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Macrophage Cell Membrane-Cloaked Nanoplatforms for Biomedical Applications

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Biomimetic approaches utilize natural cell membrane-derived nanovesicles to camouflage nanoparticles to circumvent some limitations of nanoscale materials. This emergent cell membrane-coating technology is inspired by naturally occurring intercellular interactions, to efficiently guide nanostructures to the desired locations, thereby increasing both therapeutic efficacy and safety. In addition, the intrinsic biocompatibility of cell membranes allows the crossing of biological barriers and avoids elimination by the immune system. This results in enhanced blood circulation time and lower toxicity in vivo. Macrophages are the major phagocytic cells of the innate immune system. They are equipped with a complex repertoire of surface receptors, enabling them to respond to biological signals, and to exhibit a natural tropism to inflammatory sites and tumorous tissues. Macrophage cell membrane-functionalized nanosystems are designed to combine the advantages of both macrophages and nanomaterials, improving the ability of those nanosystems to reach target sites. Recent studies have demonstrated the potential of these biomimetic nanosystems for targeted delivery of drugs and imaging agents to tumors, inflammatory, and infected sites. The present review covers the preparation and biomedical applications of macrophage cell membrane-coated nanosystems. Challenges and future perspectives in the development of these membrane-coated nanosystems are addressed.

1. Introduction

Nanoscale platforms have been investigated for the diagnosis and treatment of various diseases.[1,2] These nanoplatforms protect the encapsulated diagnostic or therapeutic payload from degradation or premature leakage, and improve their targeting ability and biodistribution.[3–5] Although the relative success of nanocarriers has resulted in significant improvements in both efficacy and safety, compared to conventional modalities, there are still numerous challenges that hamper the clinical translation of this technology. One of the most important concerns is associated with the recognition and clearance of nanomaterials by the mononuclear phagocytic system before they can fulfill their task. This issue is caused by the foreign nature of the nanomaterials and their poor biocompatibility, resulting in lower blood circulation times.[6,7] In addition, the inability of the nanomaterials to cross biological barriers

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results in reduction of the delivery efficacy of bioactive compounds to the sites of interest.[8]

The ability of nanomedications to remain undetected by the mononuclear phagocytic system, and to interact with the complex biological environment of cells and tissues, are crucial prerequisites for their effective clinical translation in vivo.[9,10] Accordingly, the design of nanoplatforms with an active targeting capability and high affinity for target cells has gained increasing attention. This may be achieved by functionalizing the nanomaterials with specific targeting ligands such as antibodies, peptides, aptamers, or even small molecules that are capable of interacting with receptors overexpressed in pathological tissues.[9,11–14] However, the immunogenicity of an artificial polymer and the complexity of bottom-up ligand synthesis remain important concerns. This highlights the need for novel surface modification approaches. This involves enhancing the performance of nanoplatforms by increasing their ability to actively target the desired sites and reducing nanoparticle (NP) uptake by the immune system.[9,15–17]

There has been a recent paradigm change in the design of nanomaterials, by employing bioinspired principles to produce more biocompatible and long-circulation cell-based delivery nanosystems that are capable of mimicking the biological features of the source cells while maintaining the physicochemical properties of NPs.[18–20] Although the use of whole cells as carriers has also been studied, current research in this field has primarily focused on cell membrane coatings for surface functionalization of NP cores.[21–23] This top-down approach consists of wrapping a nanostructure inner core with a thin layer of a natural cell membrane, such as those derived from red blood cells, platelets, white blood cells, stem cells, bacteria, cancer cells, and others.[9,24–26] This membrane cloaking approach preserves the intact proteolipid composition and the complex set of surface proteins essential for effective biointerfacing, thus endowing NPs with the desirable functionality of the parent cells.[9,23,27]

The present review summarizes the latest advances and original research covering macrophage cell membrane (MCM)-coated nanoplatforms for diagnosis, therapy, and theranostics of both cancer and noncancer diseases. First, the genesis, phenotypic diversity, heterogeneous functions, and surface markers of macrophages are discussed in detail. Second, the four classes of macrophage-based therapeutics (i.e., live macrophages, macrophage-derived extracellular vesicles, synthetic macrophage-mimicking proteolytic vesicles (leukosomes), and MCM-coated nanoplatforms) are discussed in terms of their main biomedical applications. The fabrication and characterization techniques of MCM-based nanomediations are then introduced. This is followed by a comprehensive discussion of the current applications of these biomimetic nanoplatforms in the biomedical field. These applications include cancer bioimaging, phototherapy, treatment of neurodegenerative disorders, inflammation-associated disorders, infection, immunomodulation, detoxification, as well as vaccination. Finally, the future perspective and challenges associated with the clinical translation of these nanosystems are presented.

2. Immune Cell-Membrane Coating Nanotechnology: An Overview

Immune cell membrane-coating nanotechnology is an emergent and nature-inspired approach that harnesses good biocompatibility, long blood circulation time, and enhanced specificity of immune cells to migrate to inflamed tissues and tumors.[23,28] This strategy overcomes the shortcomings of nanomaterials, improves the delivery of therapeutic agents and diagnostic compounds to sites of interest. Consequently, the strategy may enhance the clinical results achieved with NP-based systems.[15,18] By using functional and intact immune cell membranes to cloak the NPs via top-down approaches, the resulting core–shell NPs inherit the biological features of the parent cells, enabling them to replicate the cellular biofunctionality in vivo.[23,28]

White blood cells, also referred to as leukocytes, are important components of the immune system. Leukocytes are divided into two major subsets: granulocytes and agranulocytes.[23,29]

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Table 1. The main applications, advantages, and disadvantages of different immune cell membranes.

Immune cell membrane type	Clinical applications	Advantages	Disadvantages	Refs.
Macrophage	Cancer imaging and therapy, phototherapy, targeted chemotherapy, targeted antimetastasis therapy, therapy of diverse infectious and inflammation-associated disorders	Immune evasion, phagocytosis ability, antigen-presenting activities, immune and inflammatory modulation, tumor and inflammation targeting, good biological barrier penetration ability, intratumoral penetration	The mechanisms of macrophage migration and polarization should be further understood	[19,23,36,43–49]
Neutrophil	Targeted cancer therapy, enhanced CTC-capture efficiency preventing the formation of metastatic niches, targeted anti-inflammatory therapy (e.g., acute pancreatitis, inflammatory arthritis)	Immune evasion, high binding affinity to blood CTCs via their membrane proteins, earliest immune cells to migrate to inflammatory tissues via chemotaxis, most abundant blood leukocytes	Short half-life in blood circulation	[19,23,36,43,50–52]
T cell	Targeted cancer imaging and therapy (e.g., melanoma), enhanced tumor-specific drug delivery, effective PTT	Prolonged blood circulation; high affinity to tumor-specific antigens via their TCR-expressing molecules, specific tumor-homing features	Requires previous sensitization to specific antigens to induce cell death, MHC restriction	[19,23,34,43,53]
Natural killer cell	Targeted cancer immunotherapy, M1 macrophage polarization, PDT-induced immunogenic cell death	Strong cell killing ability without requiring prior antigen-specific excitation and MHC restriction	Reduced proliferation capacity of primary cells	[19,23,43,54–56]
Dendritic cell	Targeted cancer immunotherapy, effective lymph node targeting, biomimetic nanovaccines for enhanced tumor therapy	Antigen-presenting features via their MHC-expressing proteins, activation of T cells, immune modulation	MHC restriction, limited number in blood circulation	[19,23,43,57–59]

Abbreviations: CTC, circulating tumor cell; MHC, major histocompatibility complex; PTT, photothermal therapy; TCR, T cell receptor.

The growing interest in using white blood cells for cargo delivery comes from their good biocompatibility, prolonged blood circulation and unique ability to be recruited and guided by chemoattractant gradients. This enables the white blood cell-camouflaged cargo to bind to and cross the vascular wall to arrive at sites of inflammation.[17,23,30,31] Different immune cell membranes with highly optimized functions have been exploited to render higher biocompatibility, superior immune evasion, and specific cell-targeting features to synthetic nanomaterials. They include macrophages, neutrophils, T cells, natural kill cells, and dendritic cells. Neutrophils, being the first immune cells to respond to inflammatory mediators, have the ability to target sites of inflammation.[32,33] T cells have unique surface receptors (e.g., T cell receptors (TCRs)), which confer a higher binding affinity to tumor-associated antigens.[34] Natural killer cells are professional assassin cells that can destroy cancerous or infected host cells without prior activation.[35] Dendritic cells are antigen-presenting cells (APCs) in charge of eliciting potent immune responses against foreign agents.[31] Macrophages are versatile phagocytic cells that play a prominent role in regulating both innate and adaptive immune response through the recognition and removal of tumor cells and other foreign invaders (e.g., bacteria, viruses) from the human body by phagocytosis and antigen presentation. Macrophages also specifically target tumors, inflamed and infected sites via chemotaxis.[36–39] Their ability to actively recognize and bind tumor cells via cell–cell adhesion makes them attractive cells for improving cancer diagnosis and therapy, while reducing unwanted systemic toxicity.[21,40,41] Moreover, macrophages are crucial players in the tumor microenvironment that can determine cancer immunity and tumor progression according to the signals received.[36,42] Because of these unique features, the use of macrophages for drug delivery, cancer immunotherapy, and treatment of inflammatory and infectious diseases has received increasing attention. **Table 1** provides a comprehensive comparison of the clinical applications as well as advantages and disadvantages of the different types of immune cells.

3. Macrophages as Key Mediators of the Immune System

Macrophages are mononuclear phagocytes, a type of white blood cells derived from monocytes circulating in the blood. They are found in tissues throughout the body. Macrophages play a key role in immune surveillance.[23,39,42] As the major immunomodulatory cells, macrophages perform a central role in maintaining homeostasis and protecting the body by regulating both innate and adaptive immune responses. Beyond their crucial protective role, certain macrophage phenotypes are believed to be involved in the pathogenesis of various diseases by governing inflammatory responses and promoting tissue repair. Macrophages are a unique cell type with clinically significant effects in both the healthy and disease states.[60,61]

3.1. Surface Properties and Physiology

3.1.1. Biogenesis of Macrophages

Myeloid cells are derived from myeloid progenitor cells residing in the bone marrow. These cells include, among others, monocytes, macrophages, granulocytes, and dendritic cells.[30] Tissue macrophages are phagocytic immune cells in the mononuclear phagocytic system. They are either established before birth and self-sustained over time independent of monocyte recruitment,

or are derived from circulating monocytes.[30,43,62] After birth, continuous replenishment of tissue macrophages is important for homeostasis. Replenishment of tissue macrophages depends on the differentiation of monocytes from hematopoietic stem cells (HSCs) residing in the bone marrow, and their subsequent migration to damaged tissues. Within the smaged tissues, the HSCs undergo modifications to become either dendritic cells or tissue macrophages.[30,63,64] Migration of monocytes and macrophages toward inflamed or tumor tissues is mediated by chemoattractive gradients released by tumor cells within the tumor microenvironment. Molecules that create the chemoattrcive gradients include CC-chemokine ligand 2 (CCL2), CC-chemokine ligand 5 (CCL5), and colony stimulating factor-1 (CSF-1).[11,18] Because macrophages are naturally recruited to the sites of inflammation, a hallmark of neoplastic disease, they are also attracted to tumor tissues via tumor-derived inflammatory mediators.[21,23,36]

3.1.2. Phenotypic Diversity of Macrophages

Immune cells derived from the myeloid lineage are highly flexible and plastic. They can switch and adopt different phenotypes according to the environmental stimuli.[30] Heterogeneous populations of macrophages are found within the tumor microenvironment, each with distinct effects on tumor development and progression.

Tumor-associated macrophages (TAMs) are macrophages that have migrated into tumor tissues. The TAMs may be polarized into two opposite phenotypes through classical or alternative pathways according to specific signals and stimuli encountered in the tumor microenvironment. These phenotypes differ from each other in their surface receptors, cytokine and chemokine profiles, as well as inflammatory functions. They are usually classified into classically activated or M1 macrophages, and alternatively activated or M2 macrophages.[36,37,42,43] The TAMs play a central role in modulating cancer immunity and tumor development. The M1 macrophages phagocytize and kill tumor cells to suppress tumor growth. By contrast, the M2 macrophages support tumor growth and promote progression and metastasis.[42,65,66]

Bacterial lipopolysaccharides (a.k.a. endotoxins) and pro-inflammatory cytokines such as interferon gamma (IFN-γ) and tumor necrosis factor (TNF-α), can instruct nonpolarized macrophages (M0) to adopt the M1 phenotype. The latter is characterized by proinflammatory activity, enhanced phagocytic activity and antigen presentation to produce antitumor-specific T cells. M1 macrophages play a key role in activating adaptive immunity for a stronger antitumor immune response.[37,39,42,67] M1 macrophages secrete several proinflammatory cytokines, including TNF-α, IL-6, IL-12, and IL-1β. They also secrete chemokines such as CXCL-10, reactive oxygen species (ROS) and nitric oxide (NO). The latter was produced via the inducible nitric oxide synthase (iNOS) pathway. Nitric oxide can promote tissue damage but also suppress tumors. The main markers of M1 macrophages include Toll-like receptors (TLRs) such as TLR4. The TLRs are capable of binding to bacterial lipopolysaccharides, costimulatory molecules CD80 and CD86, iNOS and major histocompatibility complex II (MHC-II). These features are depicted in **Figure 1**.[60,68]

During the advanced stages of tumors, various anti-inflammatory factors capable of instructing macrophages to adopt the M2 phenotype are released in the tumor microenvironment. These factors include interleukin 10 (IL-10), interleukin 13 (IL-13), and interleukin 4 (IL-4). The M2 phenotype has protumor activity, anti-inflammatory activity, and is directly involved in fostering an immunosuppressive tumor microenvironment. This enables tumor cells to avoid elimination by the immune system, and encourages them to spread to establish

