most used techniques to date. This consists of cultivating macrophages with the desired molecules. These molecules can then be absorbed by phagocytosis [2, 7]. The problem with phagocytosis is that it could lead to the degradation of molecules [7]. Although it is important to mention that there are other techniques that avoid this problem.

- **Electroporation:** This technique consists of using an electrical stimulus to allow the known increase in cell permeability. Therefore, macrophages stimulated with an electric field can take up molecules. This technique has proven to be a fast and reliable alternative to obtain MEV [2, 7].
- **Hypotonic method:** *In this case, macrophages are subjected to osmotic shock through*

hypotonic dialysis. Which further promotes cellular swelling and the formation of pores, which allow molecules to enter macrophages [7].

While it is true that many of these techniques have shown potential, the dependence on the capacity of macrophages for MEV generation may limit production efficiency. Consequently, more updated methods have begun to emerge [2, 7].

- **Encapsulation within macrophage cell membranes:** This process involves extractig macrophage membranes through hypotonic swelling, mechanical disruption, and gradient centrifugation. Consequently, the resulting membranes are wrapped around the desired molecules using techniques like ultrasonication and mechanical extrusion. In this way, these vesicles are still coated with the transmembrane proteins that give MEVs their discussed advantages [2, 7].
- **Encapsulation within macrophage-derived vesicles:** This method relies on macrophage-induced vesicle secretion, typically facilitated by treatment with cytochalasin B. Following secretion, the vesicles are isolated, and then target molecules are loaded [2, 7].

Once the desired molecules are loaded, the second stage focuses on the isolation of MEVs [5]. The most reported technique will be discussed below.

- **Ultracentrifugation:** This technique relies on MEVs separation based on its size and density. While ultracentrifugation a widely used technique, it may lead to MEV´s aggregation or damage [5].
- **Precipitation method:** This technique involves conjugating hydrophobic polymers with MEVs, which causes consequent precipitation. However, this technique presents a notable weakness as the polymers need to be separated [5].
- **Size-based isolation:** This method, like ultracentrifugation, use MEVs size for isolation. However, this method uses filtration techniques, such as ultrafiltration or exclusion chromatography. In this way, this technique avoids the centrifugation driven aggregation or damage towards MEVs [5].
- **Immunoaffinity:** This method uses antibodies that directly target MEV-membrane proteins. This technique is quite useful as it also allows the selection of MEVs based on the desired transmembrane proteins. However, this technique has limitations within large-scale systems [5].

Use in cancer

Macrophages have been widely reported to play an important role in the tumor microenvironment [1]. Therefore, great efforts have been made to exploit the ability of MEVs to be taken up by cells within the tumor site. A study led by Haney *et al.* focused on loading MEV with chemotherapeutic agents such as paclitaxel (PTX) and doxorubicin (Dox) [1]. These formulations showed results, as MEVs efficiently accumulated within triple negative breast cancer cells (TNBC) *in vitro*, generating a substantial antiproliferative effect. Furthermore, this efficacy was observed in both animal models and human tumors, effectively restricting tumor growth [1].

Further investigations have revealed various applications for PTX-loaded MEVs. Kim *et al.* used them against drug-resistant cancer cells, which increased cytotoxicity more than 50 times (Figure 2) [8]. Furthermore, an important phenomenon was observed in a murine model which simulated lung metastases and after administration through the respiratory tract, these vesicles exhibited almost perfect colocalization with cancer cells [8]. This targeted delivery mechanism appears to be facilitated by specific proteins present on the surface of exosomes, in addition to the acidic microenvironments commonly found at tumor sites [8]. Therefore, MEVs have demonstrated remarkable potential for precise delivery of chemotherapeutic agents to the tumor site. This finding could represent a

significant advance in the way of treating cancer.

Use in inflammatory diseases

MEVs applications are also remarkable within the context of inflammation. This is due to their natural role in communication between macrophages and a wide class of different immune cells [3]. A notable application is their use for atherosclerosis treatment [9]. Gao *et al.* wrapped oxidative stress-sensitive nanoparticles (NPs) with macrophage membranes, which established a dual drug delivery system. MEVs will guide the system towards inflammation sites, while also avoiding clearance by the reticuloendothelial system [9]. Once MEVs arrive at an inflammation site, NPs will react to reactive oxygen species (ROS), triggering the release of their payload [9]. Interestingly, MEVs membrane were also able to capture pro-inflammatory cytokines, due to their transmembrane proteins [9]. In this way, the use of MEVs within this context generates a synergistic action, enhancing atherosclerosis treatment [9].