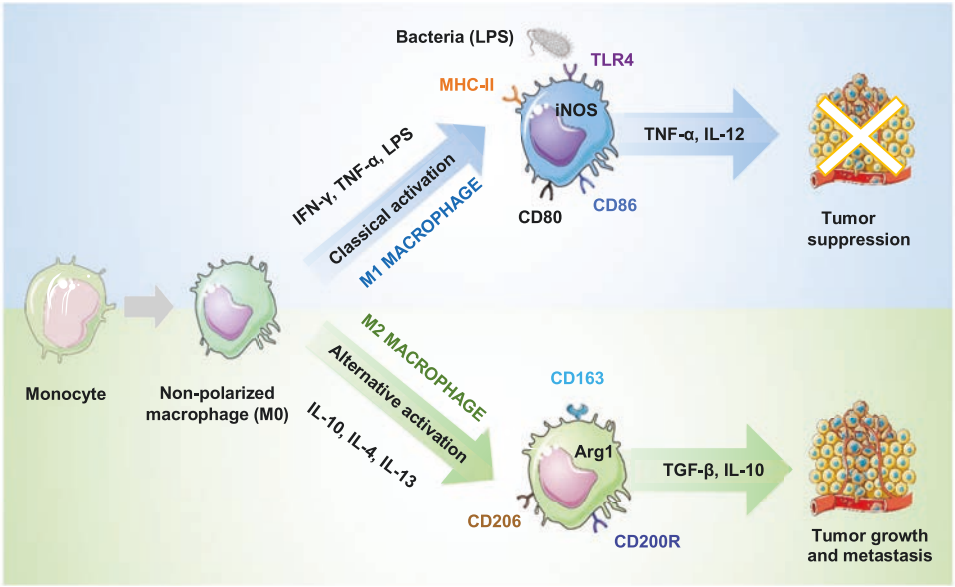

Figure 1. Schematic representation of M0 macrophage polarization pathways in response to specific mediators and the characteristic markers for each phenotype. M1 macrophages are involved in secreting inflammatory factors that kill tumor cells and are activated through the classical pathway. M2 macrophages are involved in promoting tumor growth and metastasis and are activated by an alternative pathway. Abbreviations: Arg1, arginase 1; iNOS, inducible nitric oxide synthase; LPS, bacterial lipopolysaccharide; MHC-II, major histocompatibility complex II; TLR4, Toll-like receptor 4.

a metastatic focus.[23,42,67] M2 macrophages contribute to tissue remodeling and repair, angiogenesis, tumor growth, and metastasis due to the secretion of anti-inflammatory cytokines (e.g., IL-10), TGF-β, indoleamine 2,3-dioxygenase 1 (IDO1), CCL20, and CCL22. Figure 1 shows the major markers of M2 macrophages, which include the mannose receptor (CD206), scavenger receptor (CD163), arginase 1 (Arg1), and CD200R.[60,68]

There is strong evidence that TAMs are usually found in the protumorigenic M2 phenotype instead of the antitumor M1 phenotype. This indicates that abundant macrophage recruitment to tumor sites results in a worse prognosis in most types of cancer.[64] Given the pivotal role played by TAMs in cancer immunity, exploiting the dichotomy between M1/M2 macrophage functions for modulating the immunosuppressive tumor microenvironment has received increasing attention. In this regard, depletion of TAMS or inhibition of macrophage recruitment to tumor sites may attenuate the tumor immunosuppressive response and prevent tumor progression.[37,42,43,65] Another promising strategy in cancer immunotherapy is to reprogram TAMs into the M1 phenotype, thereby exploiting the antitumor properties of M1 macrophages to inhibit tumor progression and metastasis. This approach has the potential to reinforce the antitumor activity of macrophages within the tumor microenvironment by converting the immunosuppressive tumor microenvironment into a more proinflammatory one, thereby improving the immune response against cancer.[27,42]

3.2. Cell–Cell Communication, Physiological, and Pathophysiological Processes

Macrophages are key sentinel cells of the innate immune system by virtue of their unique capability to identify, engulf, and phagocytize tumor cells, foreign particles, and invading microorganisms. This capability is derived from their crucial membrane markers that distinguish foreign particles from "self-particles."[36,69] These surface markers also play a key role in enabling communication between macrophages and their neighboring environment and different cells, such as tumor cells within the tumor microenvironment, by activating or suppressing specific signals.[19,23]

White blood cells can avoid clearance by the mononuclear phagocytic system. This feature is conferred by the "self-marker" CD47 and the leucocyte common antigen (CD45).[17,70,71] Macrophages have a natural tropism for inflammatory and tumor sites due to the presence of specific proteins on their membrane surface, such as cell adhesion molecules (e.g., integrins and selectins) and chemokine receptors. The latter can bind to specific adhesion molecules and inflammatory chemokines. These ligands are highly expressed in the inflamed endothelium to initiate transendothelial migration, a phenomenon known as diapedesis.[23,36,38,71] In addition to their inflammation-targeting properties, macrophages can also actively target metastatic cancer cells and penetrate the blood-brain barrier (BBB) via membrane receptor–ligand interactions. This renders macrophages attractive and versatile carriers for cargo delivery.[9,42,72] A summary of the main surface membrane markers involved in these multiple roles of macrophages is presented in **Table 2**.

Macrophages are crucial components of the tumor microenvironment. This microenvironment mediates complex interactions with tumor cells and controls tumor progression and cancer immunity.[18] Macrophages express on their membrane surface the signal-regulatory protein alpha (SIRPα) that binds specifically to the "self-marker" CD47, a transmembrane protein expressed in all healthy cells as well as some cancer cells.[37,73] The SIRPα/CD47 interaction creates a "don't eat me" signal that prevents the phagocytosis of healthy cells by macrophages. The "don't eat me" signal also prevents the immune clearance of CD47-overexpressing cancer cells. This creates an immunosuppressive environment that promotes tumor growth, and is responsible for the poor prognosis of several solid tumors.[37] Blocking this intercellular interaction is a powerful strategy in cancer immunotherapy for increasing the phagocytic activity of macrophages and improving their antitumor activity.[42,65]

Table 2. Overview of the macrophage cell membrane (MCM) markers and their functions.

Classification of the membrane marker	Macrophage membrane marker	Counter-receptor/ligand	Function	Refs.
"Self-recognition" protein	CD47	SIRPα (on mononuclear phagocytes)	Creates a "don't eat me signal" that inhibits immune clearance (prolonged blood circulation)	[17,70,71]
Cell adhesion molecule – selectins	L-selectin, PSGL-1	E-selectin, P-selectin (on endothelium)	Cell–cell adhesion (firm adhesion to endothelium)	[29,30,36,38]
Cell adhesion molecule – integrins	LFA-1 (αLβ2 integrin) Mac-1 (αMβ2 integrin)	ICAM-1 (on endothelium)	Cell–cell adhesion, facilitates macrophage migration across the endothelium and BBB toward inflamed and tumor tissues	[17,19,23,29]
Chemokine receptor	CCR2	CCL2	Strong chemotactic response that elicits macrophage migration to inflammatory sites and tumors	[19,38,42]
Cell adhesion molecule – integrins	VLA-4 (α4β1 integrin)	VCAM-1 (overexpressed by several metastatic cancer cells)	Cell–cell adhesion, increases macrophage uptake in VCAM-1 positive metastatic cells	[38,42]

Abbreviations: BBB, blood-brain barrier; CCL2, CC-chemokine ligand 2; CCR2, CC-chemokine receptor 2; ICAM-1, intercellular adhesion molecule 1; LFA-1, lymphocyte function-associated antigen 1; Mac-1, macrophage-1 antigen; PSGL-1, glycoprotein P-selectin ligand 1; SIRPα, signal-regulatory protein alpha; VCAM-1, vascular cell adhesion protein 1; VLA-4, very late antigen 4.

3.3. Macrophages and Macrophage-Derived Structures as Drug Delivery Systems

There is growing interest in using macrophages for diagnostic and therapeutic purposes. In the context of cancer, using TAMs as targets for cancer therapy, or biomarkers for cancer diagnosis and prognosis, has become attractive.[74] One example is the use of macrophages for early detection of cancer. In a recent study, a highly sensitive macrophage-based in vivo sensor was developed by exploiting the repolarization of macrophages to the protumorigenic M2 phenotype, to detect 4T1 breast tumors that are smaller than 50 mm^3.[75] Because tumor-penetrating M2 macrophages (TAMs) overexpress specific markers that are involved in an immunosuppressive tumor microenvironment, such as Arg1, Ym1, Mrc1, and Fizz1, the TAMs were genetically modified to express an artificial bioluminescent reporter upon activation of the Arg1 promoter. The reporter could be detected by bioluminescent imaging and blood measurement of the secreted reporter. The main advantage of this biocompatible sensor was its enhanced tropism for tumor sites. This is because macrophages have an innate ability to be recruited to tumors. This macrophage-based sensor has the potential to overcome the poor sensitivity and specificity of conventional cancer diagnostic techniques.[75]

For therapeutic applications, more attention has been given to M1 macrophages as drug carriers because of their biocompatibility, antigen presentation ability, ability to cross biological barriers, and tropism for tumors and inflammatory sites.[23,30] Moreover, macrophages may have an intrinsic antitumor potential owing to their phagocytosis ability. The potential of these immune cells for targeted drug delivery is multifaceted, as they can target both primary and metastatic tumors, and can also infiltrate deep inside hypoxic regions of tumors. This provides an opportunity for the macrophages to target these poorly perfused tumorous areas that are resistant to radiotherapy and chemotherapy.[18,62] Conventional NPs can only gain limited access to deep tumorous tissues through the enhanced permeability and retention effect, because of the elevated interstitial pressure and poorly developed tumor vasculature. By contrast, macrophages can infiltrate into these deep regions because they are unaffected by the interstitial pressure. This highlights the potential of macrophages to successfully deliver drugs or agents to deep neoplastic tissues.[18,30]

Similar to other drug delivery vehicles, macrophage-based delivery systems can protect their therapeutic payloads from recognition and clearance by the mononuclear phagocytic system. This helps to improve the pharmacokinetic properties of the loaded drugs. Reduction in both hepatic and renal excretion results in a longer circulation time in vivo.[18,30,43] As outlined in **Figure 2**, several promising macrophage-based systems have currently been studied as carriers for targeted delivery of therapeutic agents to the tumor microenvironment and sites of inflammation. These macrophage-based systems have the potential to increase therapeutic efficacy and prevent off-target toxicity. These approaches include 1) live macrophages as drug delivery vehicles, 2) macrophage-derived extracellular vesicles (EVs) and EV-like NPs as drug delivery vehicles, 3) macrophage-derived proteolipid nanovesicles as drug delivery vehicles, and 4) MCM-based NPs as drug delivery vehicles.[41,42]

3.3.1. Live Macrophages

Live macrophages are used as drug carriers because of their intrinsic phagocytosis capacity, tropism for tumor tissues and ability to penetrate deep inside tumors. Contemporary research has focused on constructing ex vivo macrophage-based drug delivery systems by incubating living macrophages with drugs or drug-loaded NPs (Figure 2A).[18] Because macrophages can naturally phagocytose foreign materials, they can engulf drugs or NPs and deliver them precisely to sites where macrophages tend to gather. The most conventional approach is to engineer macrophages to carry NPs (rather than directly transporting drugs). This is because NPs help to decrease the toxicity of therapeutic agents to macrophages. This results in better therapeutic results by increasing drug loading.[11,42,43,62] In a recent study, the unique ability of macrophages to penetrate into the hypoxic regions of tumors was exploited to enable targeted delivery of poly(lactic-*co*-glycolic acid) (PLGA) NPs containing tirapazamine, a hypoxia-activated prodrug, to the hypoxic areas of 4T1 breast tumors in vivo. Such an experimental treatment scheme enhanced accumulation of the drug-loaded macrophages within the tumors and resulted in better inhibition of tumor growth.[76] Other nanomaterials have been encapsulated within live macrophages for therapeutic purposes. One example is the encapsulation of photothermal nanoprobes (MFe_3O_4-Cy5.5) for photothermal ablation of glioma, due to intrinsic ability of the macrophages in crossing the BBB.[77] Another example is the encapsulation of magnetic NPs that capable of migrating to tumor sites under external magnetic guidance.[78] The results of these studies demonstrate the potential of living macrophages as cell-based delivery vehicles for targeted and precise cancer therapy.

Perhaps the most serious disadvantage of encapsulating NPs in living macrophages is the potential degradation of the cargo within macrophage phagosomes after engulfment. This results in reduction of drug release from the macrophages. To address this issue, NPs may be immobilized on the surface of the macrophages instead of being phagocytosed by those cells. Such a strategy helps to improve the integrity of the cargo and the targeting ability of macrophages. Thus, NPs can be internalized or attached to macrophages in a "hitchhiking" approach for targeted drug delivery.[18,62,72]

3.3.2. Macrophage-Derived Extracellular Vesicles and Extracellular Vesicle-Like Nanoparticles

M1 macrophage-derived EVs have also been investigated as potential candidates for drug delivery. The EVs secreted by M1 macrophages express surface proteins identical to those expressed on the parent cells. Accordingly, the EVs may inherit their tumor and inflammation-targeting capability.[42,65] Extracellular vesicles perform a crucial role in cell–cell communication. According to their size and origin, EVs may be classified into exosomes, microvesicles, or apoptotic bodies. As depicted in Figure 2B, macrophage-derived EVs may be utilized in different ways. For example, naturally secreted exosomes may be loaded directly with therapeutic agents for targeted delivery to tumors.

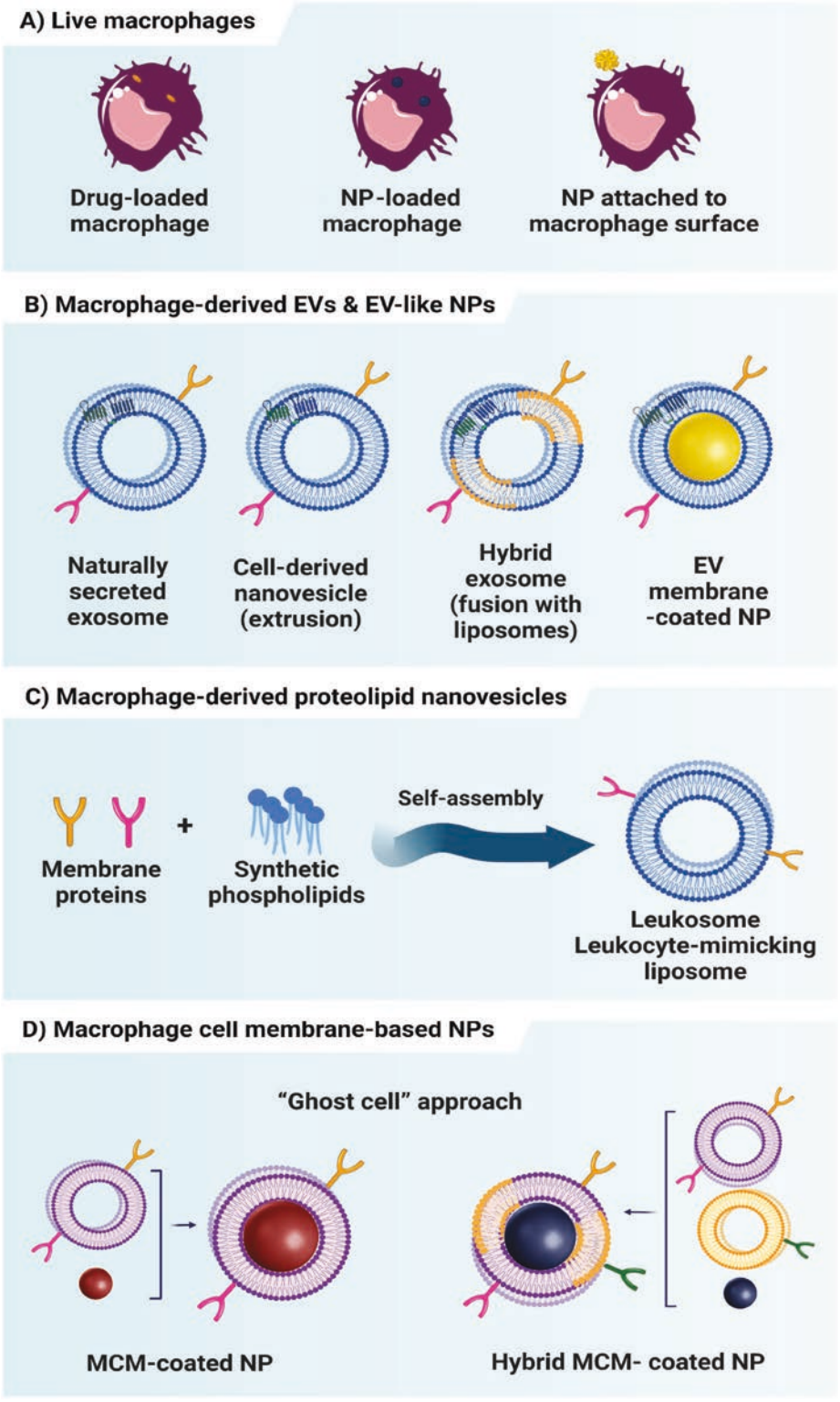

Figure 2. Bioinspired macrophage-based therapeutics for active targeted drug delivery. These therapeutic systems include: A) live macrophages (encapsulation of drugs and NPs or NP surface conjugation), B) macrophage-derived extracellular vesicles (EVs) and EV-like nanoparticles (including naturally-secreted exosomes and artificial EVs, namely, exosome-mimetic nanovesicles, obtained by directly extruding macrophage cells through porous membranes, hybrid exosomes and macrophage EV membrane-coated NPs), and C) macrophage-derived proteolipid nanovesicles, also known as leukosomes. They are prepared by self-assembly of synthetic lipid bilayers and surface membrane proteins using a synthetic approach (surface membrane proteins can be extracted from different biomembranes). D) MCM-based NPs (including single MCMs or hybrid MCMs produced by fusion of different types of cell membrane nanovesicles). Abbreviations: EV, extracellular vesicle; MCM, macrophage cell membrane; NP, nanoparticle.

Despite the promising results achieved with the use of natural exosomes, there are limitations as to how far the concept of using exosomes as drug carriers can be taken. The extraction and isolation of exosomes remain a real challenge due to the low number of EVs secreted by cells, the extremely low yield of EV isolation procedures, as well as the possibility of disrupting the integrity and function of the EVs during the isolation process.[42,79] To circumvent these drawbacks, a great deal of effort has been devoted to the study of artificial exosomes. Artificial EVs (EV-like NPs) include exosome-mimetic nanovesicles, hybrid exosomes, and EV membrane-coated NPs.[42,80] The preparation of exosome-mimetic nanovesicles relies on serial cell extrusion via top-down approaches.[80] In a recent study, exosome-mimetic nanovesicles were developed by directly extruding doxorubicin-loaded macrophages through porous membranes for drug delivery. These cell-derived doxorubicin-loaded nanovesicles can mimic the protein profile, size and tumor-homing properties of macrophage-secreted exosomes, with the added benefit of a higher production yield.[81] In another study, the tumor-targeting capability of macrophage-derived exosome-mimetic nanovesicles was harnessed to codeliver AB680 (a small molecule CD73 inhibitor) and the monoclonal antibody against programmed cell death ligand 1 (PDL1, an immune checkpoint inhibitor) for targeted immunotherapy for bladder cancer.[82] These macrophage-derived nanovesicles preserve the protein content of natural exosomes as well as their intrinsic targeting property to tumor tissues, while providing a superior production yield.[82]

Another promising strategy to address the aforementioned shortcomings of EVs is the use of hybrid exosomes. These entities are prepared by fusing macrophage-derived exosomes with synthetic liposomes through membrane extrusion. The objective of this fusion process is to combine the tumor-homing features of natural exosomes with the biopharmaceutical benefits of liposome-based drug delivery systems, such as enhanced flexibility for surface modification and large-scale production.[42,79] As an example, macrophage-derived hybrid exosomes with intrinsic tumor-targeting ability, superior drug loading capacity and a pH-sensitive drug release property were employed as a biomimetic nanocarrier to deliver doxorubicin to breast cancer cells. Such an experimental strategy to overcome the issues are related to low production yield and poor modification flexibility of EVs.[79]

The application of macrophage-derived EV membrane-coated NPs has been reported for the treatment of rheumatoid arthritis and the management of lung metastasis from orthotopic breast cancer.[83,84] In both studies, the enhanced ability of the NPs to target inflammatory sites and tumors was attributed to their membrane coating. It is because the membrane-derived from macrophage-secreted EVs expresses a protein profile similar to that of macrophages. This bestows the NPs with the desirable macrophage functionality.[83,84]

3.3.3. Macrophage-Derived Proteolipid Nanovesicles

Macrophage-derived proteolipid nanovesicles can be synthesized to closely mimic the complex surface protein composition and unique features of the macrophage membrane. These nanovesicles have been investigated as drug delivery platforms for targeted therapy in cancer, viral infections, sepsis, and other inflammatory disorders.[41,85,86] Macrophage-derived proteolipid nanovesicles are prepared by a bottom-up approach, using the self-assembly of synthetic lipid bilayers (liposomes) with key surface membrane proteins extracted from leukocytes (Figure 2C).[80] In a recent study, leukocyte-mimicking liposomes (a.k.a. leukosomes) were designed by anchoring surface membrane proteins isolated from murine J774 macrophages into a synthetic phospholipid bilayer for delivery of dexamethasone to inflamed sites, or doxorubicin to treat both melanoma or breast cancer.[87,88] The resulting nanovesicles preserved the expression of CD45, CD47, LFA-1, Mac-1, and PSGL-1. These nanovesicles demonstrated enhanced ability to recognize and adhere to inflamed vasculature and reduced immune clearance. The dexamethasone-loaded leukosomes had a fivefold increased accumulation at sites of inflammation compared to liposomes. Likewise, the doxorubicin-loaded leukosomes showed superior targeting ability to 4T1 breast cancer cells and B16 melanoma cells, with better tumor accumulation compared to free doxorubicin. These studies illustrate the potential of leukosomes for management of localized inflammation and for chemotherapeutic drug delivery to 4T1 and B16 tumors.[87,88]

In addition to modifying liposomes with cell membrane proteins, a novel strategy consisting of fusing synthetic liposomes to cell membrane fragments has also been reported. Such a strategy has been successfully employed to confer biomimetic and site-specific targeting properties.[89] For instance, a biomimetic liposome-based platform was recently designed for targeted chemophototherapy against 4T1 breast cancer. This liposome-based platform was produced by fusing liposomes containing both platinum NPs, a chemotherapeutic agent and verteporfin (a photosensitizer) with murine RAW 264.7 cell-derived membranes using a freeze-thaw method and extrusion.[89] The hybridization of the MCM with the liposomal membrane endowed the engineered liposomes with improved immune evasion, tumor targeting, and prolonged systemic circulation. This resulted in improved antitumor efficacy and extended survival of tumor-bearing mice.[89]

Incorporation of membrane fragments from different cell types, such as HN12 cancer cells or J774A.1 macrophages, into paclitaxel-loaded liposomes via extrusion has also been reported. This strategy combined immune evasion and homotypic tumor-targeting properties of macrophages and cancer cells, respectively.[90] The liposomes fused with both macrophage and cancer cell membranes, producing what is known as leutusomes. The leutusomes demonstrated superior antitumor effects and inhibited tumor growth in vivo.[90]

3.3.4. Macrophage Cell Membrane-Based Nanoparticles

Despite the exciting potential of macrophage-based drug delivery vehicles, current research has focused on MCM-coated NPs as drug delivery vehicles for biomedical applications. This emergent top-down approach aims at designing biomimetic nanocarriers that combine the biofunctionality of macrophages and the biopharmaceutical advantages of nanomaterials. Indeed, the site-specific targeting and immune evasion ability exhibited by macrophage cells are essentially a consequence

of their surface membrane proteins. These membrane proteins may be preserved and transferred to the NP surface by wrapping them with macrophage-derived membranes, using a "ghost cell" approach.[9,18]

In addition to using single cell-derived membranes, other coating materials have been investigated in this biomimetic approach. These coating materials include hybrid cell membranes that incorporate multiple functionalities derived from different cell membranes (Figure 2D).[15,91,92] In a recent study, a macrophage-4T1 breast cancer cell hybrid membrane was prepared by fusing both types of cell membranes. The hybrid membrane was used to transport PLGA NPs containing doxorubicin, for targeted drug delivery to pulmonary metastases that originated from breast cancer.[93] The authors showed that the hybrid cell membrane retained the protein markers of both macrophages and cancer cells, as well as their specific biological properties. This enabled the resulting NPs to be endowed with the homotypic tumor-targeting capability of cancer cells and the metastasis-targeting ability of macrophages. Because of these multifunctional properties, the biomimetic nanoplatform demonstrated substantial reduction in the number of lung metastatic nodules and efficiently prolonged survival time in vivo. The results suggest that biomimetic NPs coated with a macrophage-4T1 cancer cell hybrid membrane is an exciting therapeutic approach for treating pulmonary metastasis that own its origin from breast cancer.[93]

Apart from cancer cell membranes, platelet membranes were recently used to fuse with the membrane of RAW 264.7 macrophages for creating a hybrid membrane that incorporated the intrinsic targeting ability of both cell membranes to triple-negative breast cancer cells.[94] In this study, the hybrid membranes were successfully coated onto dendritic large-pore mesoporous silicon NPs. Those assemblies were coloaded with doxorubicin and the near-infrared (NIR) fluorescent dye IR780, a phototherapeutic agent, for targeted chemophototherapy against triple-negative breast cancer.[94]

4. Fabrication of Macrophage Cell Membrane-Coated Nanomaterials

The fabrication of MCM-coated NPs requires a few sequential steps. This is achieved by coating the previously prepared NP inner core with an MCM-derived nanovesicle. The preparation steps involve 1) extraction of the outer membrane and isolation of membrane vesicles (often referred to as "ghost cells"), 2) selection and fabrication of the NP core, and 3) fusion of the MCM nanovesicles with the NP core either by coextrusion, sonication or electrostatic interaction to produce MCM-coated NPs. In the following sections, an overview of these three fundamental steps will be presented. **Figure 3** shows a schematic of the three steps used to synthesize MCM-coated NPs.

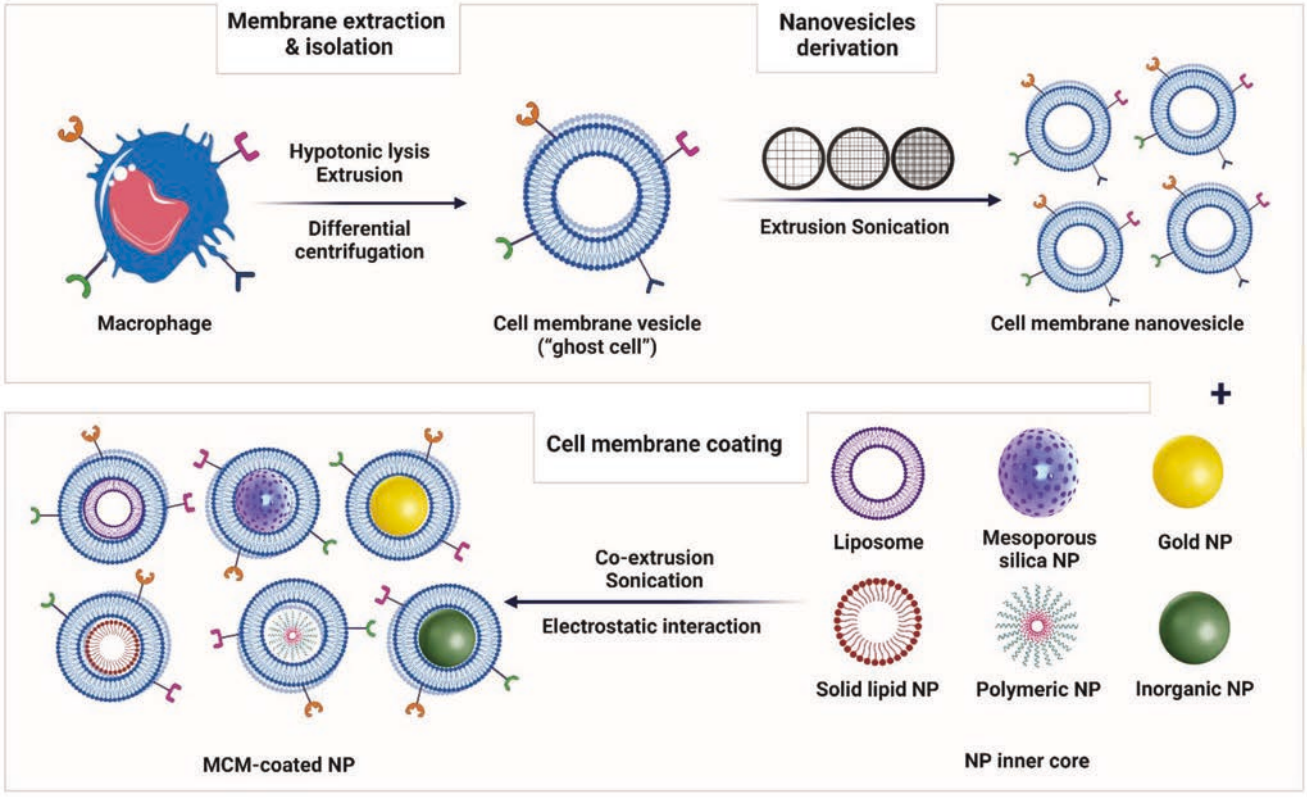

Figure 3. Schematic of the steps required for coating different types of NPs with MCM nanovesicles. These steps include extraction of the outer membrane from macrophages, preparation of nanovesicles, preparation of the NP inner core, and fusion of the NPs to a cell membrane nanovesicle via a top-down approach. Abbreviations: MCM, macrophage cell membrane; NP, nanoparticle.