Another widely reported and promising

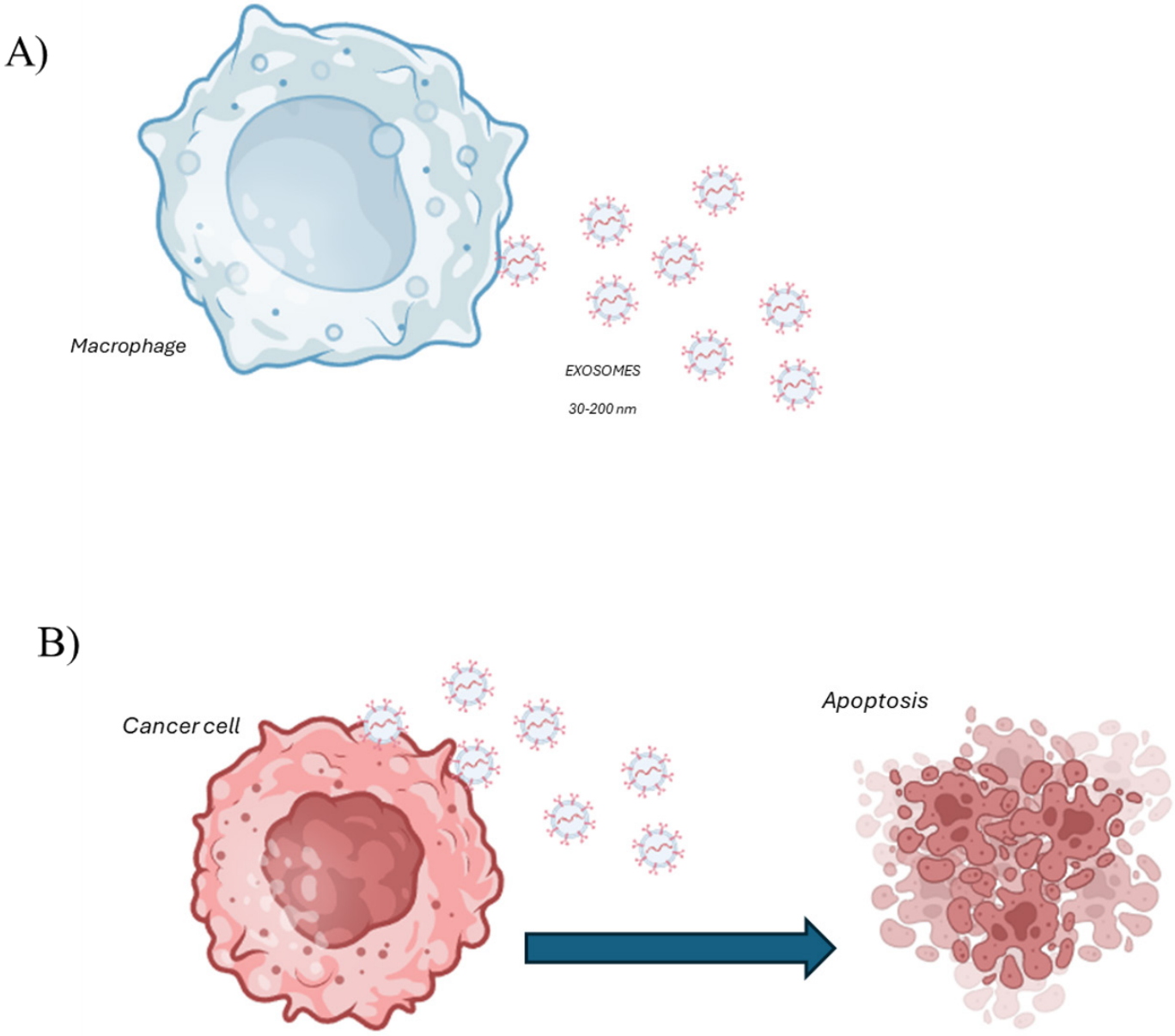

Figure 2. Schematic representation of the use of MEV against cancer. A) MEV loaded with chemotherapeutic agents and subsequently isolated, B) MEV administered so that they can bind to cancer cells and release the chemotherapeutic agents to induce apoptosis.

application lies in using MEVs to modulate macrophage polarization. Specifically, the transition from pro-inflammatory (M1) to an anti-inflammatory (M2) state, or the other way around [10]. Naturally derived MEVs from M1 macrophages possess the ability to polarize macrophages towards M1, while MEVs derived from M2 will promote M2 polarization [10]. M1 and M2 control different phenomena within our bodies. The pro-inflammatory properties of M1 are useful within the context of cancer, while modulating macrophage polarization towards M2 will reduce the inflammatory state of many diseases [10]. This strategy presents a unique avenue for manipulating immune system responses.

Conclusions

In conclusion, MEVs represent a promising strategy for drug delivery, as they are naturally used as key messengers. MEVs present advantages that other methods don't possess, such as the presence of CD47, LFA-1, SNARE proteins, and other surface markers. Which respectively allow MEVs to evade clearance mechanisms, traverse the BBB, facilitate vesicle fusion, and bind to specific cellular targets with high precision. In addition, MEVs loading, and isolation methods are relatively easy to perform. Making them more accessible than other vesicle engineering techniques. All these advantages are followed with promising results on treating cancer and modulating inflammatory related diseases. In this way, MEVs have an unparalleled potential to revolutionize drug delivery systems.

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