4.1. Cell Membrane Extraction and Nanovesicle Preparation

Conventional methods used to extract biomembranes from natural cells are mainly based on cell disruption and lysis. However, the choice of the exact methodology is governed by the cell type.[15,23] Several gentle techniques that disrupt the cell structure and induce cell lysis have been used to empty the source cells and remove their intracellular contents. These techniques include hypotonic lysis buffer, freeze–thaw cycles, sonication, extrusion, and the use of Dounce homogenizer. These techniques keep the surface membrane proteins intact to maintain the biofunctionality of the cell membranes for successful biological interactions.[15,95]

The first step in fabricating MCM-coated NPs involves extracting the outer membranes from previously isolated macrophage cells. Because white blood cells have complex intracellular components and are nucleated, the membrane extraction procedures are more complicated than for red blood cells. The technique first involves cell disruption to remove the nucleus and cytoplasmic components, such as hypotonic lysis treatment and extrusion. This is followed by differential centrifugation and purification of the isolated membranes.[15,23,96,97] The retrieved membranes are then mechanically extruded through variable-sized pores on a polycarbonate membrane. The extruded membranes are then sonicated to produce a nanosized vesicle that mimics the complete proteolipid composition of the original MCMs.[96–98]

4.2. Nanoparticle Core Preparation

The next step includes the preparation of the NP core and loading the required payload onto the NPs. The payload may be a diagnostic or a therapeutic agent.[15] Since the first description of cell membrane-coating nanotechnology, a wide variety of nanoconstructs composed of different materials has been used as the inner core. These materials include gold-based NPs, lipid-based NPs, inorganic NPs, mesoporous silica NPs, upconversion NPs (UCNPs) or magnetic iron oxide NPs (Fe_3O_4 NPs). Organic polymeric NPs, such as chitosan NPs, albumin NPs or PLGA NPs have also been reported.[96,99,100] The selection of NP material is based on the specific characteristics and requirements of the payload. Regardless of the core material, a negative zeta potential of the NP core is necessary to correctly orientate the cell membrane around the NP surface. This is because of the electrostatic repulsive forces between the negatively charged NP core and the negatively charged extracellular membrane constituents.[69,101]

4.3. Coating Nanoparticle Cores with Extracted Cell Membranes

After separately obtaining the cell membrane nanovesicles and the NP inner cores, both components must be fused so that a membrane coating can be formed on the NP surface, resulting in a core–shell nanostructure. For this purpose, different coating techniques have been suggested in the literature. These coating techniques include membrane extrusion through a porous membrane, sonication, electroporation or electrostatic interactions.[66,97,101]

The first and most common coating method is based on physical extrusion. In this method, the NP cores and cell membrane vesicles are coextruded several times through a porous membrane to produce the final MCM-coated NPs.[97,98] Lately, a sonication coating approach has been described, in which both components are mixed and coincubated under the influence of ultrasound. Here, they are exposed to disruptive forces derived from ultrasonic energy to form MCM-coated NPs, with the additional advantage of losing less material compared to physical extrusion.[9,98] Another reported method for coat NPs with cell membranes relies on electrostatic interactions between the positively charged NP inner core and negatively charged membrane vesicles, resulting in spontaneous generation of membrane-coated NPs. In this approach, the strong electrostatic attraction induces disruption of cell membranes, which is required for successful membrane coating.[101]

Apart from these fusion methods, an electroporation technique using live macrophages (instead of purified cell membranes) has very recently been reported for preparing MCM-coated inorganic NPs. This technique helps to solve problems related to loss of cell membrane integrity when using extrusion or sonication approaches.[102] In this approach, the NPs are first incubated with and phagocytosed by macrophages. The resulting NPs-loaded macrophages are subsequently exposed to an external electric field to open pores on the cell membrane through which only the cellular contents (but not the NPs) are released.[102] In situ packaging of NPs has also been described to yield high quality cell membrane-coated NPs.[9,39] This method consists of incubating living cells with NPs for collecting cell-secreted vesicles containing the exogenous NPs. The in situ packaging technique has the advantage of preserving the integrity of cell membranes and surface proteins.[9,39] **Table 3** summarizes the principle of these coating techniques and their main limitations.

5. Biomedical Applications

Macrophage cell membrane-coated NPs have recently been designed to combine the advantages of macrophages and the biopharmaceutical effects of nanomaterials. These MCM-coated NPs have been used successfully for a variety of biomedical applications, ranging from cancer bioimaging and therapy, to the management of inflammatory disorders and infections.[19,23,28] Because of their immune evasion properties, enhanced biocompatibility, superior ability to target inflammatory and tumor sites, and to bind to tumor cells, MCM-coated NPs have been used extensively as carriers of imaging agents, therapeutic drugs, immunomodulators, photosensitizers or photothermal agents for cancer therapy, imaging and theranostics. Moreover, MCM-coated NPs have also been recently used for diagnosis and treatment of diseases other than cancer, namely, vascular disorders characterized by inflammation (atherosclerosis, acute ischemic stroke, and vascular intimal hyperplasia), inflammatory osteolysis, age-related macular degeneration, Alzheimer´s disease, and infectious diseases (bacterial and viral infections). A schematic of some of the biomedical applications of MCM-coated nanosystems is presented in **Figure 4**. **Tables 4** and **5** summarize the biomedical applications of MCM-coated nanosystems.

Table 3. Coating techniques for preparing macrophage cell membrane (MCM)-coated nanosystems.

Fusion method	Principle of the technique	Key limitations	Refs.
Physical extrusion or coextrusion (inspired by liposome synthesis)	The mechanical force of extrusion provokes the disruption of cell membrane structure, allowing its reconstruction around the NP core	• Difficult scalability • Time-consuming • Possible disruption of cell membrane integrity	[15,99,101,103]
Sonication	The ultrasonic energy induces the spontaneous reconstruction of the cell membrane around the NP core	• The final coated NPs exhibit a huge variation in size • Ultrasonic parameters (power, frequency, duration) should be optimized to enhance fusion efficacy • Nonuniform membrane coating might be formed onto the NPs • Possible disruption of cell membrane integrity	[15,99,101,103]
Electrostatic interaction	Spontaneous assembly through electrostatic attractions between the positively charged NP inner core and negatively charged membrane vesicles	• Noncomplete membrane coating might be formed onto the NPs	[101]
Electroporation	Membrane coating onto NPs through the use of an external electric field to open transient pores on the cell membrane	• Not suitable for larger nanomaterials	[39]
In situ production	Incubation of living cells with NPs and collection of cell-secreted vesicles containing the NPs	• Reduced fusion efficiency (<1% of the NPs possess a cell membrane coating)	[39]

Abbreviation: NP, nanoparticle.

5.1. Cancer Diagnosis and Therapy

Contemporary experimental cancer therapy has exploited the unique ability of macrophages to migrate to tumor sites to improve antitumor effects against both primary tumors and metastatic cancer.[72] Recent research in this area has shown promising results for MCM-coated nanosystems to deliver chemotherapy drugs to primary tumors. Other applications of MCM-coated nanosystems include antimetastatic therapy, antiangiogenic therapy, antiproliferative cancer therapy, cancer immunotherapy, phototherapy, cancer bioimaging, cancer theranostics, and capture of circulating tumor cells (CTCs).

5.1.1. Cancer Bioimaging

Accurate localization of tumors prior to cancer treatment, achieved by the imaging of the tumor area, is crucial for effective and localized cancer therapy. Cancer imaging is a noninvasive modality for the early detection of cancer, monitoring of tumor progression and detection of metastasis.[104,105] Different optical imaging techniques have been investigated for cancer diagnosis. These imagining techniques include magnetic resonance imaging (MRI), positron emission tomography, computerized tomography, photoacoustic tomography, and fluorescence imaging (which employs fluorescent probes

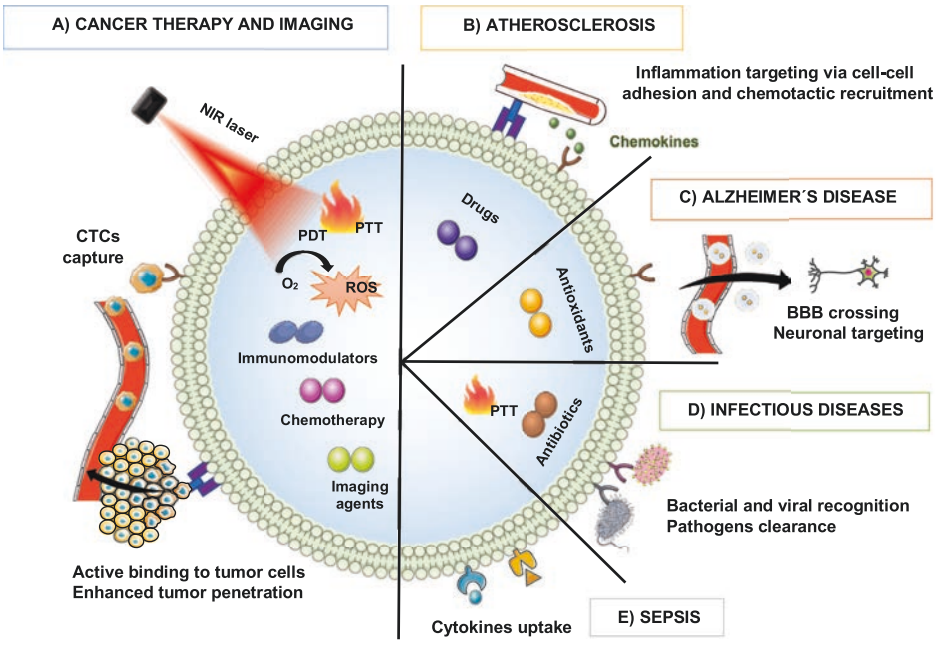

Figure 4. Biomedical applications of MCM-coated nanosystems: A) cancer therapy and imaging, B) atherosclerosis, C) Alzheimer's disease, D) infectious diseases, and E) sepsis. Abbreviations: BBB, blood-brain barrier; CTC, circulating tumor cell; MCM, macrophage cell membrane; NIR, near-infrared; PDT, photodynamic therapy; PTT, photothermal therapy; ROS, reactive oxygen species.

Table 4. Overview of nanosystems coated by a single macrophage cell membrane (MCM) or hybrid MCM for cancer diagnosis, therapy, and theranostics.

Application	Cell membrane coating	Inner core	Cargo(es)	Coating method	Size/zeta potential	Mouse model	Principal outcomes	Refs.
Cancer bioimaging	RAW 264.7 macrophage cell membrane	Upconversion nanoparticle (UCNP)	–	Coextrusion through 200 nm porous membrane	100.0 nm −20.0 mV	Xenograft mouse model of MCF-7 breast cancer	• ↑ biocompatibility • Immune evasion properties • ↑ active tumor-targeting ability • Good performance of the fluorescent UCNP, resulting in efficient cancer imaging in vivo	[44]
Chemotherapy delivery to primary tumors	RAW 264.7 macrophage cell membrane	Mesoporous silica nanocapsule	Doxorubicin	Coextrusion through 100 nm porous membrane (20 times)	65.1 nm −16.9 mV	4T1 breast cancer mouse model	• ↑ biocompatibility • ↑ blood circulation time • Specific uptake by tumor cells • ↑ cancer ablation with chemotherapy	[107]
	RAW 264.7 macrophage cell membrane	Albumin nanoparticle (NP)	Paclitaxel	Coextrusion through 200 nm porous membrane	188.7 nm −10.5 mV	B16F10 melanoma mouse model	• Immune evasion properties • Specific uptake by tumor cells • Tumor-targeted chemotherapy against malignant melanoma in vivo	[46]
	Macrophage cell membrane	Poly(lactic-*co*-glycolic acid) (PLGA) NP	Gemcitabine	Coextrusion (20 times)	≈192.0 nm −16.8 mV	Xenograft mouse model of human pancreatic cancer (PANC-1)	• Immune evasion properties • Tumor-targeted delivery of gemcitabine with minimal toxicity • Synergistic antitumor effects of gemcitabine and erlotinib by downregulation of PI3K/AKT and MEK/ERK signaling pathways	[108]
	Interleukin 4 (IL-4)-induced M2 macrophage cell membrane	Polyfluorocarbon NP	Cabazitaxel	Coextrusion (20 times)	73.6 nm −9.2 mV	4T1 and MCF-7 breast cancer mouse models	• Intrinsic targeting properties to tumor tissue • ↑ intratumoral penetration • ↓ number of both cancer cells and cancer stem cells • ↓ tumor growth and progression	[109]
	Macrophage cell membrane	Polymer-based NP functionalized with a cationic ligand (PPiP) and an IGF1R-targeting ligand	Paclitaxel	Coextrusion	200.1 nm −31.3 mV	Orthotopic MDA-MB-231 breast cancer mouse model	• Multitargeting properties • ↑ biocompatibility • Active tumor-targeting ability • Controlled and stepwise release of paclitaxel in the acidic pH within the tumor microenvironment	[110]
Therapy for metastatic tumors	RAW 264.7 macrophage cell membrane	Liposome	Emtansine	Coextrusion through 400 and 200 nm porous membrane	115.4 nm 26.2 mV	4T1 breast cancer mouse model with lung metastasis	• Encapsulation efficiency of 96.7% • ↑ biocompatibility • ↓ macrophage uptake • ↑ drug uptake by metastatic 4T1 breast cancer cells in the lung tissue • Efficient suppression of lung metastasis from breast cancer	[45]
	RAW 264.7 macrophage cell membrane	DNA tetrahedron dendrimer-liposome	DOX prodrug (DOX-MPK)	Coextrusion through 200 nm porous membrane	91.0 nm −22.6 mV	4T1 breast cancer mouse model with lung metastasis	• Selective accumulation at sites of lung metastasis • 2.1-fold increase in lung accumulation compared to uncoated NPs after 4 h of administration • Controlled drug release in response to the acidic pH within the tumor microenvironment • ↓ number of metastatic nodules in the lung tissue	[111]

Table 4. Continued.

Application	Cell membrane coating	Inner core	Cargo(es)	Coating method	Size/zeta potential	Mouse model	Principal outcomes	Refs.
Antiangiogenic therapy	T7 peptide-inserted macrophage cell membrane	PGLA NP	Saikosaponin D	Coextrusion	222.0 nm ≈−20.0 mV	4T1 breast cancer mouse model	• Targeted antiangiogenic therapy with minimal side effects • Suppression of primary breast cancer growth and lung metastasis	[112]
Antiproliferative cancer therapy	TNF-α- attached macrophage cell membrane	Chitosan NP	–	Coextrusion through 200 nm porous membrane	–	MDA-MB-231, MCF-7 and HeLa cancer cell lines	• ↑ biocompatibility • ↑ cytotoxic effects against different cancer cells lines in vitro	[113]
Cancer immunotherapy	Azide-attached macrophage cell membrane (dual-functionalized with the T-cell stimulatory signals pMHC-1 and anti-CD28)	Fe_3O_4 magnetic nanocluster	–	Electrostatic interaction	380.0 nm −25.0 mV	EG-7 tumor-bearing mouse model	• Promising as antigen-presenting cell to CD8 + T cells ex vivo • ↑ accumulation at tumor sites with magnetic guidance in vivo • Good magnetic resonance imaging (MRI) properties in vivo	[117]
	Bacterial lipopolysaccharide-induced M1 macrophage cell membrane	PLGA NP	Fe_3O_4 NP and imiquimod (R87)	Coextrusion through 200 nm porous membrane	166.2 nm −22.8 mV	Orthotopic 4T1 breast cancer mouse model	• ↑ biocompatibility • ↑ M2-to-M1 macrophage phenotypic conversion efficiency in tumor tissue • Synergistic effects to enhance antitumor immunity and eradicate cancer	[118]
	RAW 264.7 macrophage cell membrane	Polydopamine NP	Repolarization agent TMP195	Coextrusion through 200 nm porous membrane	159.6 nm −20.0 mV	4T1 breast cancer-bearing mouse model	• ↑ biocompatibility • Tumor-targeting ability • ↑ M2-to-M1 macrophage phenotypic conversion efficiency in tumor tissue • Synergistic effects of photothermal therapy (PTT) and immunotherapy to enhance tumor ablation	[119]
Photothermal therapy (PTT)	RAW 264.7 macrophage cell membrane	Iron oxide (Fe_3O_4) NP	–	Coextrusion (11 times)	100.0 nm −18.0 mV	Xenograft mouse model of MCF-7 breast cancer	• ↑ biocompatibility • ↓ macrophage uptake • Intrinsic targeting properties to tumor tissue • Selective uptake by tumor cells • ↑ near-infrared (NIR) absorption capacity for photothermal cancer ablation in vivo	[122]
	Macrophage cell membrane	Gold nanoshell (AuNS)	NIR fluorescent dye cyanine 7 (Cy7)	Sonication and extrusion through 200 nm porous membrane	100.0 nm −20.8 mV	4T1 breast cancer-bearing mouse model	• Potential for fluorescence imaging and tumor-targeted PTT in vivo • Efficient suppression of 4T1 tumor growth (almost complete tumor eradication after 25 days of treatment)	[123]
	Macrophage cell membrane	Janus mesoporous silica nanomotor	–	Sonication (100 W)	80.6 nm −21.4 mV	–	• Immune evasion properties • ↑ uptake by 4T1 tumor cells in vitro • Enhanced tumor penetration • Photothermal effects in vitro	[124]

Table 4. Continued.

Application	Cell membrane coating	Inner core	Cargo(es)	Coating method	Size/zeta potential	Mouse model	Principal outcomes	Refs.
	Macrophage cell membrane	DSPE-PEG liposome	NIR-Ib fluorescence IR-792 dye	Coextrusion (20 times)	137.0 nm −20.8 mV	Orthotopic U87L glioblastoma mouse model	• ↑ biocompatibility • Efficient blood-brain barrier (BBB) penetration ability • ↑ accumulation at glioblastoma sites • Photothermal cancer ablation with NIR-Ib fluorescence imaging guidance • Extended survival time of glioblastoma-bearing mice to 22 days (superior to other groups)	[125]
	Macrophage cell membrane	Hollow bismuth selenide NP	Quercetin	Coextrusion	155.3 nm −19.1 mV	4T1 breast cancer mouse model with lung metastasis	• Immune evasion properties • Dual tumor-targeting ability through the CCR2/CCL2 chemotactic recruitment and $\alpha 4\beta 1$ integrin/VCAM-1 interaction • Good in vivo X-ray computed tomography (CT) and infrared thermal (IRT) imaging performance • Photothermal effects • Synergistic effects for potentiating PTT in vivo and inhibiting lung metastasis from breast cancer	[127]
	Peritoneal macrophage cell membrane functionalized with an anti-PDL1 antibody	Hollow gold nanocage nanocomposites	Galunisertib	Coextrusion	57.0 nm −18.0 mV	CT26 colon carcinoma-bearing mouse model	• Specific uptake by tumor cells • Synergistic antitumor effects of PTT and immunotherapy • ↓ primary tumor growth • ↓ number of metastatic lung nodules • Extended overall survival of tumor-bearing mice	[128]
	RAW 264.7 macrophage-H22 hepatic cancer cell hybrid membrane	Cooper sulfide (CuS) NP	Sorafenib	Sonication	≈210.0 nm	Hepatocellular carcinoma-bearing mouse model	• ↑ biocompatibility • Immune evasion properties • Homotypic tumor-targeting ability • ↑ NIR absorption capacity for photothermal tumor ablation • Synergistic chemo-PTT	[129]
Photodynamic therapy (PDT)	Bacterial lipopolysaccharide-induced M1 macrophage cell membrane	PEGylated bilirubin NP	Doxorubicin, indoximod (IND) and chlorin e6 (Ce6)	Sonication (100 W, 2 min) and extrusion	117.0 nm	B16F10 and 4T1 cancer-bearing mouse models	• Prolonged systemic circulation • Tumor-targeted codelivery of doxorubicin, IND and Ce6 • ↑ reactive oxygen species (ROS) generation ability upon NIR light irradiation • Synergistic effects of immunotherapy, chemotherapy and PDT to induce strong antitumor immune responses	[133]
	Bacterial lipopolysaccharide-induced M1 macrophage cell membrane	Bilirubin/Ce6 core	Paclitaxel, IND and Ce6	Sonication (65W, 5 s)	–	4T1 breast cancer-bearing mouse model	• Tumor-targeted codelivery of paclitaxel, IND and Ce6 • Promising for multimodal therapy through immunotherapy, chemotherapy and PDT, resulting in efficient suppression of primary tumor growth and lung metastasis	[135]

Table 4. Continued.

Application	Cell membrane coating	Inner core	Cargo(es)	Coating method	Size/zeta potential	Mouse model	Principal outcomes	Refs.
	RAW 264.7 macrophage cell membrane	CuS NP	Paclitaxel	Coextrusion through 200 nm porous membrane (20 times)	191.8 nm −33.4 mV	4T1 breast cancer-bearing mouse model	• Synergistic targeting effects of $\alpha 4\beta 1$ integrin and iRGD peptide • ↑ uptake by 4T1 cancer cells • ↑ NIR absorption capacity for ROS and heat generation • Effective tumor eradication through PTT, PDT, and chemotherapy	[136]
	Macrophage cell membrane	Mesoporous silica nanorod functionalized with folic acid	Doxorubicin, L-menthol and indocyanine green	Coextrusion	−27.9 mV	Tumor-bearing mice model	• Multitargeting properties • Immune evasion properties • ↑ tumor accumulation and uptake by tumor cells in the acidic tumor microenvironment • Synergistic antitumor effects of PTT, PDT, and chemotherapy	[137]
Capture of circulating tumor cells (CTCs)	Azide-attached macrophage cell membrane (functionalized with the anti-EpCAM antibody)	Fe_3O_4 magnetic nanocluster	–	Electrostatic interaction	240.0 nm −20.0 mV	–	• ↑ CTCs capture ability in vitro without interference from leucocytes interaction (capture of ≈90% tumor cells in 15 min)	[138]
Cancer theranostics	RAW 264.7 macrophage cell membrane	Liposome	Doxorubicin and quaternary quantum dots (QDs)	Coextrusion through 200 nm porous membrane	146.0 nm −25.9 mV	4T1 breast cancer mouse model with lung metastasis	• Immune evasion properties • Intrinsic targeting properties to metastatic tumor tissue • Tumor-targeted fluorescent imaging in vivo and efficient tumor ablation with chemotherapy • ↓ number of metastatic nodules in the lungs	[143]
	J774A.1 macrophage cell membrane	Persistence luminescence NP (PLNP)-based inner core	Paclitaxel	–	142.0 nm −28.4 mV	SCC-7 squamous epithelial cancer mouse model	• Prolonged blood circulation • Intrinsic targeting properties to tumor tissue • Tumor-targeted drug delivery • Effective chemotherapy with luminescence imaging guidance	[144]
	Macrophage cell membrane	Silver nanocluster (AgNC)	–	Sonication (1 h)	256.5 nm −58.9 mV	Dalton Lymphoma Ascites (DLA) tumor-bearing mouse model	• Potential for DLA tumor-targeted theranostics • Intrinsic cytotoxic effects against DLA tumor cells in vitro • Strong fluorescence intensity at DLA tumor sites in vivo	[145]

Symbol definition: ↑ indicates enhancement; ↓ indicates reduction.

capable of converting near-infrared light excitation into visible light emission).[104,105] Among the materials investigated, β-NaYF_4:Er^{3+},Yb^{3+} UCNPs have captured attention because of their remarkable optical properties, low toxicity, high photostability, and good light penetration depth. However, the main drawbacks of these tumor imaging techniques arise from the lack of specific in vivo tumor-targeting and localized delivery of contrast agents to tumors.[44]

Cell membrane-coated NPs have been studied as biomimetic nanocarriers to deliver imaging agents to targeted tumor sites for localized cancer bioimaging.[104,105] In a recent study designed to improve cancer targeting and imaging, UCNPs were camouflaged with macrophage-derived membrane vesicles. The membrane coating enhanced the performance of these imaging agents in vivo because of its intrinsic ability to target neoplastic tissues (**Figure 5**A).[44] The resulting MCM-coated UCNPs (MM-UCNPs)

Table 5. Biomedical applications of nanosystems coated by a macrophage cell membrane (MCM) for inflammatory and infectious diseases.

Application	Cell membrane coating	Inner core	Cargo(es)	Coating method	Size/zeta potential	Mouse model	Principal outcomes	Refs.
Atherosclerosis	RAW 264.7 macrophage cell membrane	Poly(lactic-*co*-glycolic acid) nanoparticle (PLGA NP)	Rapamycin	Sonication (100 W for 3 min) and extrusion (10 times)	110.8 nm −41.7 mV	ApoE deficient (Apo $E^{-/-}$) mouse model with atherosclerosis	• ↑ biocompatibility • Intrinsic targeting properties to atherosclerotic plaques (inflammatory tissues) • ↓ atherosclerosis progression without significant toxicity	[146]
	RAW 264.7 macrophage cell membrane	Reactive oxygen species (ROS)-responsive NP	Atorvastatin	Extrusion (≥20 times) and sonication (100 W for 2 min)	≈227.0 nm	Apo $E^{-/-}$ mouse model with atherosclerosis	• Intrinsic targeting properties to atherosclerotic plaques • Targeted release of atorvastatin in response to locally produced ROS • ↓ inflammatory cytokine levels, contributing to suppress the inflammatory responses	[48]
	Simvastatin-embedded apolipoprotein A-I mimetic 4F peptide (AP)-attached J774A.1 macrophage cell membrane	Fe_3O_4 magnetic nanocluster	–	Electrostatic interaction	–	Apo $E^{-/-}$ mouse model with early atherosclerotic lesions	• Multitargeting properties • Promising for targeted atherosclerosis theranostics • Early detection of atherosclerosis via magnetic resonance imaging (MRI) • Synergistic effects of AP and simvastatin for efficient atherosclerosis treatment	[147]
Acute ischemic stroke	Macrophage cell membrane	Manganese dioxide nanosphere	Fingolimod	Coextrusion through 200 nm porous membrane (30 times)	144.0 nm −21.4 mV	Mouse model of transient middle cerebral artery occlusion/reperfusion (tMCAO/R)	• Intrinsic targeting properties to ischemic brain lesions • ↑ ROS-to-O_2 conversion efficiency, contributing to reduce the ischemia-associated oxidative stress • Efficient suppression of inflammation by converting the M1 microglia in the anti-inflammatory M2 phenotype	[148]
Vascular intimal hyperplasia	RAW 264.7 macrophage cell membrane	ROS-responsive amphiphilic molecule (PCM)	Rapamycin	Sonication (100 W for 3 min) and extrusion (16 times)	129.6 nm	Mouse model of carotid artery injury	• ↑ biocompatibility • Immune evasion properties • Dual-targeting properties to inflammatory vascular lesions via their surface-expressed $\alpha4\beta1$ integrin and CCR2 (chemokine receptor) • Targeted and controlled drug release in response to locally produced ROS • ↓ proliferation of vascular smooth muscle cells (VSMCs) in vitro and in vivo	[149]
Alzheimer's disease	Peritoneal macrophage cell membrane dual-functionalized with rabies virus glycoprotein (RVG29) and triphenylphosphine (TPP)	Solid lipid NP	Genistein	Coextrusion through 100 nm porous membrane (≥5 times)	123.2 nm 19.1 mV	Alzheimer's disease mouse model (APP/PS1 transgenic mice model)	• Multitargeting properties • ↑ blood-brain barrier (BBB) penetration ability • ↑ targeting capability to the neuronal mitochondria in vivo • ↑ elimination of mitochondrial ROS for treatment of Alzheimer's disease	[150]

Table 5. Continued.

Application	Cell membrane coating	Inner core	Cargo(es)	Coating method	Size/zeta potential	Mouse model	Principal outcomes	Refs.
Inflammatory osteolysis	RAW 264.7 macrophage cell membrane	Porous Se@SiO_2 nanospheres	–	Sonication (3 min) and extrusion through 100 nm porous membrane	≈98.0 nm −48.3 mV	Air pouch and calvarial osteolysis mouse model	• Neutralization of bacterial lipopolysaccharide and proinflammatory cytokines • Efficient suppression of inflammation by polarizing M1 macrophages toward the anti-inflammatory M2 phenotype • ↓ inflammatory cytokine levels, contributing to induce osteogenic differentiation and suppress osteolysis	[152]
Age-related macular degeneration (AMD)	RAW 264.7 macrophage cell membrane	PLGA NP	Rapamycin	Sonication (3 min)	101.3 nm −29.6 mV	Laser-induced choroidal neovascularization (LCNV) mouse model	• Immune evasion properties • Intrinsic targeting properties to inflamed lesions in the eye • ↑ ability to cross the blood-retinal barrier (BRB) • Targeted delivery of rapamycin to choroidal neovascularization (CNV) lesions via chemotactic recruitment • Downregulation of the mTOR signaling pathway	[153]
Bacterial infections	Macrophage cell membrane pretreated with *Staphylococcus aureus*	Gold–silver nanocage	–	Coextrusion through 200 nm porous membrane	–	Local infection mouse model caused by *S. aureus*	• Prolonged blood circulation • ↑ bacterial recognition ability • ↑ near-infrared (NIR) absorption capacity for enhanced photothermal therapy (PTT) • Promising for treatment of local bacterial infection	[154]
	J774A.1 macrophage cell membrane	Antimicrobial-conjugated NP (ANP)	Triclosan and ciprofloxacin	Sonication (40 s)	≈110.0 nm	Mouse acute peritoneal infection model	• ↑ biocompatibility • Efficient treatment of intracellular *S. aureus* infection • ↑ antibacterial efficacy with a fourfold reduction in peritoneal bacterial burden compared to control group	[155]
	J774A.1 macrophage cell membrane	PLGA NP	*Pseudomonas aeruginosa* secretions (PaS)	Sonication and incubation (37 °C, 15 min)	–	Pneumonia mouse model caused by *P. aeruginosa*	• Potential for vaccination against antibiotic resistant *P. aeruginosa* infections • Twofold reduction in bacterial burden in the lungs after 35 days of subcutaneous vaccination	[156]
	RAW 264.7 macrophage cell membrane	Magnetic composite NP composed of Fe_3O_4 NP, titanium dioxide (TiO_2) and calcium phosphate ($Ca_3(PO_4)_2$)	–	Direct internalization by macrophages and electroporation (200–300 V)	–	Bone infection model caused by drug-resistant bacteria	• ↑ bacterial recognition ability • ↑ neutralization of inflammatory cytokines and bacterial toxins • ↑ ROS generation ability upon ultraviolet light irradiation • ↑ Bone tissue regeneration	[102]
	J774A.1 macrophage cell membrane	PLGA NP	–	Sonication (100 W for 2 min)	102.0 nm −26.7 mV	Mouse bacteremia model caused by *Escherichia coli*	• Dual mechanism for sepsis management through neutralization and capture of bacterial lipopolysaccharide and proinflammatory cytokines • ↓ inflammatory cytokine levels • Extended survival time of mice	[49]

Table 5. Continued.

Application	Cell membrane coating	Inner core	Cargo(es)	Coating method	Size/zeta potential	Mouse model	Principal outcomes	Refs.
Viral infections (SARS-Cov-2)	Alveolar macrophage cell membrane	PLGA NP	2TPE-2NDTA	Sonication	98.6 nm −23.1 mV	Surrogate model of COVID-19 by murine coronavirus	• ↑ viral recognition ability • ↓ inflammatory cytokine levels, contributing to reduce inflammation caused by coronavirus infection • Photothermal viral ablation upon NIR light irradiation	[47]
	RAW 264.7 macrophage cell membrane	PLGA NP	Lopinavir	Sonication (100 W for 5 min)	102.2 nm −12.4 mV	Mouse model of coronavirus infection	• ↑ viral recognition ability • ↓ inflammatory cytokine levels, contributing to reduce inflammation caused by coronavirus infection • Targeted drug delivery to sites of infection, contributing to enhance antiviral therapy	[160]

Symbol definition: ↑ indicates enhancement; ↓ indicates reduction.

displayed good uptake by tumor cells in vitro, good biocompatibility in vivo, long circulation time, and superior fluorescence intensity in tumor tissue, compared with noncoated UCNPs (Figure 5B,C). These results are consistent with other studies and suggest that macrophage-like imaging nanoprobes have great potential for improving in vivo fluorescence imaging of tumors.[44]

5.1.2. Delivery of Chemotherapeutics to Primary Tumors

Chemotherapy is a common approach in cancer treatment. However, the application of this conventional treatment approach in clinical practice is limited due to poor tumor specificity. This results in extensive damage to surrounding healthy cells, causing severe systemic side effects.[25,106] Hence, the application of MCM-coated NPs as chemotherapeutic drug carriers has been investigated to improve tumor-targeted delivery and reduce off-target toxicity. In a recent study, macrophage-derived membrane nanovesicles were wrapped onto doxorubicin-loaded mesoporous silica nanocapsules for tumor-targeted chemotherapy.[107] In vivo studies showed prolonged blood circulation time compared with uncoated NPs, enhanced ability to target tumor cells, and selective accumulation at tumor sites. These favorable results are likely attributed to the enhanced

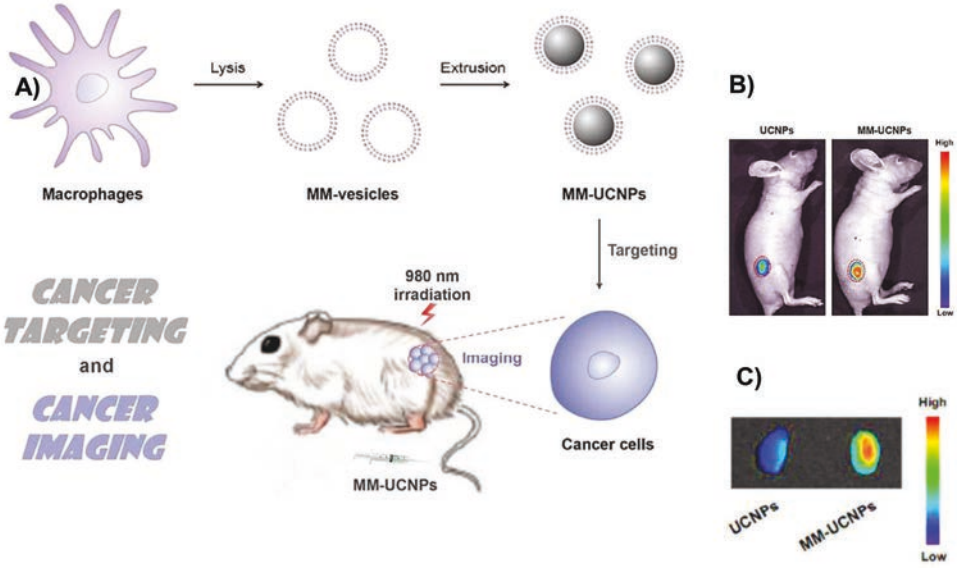

Figure 5. A) Schematic of MCM-coated upconversion nanoparticles (MM-UCNPs) preparation by coating UCNP cores with macrophage-derived membrane vesicles to improve in vivo cancer targeting and imaging. B) In vivo images of MCF-7 tumor-bearing mice 48 h after intravenous injection with UCNPs and MM-UCNPs. C) Ex vivo images of MCF-7 tumors 48 h after intravenous injection with UCNPs and MM-UCNPs. Reproduced with permission.[44] Copyright 2017, John Wiley and Sons, Inc. Abbreviations: MM-UCNP, macrophage cell membrane-coated UCNP; MM-vesicle, macrophage-derived membrane vesicle; UCNP, upconversion nanoparticle.

ability of the MCM-coating to recognize the tumor vasculature and actively target tumor cells. The biomimetic nanostructure successfully guided the anticancer drug doxorubicin to tumor tissues, resulting in highly effective chemotherapy and ablation of breast cancer.[107] Another study utilized MCM-coated albumin NPs for the delivery of paclitaxel to melanoma cells via an active targeting mechanism. In vivo results demonstrated superior uptake by tumor cells, producing impressive antitumor effects and highly-effective tumor eradication.[46] More recently, other chemotherapeutic drugs have also been encapsulated in MCM-coated nanosystems, such as gemcitabine for pancreatic cancer therapy,[108] or cabazitaxel for deep penetration into tumor tissue for targeting both cancer cells and cancer stem cells in 4T1 breast tumors.[109]

Despite the promising use of MCM-coated nanosystems to carry chemotherapeutic drugs to tumors, challenges still remain for this biomimetic approach. This is especially so regarding the release of therapeutic payloads through the barrier formed by the cell membrane coating, after the nanosystems are internalized by tumor cells.[110] To tackle this issue, a paclitaxel-loaded polymer-based pH-responsive NP preparation (lower pH in the tumor inflammatory microenvironment) was coated with a macrophage-derived membrane, to design a biomimetic platform (cskc-PPiP/PTX@Ma).[110] Based on the proton sponge effect, the polymeric cores were functionalized with a cationic 2-aminoethyldiisopropyl ligand (PPiP) so that the internal NP could behave as a sponge for H^+ in the mildly acidic extracellular tumor microenvironment. This ultimately provoked the expansion and rupture of the membrane coating. The incorporation of this cationic chemical group in the polymer enabled the removal of the external membrane cloak, after being exposed to the acidic conditions of the tumor microenvironment. This enabled the NPs to escape from the disrupted membrane coating and efficiently deliver drugs to the tumor site (**Figure 6**A,B). The authors also conjugated a targeting ligand on the surface of polymeric NPs to further enhance tumor accumulation. This conjugated ligand has high affinity for the insulin-like growth factor 1 receptor (IGF1R), which is abnormally overexpressed by tumor cells. In vivo study showed enhanced tumor uptake and retention, resulting in superior tumor ablation (Figure 6C,D). The biomimetic NPs combined the buffering properties of PPiP materials and the active tumor-targeting ability of MCMs, thus providing enhanced biocompatibility, preferential accumulation in tumors and sustained drug release in response to external and internal pH stimulation in the tumor microenvironment.[110]

5.1.3. Therapy for Metastatic Tumors

The aforementioned studies were focused on primary tumors. Other macrophage-mimetic nanosystems have been designed to target metastatic tumors.[9] In a recent study, the higher binding affinity of macrophages expressing $\alpha 4\beta 1$ integrin to metastatic 4T1 breast cancer cells, which overexpress VCAM-1, was harnessed to actively target and suppress lung metastases that originated from breast cancer.[45] To achieve this, pH-sensitive liposomes containing the anticancer drug emtansine were cloaked with macrophage-derived membrane vesicles, creating a macrophage-like nanosystem named MEL. Because of its biomimetic properties conferred by the membrane coating, the nanosystem displayed superior uptake by tumor cells in vitro, and longer blood circulation time compared to noncoated liposomes in vivo. The MEL macrophage-like nanosystem selectively targeted sites of pulmonary metastasis. This provided more efficient drug delivery to the lungs, better antimetastatic efficacy, as well as efficient suppression of lung metastases.[45]

In a similar study aimed at suppressing lung metastatic nodules that originated from breast cancer, a biomimetic nanoplatform known as Dox-MPK@MDL was formed by coating liposomes with membranes derived from macrophages for controlled and localized drug release to lung metastases.[111] The researchers first loaded a pH-sensitive doxorubicin prodrug (Dox-MPK) into DNA tetrahedron dendrimers. They subsequently cloaked the drug-loaded dendrimers sequentially with a lipid bilayer and a macrophage-derived membrane, using a self-assembly technique (**Figure 7**A). The DNA tetrahedron dendrimers helped to improve the stability of the nanosystem due to their biocompatibility, high loading capacity and stable structure.[111] In vitro and in vivo studies showed enhanced biocompatibility, prolonged blood circulation time, superior uptake by lung metastatic cells, as well as pH-induced drug release from the prodrugs under the acidic conditions of the tumor microenvironment. This resulted in an improvement in the efficacy of antimetastatic therapy and reduction in the cardiotoxicity of doxorubicin by preventing nonspecific drug release (Figure 7B,C). The Dox-MPK@MDL system markedly reduced lung metastatic nodules and doxorubicin-triggered cardiotoxicity by preferentially accumulating in the tumor tissue, therefore causing less damage to the heart (Figure 7D).[111]

5.1.4. Antiangiogenic Therapy

Chemotherapy is the standard therapy for metastatic breast cancer. However, concerns on its systemic toxicity has led to the development of an alternative approach through modulation of the angiogenic pathway.[112] Taking advantage of the anticancer effects of saikosaponin D, a triterpene saponin, saikosaponin D-loaded PLGA NPs were camouflaged with T7 peptide-loaded MCM vesicles. This arrangement resulted in a biomimetic nanoplatform with potential for targeted antiangiogenic therapy.[112] In vitro and in vivo studies showed that the MCM-coated PLGA NPs avoided macrophage clearance and selectively targeted the tumor cells. This was attributed to their membrane coating and specific recognition of overexpressed transferrin receptors by T7 peptide. The MCM-coated PLGA NPs inhibited both in situ tumor growth and breast cancer-derived metastases. The remarkable suppression of tumor growth by saikosaponin D was mediated by downregulation of angiogenesis-associated pathways, in particular the MAPK/ERK and the PI3K/AKT pathways. This approach highlights the potential use of a biomimetic nanoplatform for the management of advanced stages of breast cancer with disseminated metastasis. The favorable outcome is likely due to the enhanced ability of the nanoplatform to target tumor sites and modulate angiogenesis-related factors, including vascular endothelial growth factor (VEGF).[112]

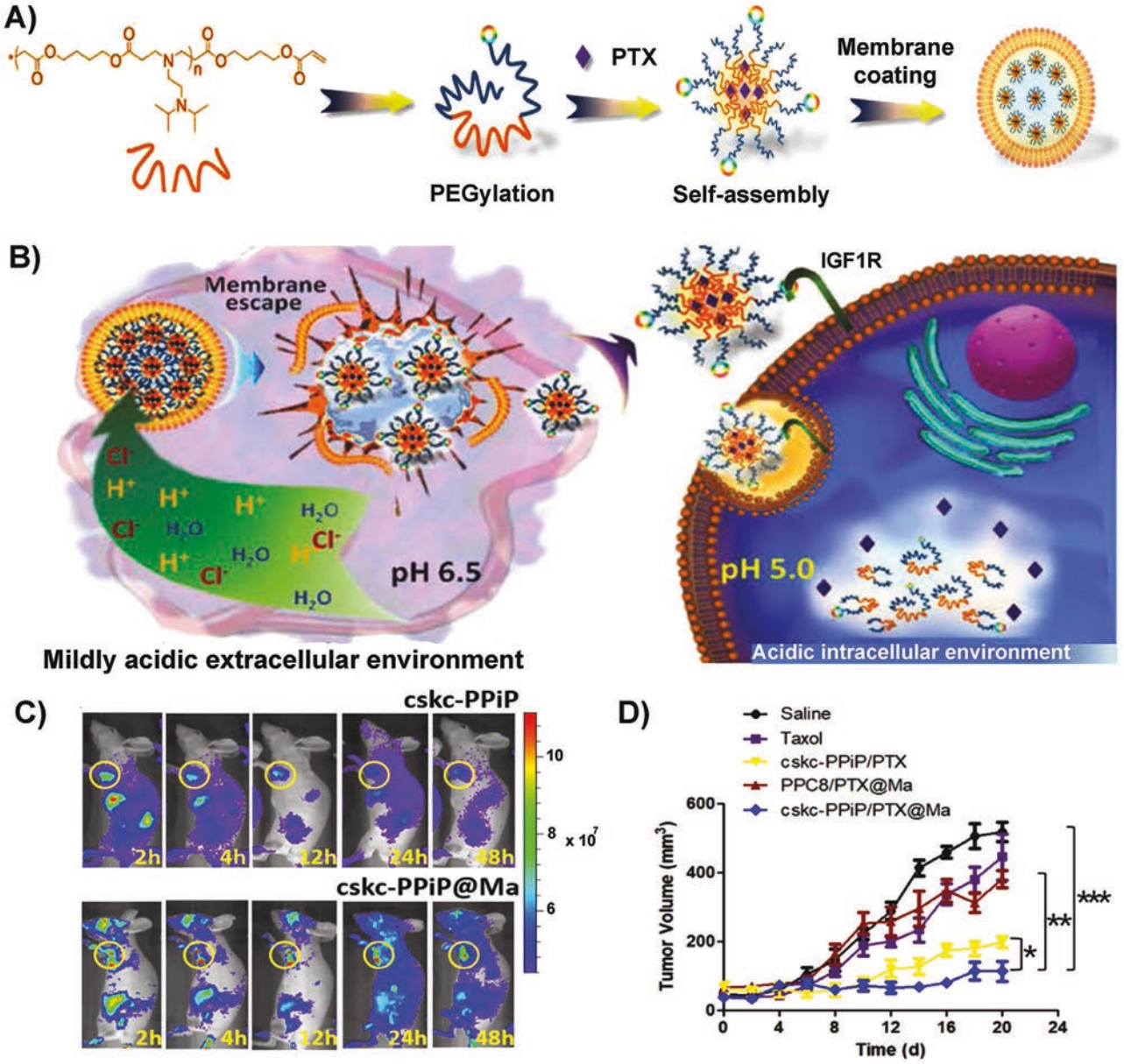

Figure 6. A) Schematic of cskc-PPiP/PTX@Ma preparation by coating a polymeric core previously functionalized with a cationic ligand (PPiP) and surface modified with an IGF1R-targeting ligand and a macrophage membrane. B) Representation of cell membrane disruption under the acidic conditions of the tumor microenvironment, membrane escape, active tumor-targeting via IGF1R signaling and drug delivery to tumor cells. C) IVIS images obtained at different times after injection of near-infrared probe-loaded cskc-PPiP and cskc-PPiP@Ma in mice. D) Tumor volume was examined during the first 3 weeks of different treatments. Reproduced with permission.[110] Copyright 2018, American Chemical Society. Abbreviations: cskc-PPiP/PTX, PTX- loaded pH-sensitive polymer; cskc-PPiP/PTX@Ma, macrophage membrane-coated pH-sensitive polymer; IGF1R, insulin-like growth factor 1 receptor; NIR, near- infrared; PCC8/PTX@Ma, macrophage membrane-coated pH-insensitive polymer; PTX, paclitaxel.

5.1.5. Antiproliferative Cancer Therapy

Macrophages are specialized immune cells characterized by the secretion of a wide range of cytokines, such as TNF-α. The transmembrane TNF-α precursor present on the cell membrane is cleaved upon exposure to bacterial lipopolysaccharide or other factors.[19] Based on the antiproliferative activity of the transmembrane TNF-α and its potential to kill tumor cells, biodegradable and biocompatible polymeric chitosan NPs were wrapped with an engineered TNF-α-coupled MCM.[113] First, THP-1 human monocytes (a monocytic leukemia cell line) were differentiated into macrophages after exposure to phorbol 12-myristate 13-acetate. The differentiated macrophages were stimulated with bacterial lipopolysaccharide to produce TNF-α. In this study, the ability of macrophages to produce TNF-α upon bacterial lipopolysaccharide induction was harnessed to produce a TNF-α-embedded membrane preparation.[113] In vitro studies demonstrated enhanced cytotoxicity of the membrane-coated NPs against different cancer cell lines. When used to treat tumor spheroids, the NPs were able to efficiently suppress tumor

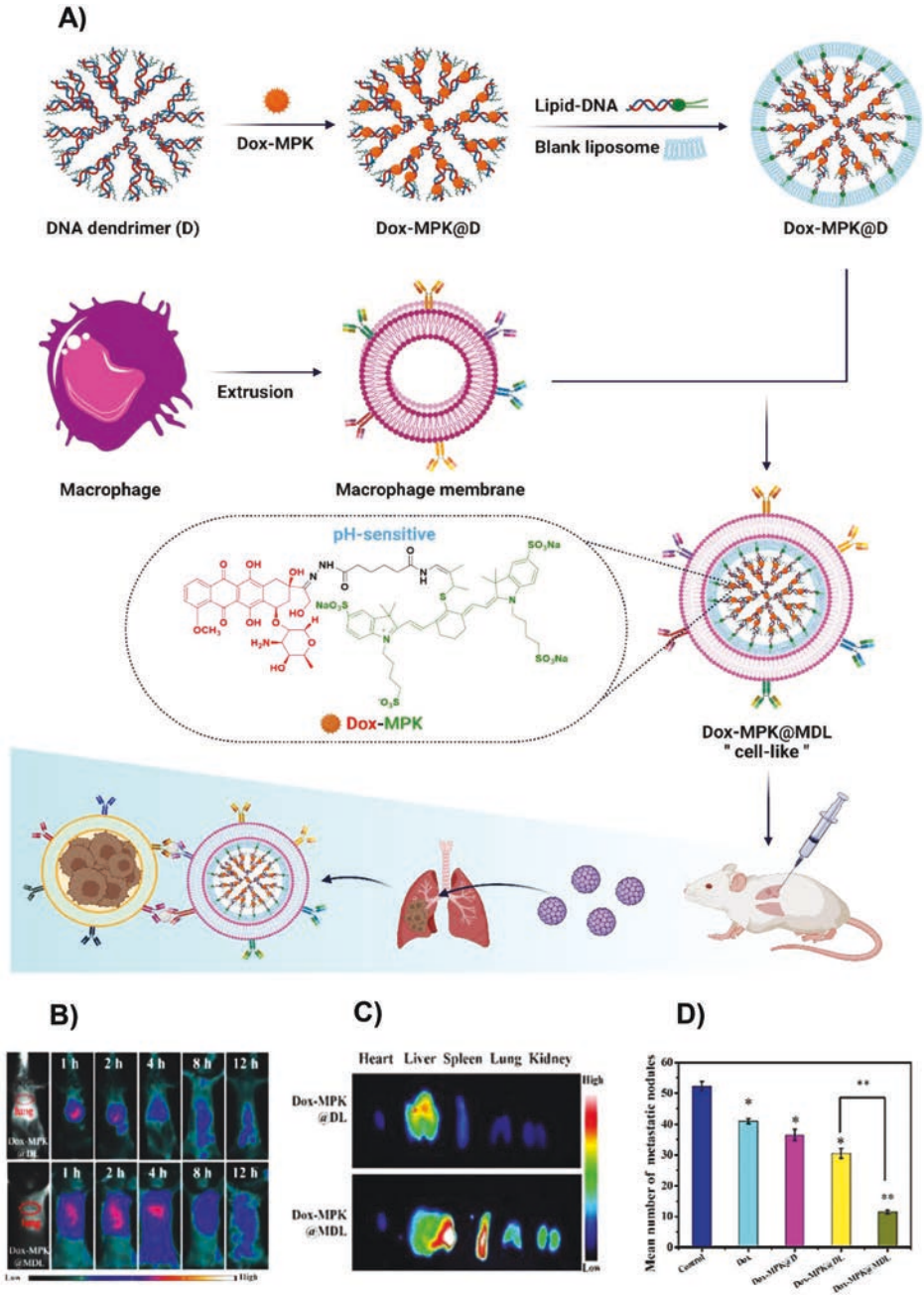

Figure 7. A) Schematic of macrophage-mimicking nanosystem (Dox-MPK@MDL) preparation by a self-assembly technique to actively target metastatic 4T1 tumor cells and suppress lung metastasis from breast cancer. B) In vivo distribution of the nanosystems assessed by fluorescence imaging. C) Ex vivo images of the main organs (heart, liver, spleen, lung, kidney) of mice for each treatment after 4 h of administration. D) Mean number of metastatic nodules in the lungs were investigated after 14 days of treatment. (B–D) Reproduced with permission.[111] Copyright 2019, Royal Society of Chemistry. Abbreviations: Dox, doxorubicin; Dox-MPK, Dox prodrug; Dox-MPK@D, Dox-MPK inserted into DNA tetrahedron dendrimers; Dox-MPK@DL, Dox-MPK@D coated with a lipid bilayer; Dox-MPK@MDL, Dox-MPK@D sequentially coated with a lipid bilayer and a macrophage membrane.

growth and reduce cell viability in a dose-dependent manner. This is effectuated by inducing apoptosis of tumor cells.[113]

5.1.6. Cancer Immunotherapy

Immunotherapy is a type of cancer therapy that attempts to trigger the patient's own immune system to recognize and eliminate tumor cells. The applications of immunotherapy ranges from cancer vaccines to adoptive T-cell transfer therapy and immune checkpoint blockade. The objective is to elicit robust antitumor immune responses by the subject's body to eradicate cancer.[114–116]

Adoptive T-cell transfer therapy consists of stimulating autologous T cells ex vivo by incubating them with APCs. The stimulated T cells are then transferred into the patient to

accumulate in the tumor and destroy tumor cells. However, using natural APCs is often time-consuming and laborious.[117] To overcome these hurdles, artificial APCs such as magnetosomes were constructed by coating Fe_3O_4 magnetic nanoclusters (MNCs) with an engineered azide-attached membrane derived from macrophages through electrostatic interactions. This enabled the incorporation of dibenzocyclooctyne-linked T cell stimulatory signals to the nanosystem surface using a click chemistry reaction.[117] Ex vivo studies showed efficient CD8+ T-cell stimulation. When injected into tumor-bearing mice, the nanoplatform effectively guided the activated T cells to the tumor site under the influence of an external magnetic field. Accumulation of the activated T cells within the tumor was monitoring in vivo by magnetic resonance imaging. The magnetosomes were endowed with stealth properties derived from the MCM coating, as well as magnetic and superparamagnetic properties derived from the MNCs. This resulted in efficacious tumor eradication in vivo without significant toxicity.[117]

A novel TAM repolarization approach has been proposed to convert M2 TAMs toward the antitumorigenic M1 phenotype. This approach is designed to reverse the immunosuppressive tumor microenvironment to induce stronger antitumor immune responses.[118] Encouraged by the macrophage polarization ability of Fe_3O_4 NPs and imiquimod (R837), a Toll-like receptor 7 (TLR 7) agonist, polymeric PLGA NPs containing both R837 and magnetic Fe_3O_4 NPs were wrapped with membranes derived from bacterial lipopolysaccharide-induced M1 macrophages (**Figure 8**A).[118] The NPs were highly efficient in inhibiting breast cancer growth when injected in vivo into tumor-bearing mice. This was attributed to the synergistic effects of R837 and magnetic Fe_3O_4 NPs that enabled the repolarization of TAMs to M1 macrophages by activating the NF-kB and IRF5 pathways (Figure 8B). The biomimetic NPs induced polarization of macrophages toward the M1 phenotype by 2.88-fold, producing remarkable antitumor effects by reshaping the tumor microenvironment and inducing a strong immune response against cancer (Figure 8C–E).[118]

Other TAM-repolarizing agents such as TMP195 (an epigenetic HDAC inhibitor) have recently been encapsulated within MCM-coated nanosystems to enhance antitumor efficacy.[119] In this study, polydopamine NPs containing TMP195 were wrapped with a macrophage-derived membrane. This resulted in improved tumor accumulation and greater suppression of tumor growth. The favorable results were attributed to the intrinsic targeting ability of the MCM coating, as well the M2-to-M1 macrophage repolarization ability of TMP195.[119]

5.1.7. Photothermal Therapy

Photothermal cancer therapy (PTT) is another application of MCM-coated NPs. In this therapeutic regime, the NP core is a photothermal agent capable of absorbing light in the near infrared. range and converting it into heat. The generated heat effectively damages cancer cells and induces cell death through hyperthermia.[120] In recent years, much research has been devoted to PTT as a minimally invasive type of cancer phototherapy. This is attributed to the potential of PTT in selectively destroying cancer cells via thermal ablation, without causing significant damage to non-irradiated normal tissues.[121]

Inorganic Fe_3O_4 NPs have been extensively studied for PTT applications due to their remarkable photoabsorption properties and their ability to generate hyperthermia when irradiated with an near-infrared laser.[122] In a recent study, macrophage-derived membrane vesicles were wrapped onto Fe_3O_4 NPs to allow PTT of breast cancer.[122] Unlike noncoated NPs that exhibited poor tumor accumulation, the biomimetic nanoplatform, Fe_3O_4 NPs@MM, demonstrated good uptake by tumor cells and selective accumulation in neoplastic tumors. In vivo administration of Fe_3O_4 NPs@MM followed by irradiation of the tumor area with an near-infrared laser successfully induced hyperthermia in the neoplastic cells, resulting in inhibition of tumor growth and nearly complete tumor elimination. These findings provide important insights on the ability of Fe_3O_4 NPs@MM to enhance the in vivo efficiency of PTT by combining the biocompatible, long circulation time, and tumor-targeting ability of MCMs with the photothermal property of the Fe_3O_4 NPs cores.[122]

Gold nanoshells (AuNSs) with an ideal near-infrared absorption capability have also been harnessed for PTT cancer therapy.[123] For instance, the near-infrared fluorescent dye cyanine 7 (Cy7) was loaded into AuNSs and coated with macrophage-derived membranes to produce a biomimetic nanoplatform with the potential for both fluorescent imaging and PTT.[123] In vitro and in vivo studies demonstrated good uptake by tumor cells, longer blood circulation, as well as efficient hyperthermia-induced tumor cell death. By capitalizing on the enhanced tumor tropism of MCMs and the photothermal properties of AuNSs, the efficacy of PTT in vivo could be greatly improved.[123]

To explore the near-infrared light-activated propulsion of Janus mesoporous silica nanomotors and their potential for PTT, these nanomaterials were camouflaged with a macrophage-derived membrane only on one of the sides. The biomimetic nanoplatform actively targeted cancer cells and circulated in the blood undetected by the immune system. This resulted in superior accumulation in tumor cells.[124] In vitro studies showed that the nanosystem opened and perforated the tumor cell membranes. Once irradiated with near-infrared light, there was highly efficient killing of cancer cells caused by irreparable damage to cell membranes. This near-infrared light-driven biomimetic nanosystem appeared to be a promising therapeutic approach for PTT in vitro.[124] Regrettably, nothing is available on its in vivo treatment efficacy.

The major obstacle associated with the treatment of neurological disorders is the BBB. The BBB hinders the passage of therapeutic agents into the central nervous system. Hence, novel strategies capable of bypassing the BBB and targeting tumor sites have to be developed for the treatment of orthotopic glioblastoma.[125,126] To tackle this challenge, a biomimetic nanoplatform with both diagnostic and therapeutic features was prepared by wrapping NIR-Ib fluorescence IR-792 dye-loaded liposomes with macrophage-derived membranes.[125] The resulting nanosystem efficiently overcame the BBB hurdle due to specific binding of the macrophage surface markers (Mac-1 and $\alpha 4\beta 1$ integrin) to their respective receptors overexpressed by brain endothelial cells (ICAM-1 and VCAM-1), and to actively

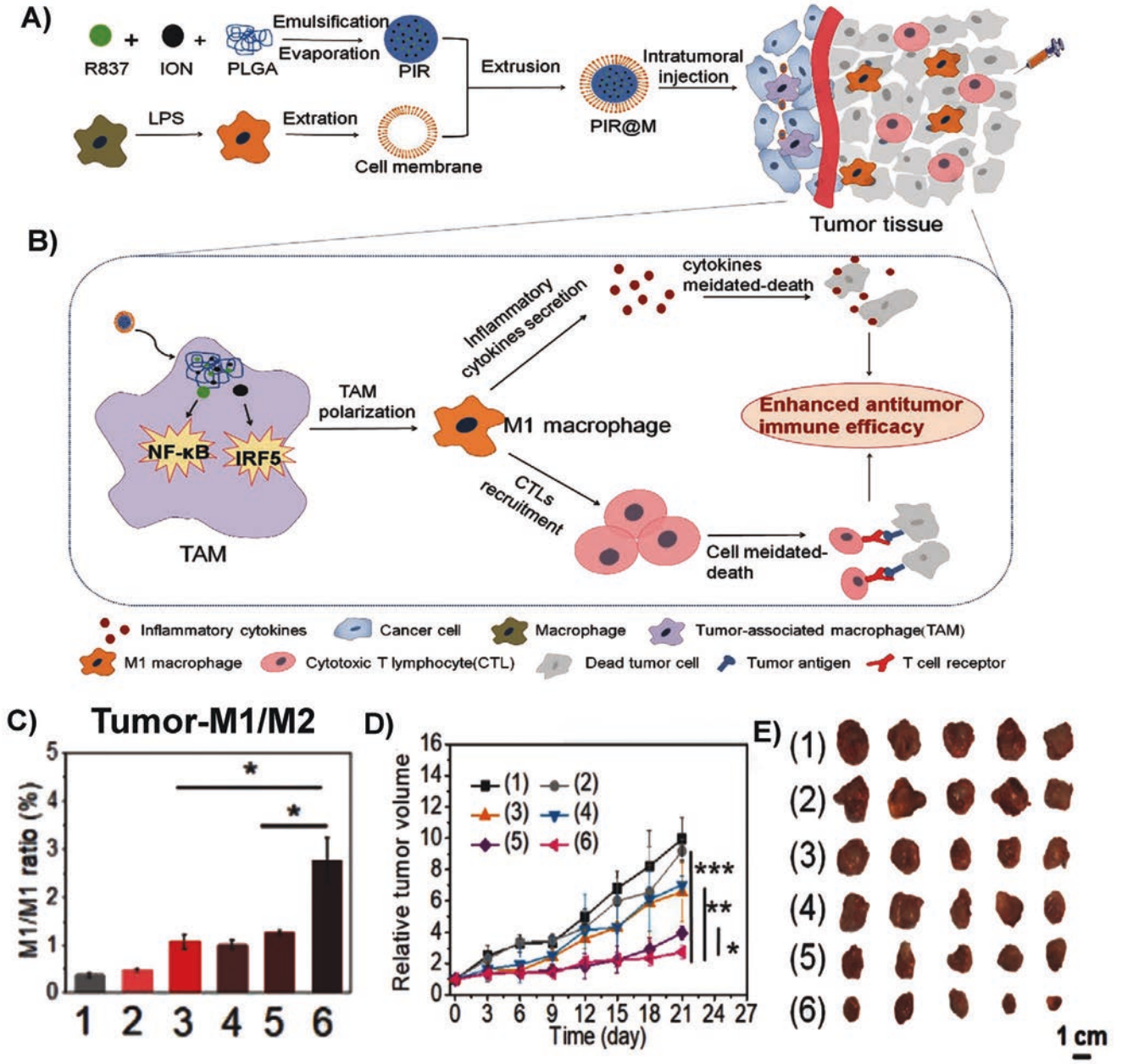

Figure 8. A) Schematic of macrophage-mimicking nanosystem (PIR@M) preparation. B) Schematic illustrating the PIR@M-induced repolarization of TAMs to M1 macrophages by activating the NF-kB and IRF5 pathways. C) The ratio of M1/M2 macrophages at tumor sites after different treatments. (1), (2), (3), (4), (5), and (6) means saline, P@M, PI@M, Vc + PI@M, PR@M, and PIR@M). D) Growth curves of tumor volume after different treatments. E) Photographs of tumor tissues after different treatments. Reproduced with permission.[118] Copyright 2020, Wiley-VCH GmbH. Abbreviations: ION, iron oxide nanoparticle; LPS, bacterial lipopolysaccharide; PIR@M, macrophage membrane-coated PLGA NP containing both R837 and magnetic Fe_3O_4 NP; PLGA, poly(lactic-*co*-glycolic acid).

target tumor cells. Such properties enabled efficient delivery of IR-792 dye to the glioblastoma site. The IR-792 dye performed a dual function; it acts as a fluorescent imaging agent and a heat generator for PTT. In vivo study reported enhanced cytotoxicity against tumor cells upon near-infrared radiation with near-infrared-Ib fluorescence imaging guidance. This resulted in remarkable antitumor efficacy and prolonged survival time of glioblastoma-bearing mice.[125]

Hollow bismuth selenide (Bi_2Se_3) NPs were investigated because of their promising properties for PTT, X-ray computed tomography, and infrared thermal imaging. These properties render them interesting nanomaterials for the development of multimodal systems.[127] In this regard, Bi_2Se_3 NPs were loaded with quercetin prior to coating with macrophage-derived membranes. The biomimetic NPs possessed immune escape ability, good biocompatibility, and active tumor-targeting capability. They also possessed the ability to be recruited to tumor sites in response to chemotactic signals such as CCL_2.[127] In experimental studies, this heat-generating platform showed the most striking effect in reducing tumor growth and induced cell death

upon near-infrared light radiation. This was partly attributed to the role of quercetin in sensitizing cancer cells to PTT by depleting thermoresistance-linked chaperones, such as heat shock protein 70 (Hsp70), that protects cancer cells from hyperthermia-induced apoptosis. The biomimetic nanosystem was efficacious in suppressing lung metastasis that originated from breast cancer. This is attributed to the ability of the nanosystem to negatively regulate metalloproteinase 9 and protein kinase B. Both of these enzymes are implicated in tumor growth, invasion and metastasis. This novel strategy improved PTT efficacy by combining two therapeutic agents with synergistic antitumor effects.[127]

Combination cancer therapies seek to achieve therapeutic outcomes that are more effective than individual therapies. For example, PTT was combined with immunotherapy to produce synergistic antitumor effect against primary and metastatic colorectal cancer.[128] In this study, hollow gold nanocage nanocomposites loaded with galunisertib, a TGFβ inhibitor drug, were coated with a macrophage-derived membrane; that was functionalized with an anti-PDL1 antibody.[128] The biomimetic nanosystem demonstrated potential for combined PTT-immunotherapy. This is because PTT can induce the release of tumor-associated antigens after near-infrared light irradiation. This helps to trigger a potent antitumor immune response by stimulating the activation of antigen presenting cells and the infiltration of cytotoxic CD8+ T cells into the tumor. These activities, in turn, amplify the antitumor effects of the anti-PDL1 antibody and galunisertib. In vivo data showed acceptable tumor accumulation with suppression of tumor growth that extended the overall survival of tumor-bearing mice.[128]

The combination of PTT and chemotherapy has also been shown to be an effective therapeutic approach for experimental treatment of hepatocellular carcinoma in animals.[129] In this study, sorafenib-loaded copper sulfide (CuS) NPs were coated with a macrophage-hepatic cancer cell (H22) hybrid membrane. Anti-VEGF receptor (VEGFR) antibody was attached on the surface of the hybrid membrane to produce a hybrid nanosystem known as CuS-SF@MCV. Combination of surface markers from both macrophages and cancer cells conferred immune escape ability and enhanced tropism for homotypic tumor cells (**Figure 9**A,B).[129] The combination of CuS-SF@MCV with near-infrared irradiation substantially suppressed tumor growth. This was attributed to the photothermal effects of CuS NPs and the synergistic antimetastatic effect of anti-VEFGR antibody and sorafenib. The latter is a chemotherapeutic drug that inhibits protein kinases in the MEK/ERK and PI3K/AKT pathways (Figure 9C,D). This synergistic chemotherapeutic-PTT approach increased the survival rate of tumor-bearing mice (note, not humans), with no loss in body weight. The results are indicative of the biosafety of the nanosystem and its therapeutic potential (Figure 9E,F).[129]

5.1.8. Photodynamic Therapy

Recent studies have extended the application of MCM-coated NPs to photodynamic therapy (PDT). This therapeutic regime requires the delivery of photosensitizers to tumor sites and their subsequent activation by laser irradiation. Laser activation results in the generation of ROS, especially singlet oxygen ($^1O_2$), from the surrounding ground-state oxygen molecules (O_2) within the tumor tissue.[130–132] The ROS play a crucial role in amplifying antitumor immunity by inducing photo-oxidative damage to tumor cells. Photo-oxidative damage increases the uptake, processing, and presentation of tumor-associated antigens by antigen-presenting cells to naïve T cells.[133,134]

A synergistic biomimetic approach against melanoma and breast cancer was achieved by combining PDT, immunotherapy and chemotherapy. Doxorubicin-attached PEGylated bilirubin NPs containing both the photosensitizer chlorin e6 (Ce6) and the IDO1 inhibitor, indoximod (IND) were coated with membranes derived from M1-polarized macrophages.[133] In vitro and in vivo data showed good tumor accumulation, long circulation time, and efficient ROS-induced tumor cell death. By blocking the IDO1 pathway, which has a well-defined role in immune suppression, and triggering immunogenic cell death (ICD) by PDT and chemotherapy, the nanosystem markedly inhibited primary tumor growth in murine models of B16F10 and 4T1 tumors. The nanosystem also exerted excellent preventive effects on tumor recurrence and metastasis.[133]

In another similar study, a macrophage-like nanosystem capable of releasing paclitaxel, Ce6, and IND in a near-infrared laser-triggered manner was used to produce combined chemo-photoimmunotherapy for experimental treatment of breast cancer in nonhuman subjects.[135] The authors used cell membranes derived from bacterial lipopolysaccharide-induced M1 macrophages to coat a hydrophobic bilirubin/Ce6 core containing both paclitaxel and IND. The combination of IND immunotherapy, Ce6-mediated PD, and paclitaxel chemotherapy provided by the final nanoassembly strongly induced antitumor immunity and exhibited remarkable antitumor activity in vivo. The assembly also inhibited lung metastasis.[135] Synergistic combination of therapies into a single nanosystem appears to be capable of inducing strong antitumor immune responses in nonhuman subjects via a combinatorial mechanism.

Photoabsorbing nanomaterials function as chemotherapeutic carriers and generators of both heat and ROS.[136] In a recent effort to treat metastatic breast cancer using a chemophototherapy approach, macrophage-derived membranes were cloaked onto near-infrared light-absorbing CuS NPs containing paclitaxel. This resulted in the production of a biomimetic nanosystem named PTX@CuS@MMNP that was capable of destroying and eliminating 4T1 tumors through a triple combination of chemotherapy, PDT and PTT (**Figure 10**A).[136] Given the tumor-targeting ability of the MCMs, the final PTX@CuS@MMNP actively targeted 4T1 cancer cells and were internalized by those cells via the $\alpha4\beta1$ integrin/VCAM-1 interaction; the latter was reinforced by systemic coadministration of the tumor-targeting peptide iRGD (Figure 10B,C). Treatment with PTX@CuS@MMNP, iRGD, and near-infrared light in vivo markedly increased the tumor temperature. This resulted in significant tumor eradication with minimal off-target toxicity. The anticancer efficacy was superior to non-coated NPs because of the multiple properties and functions provided by the nanosystem (Figure 10D,E).[136]

Another biomimetic nanosystem was designed to create a synergistic PTT-chemotherapy approach for cancer therapy. This nanosystem was supposed to overcome the hurdle of

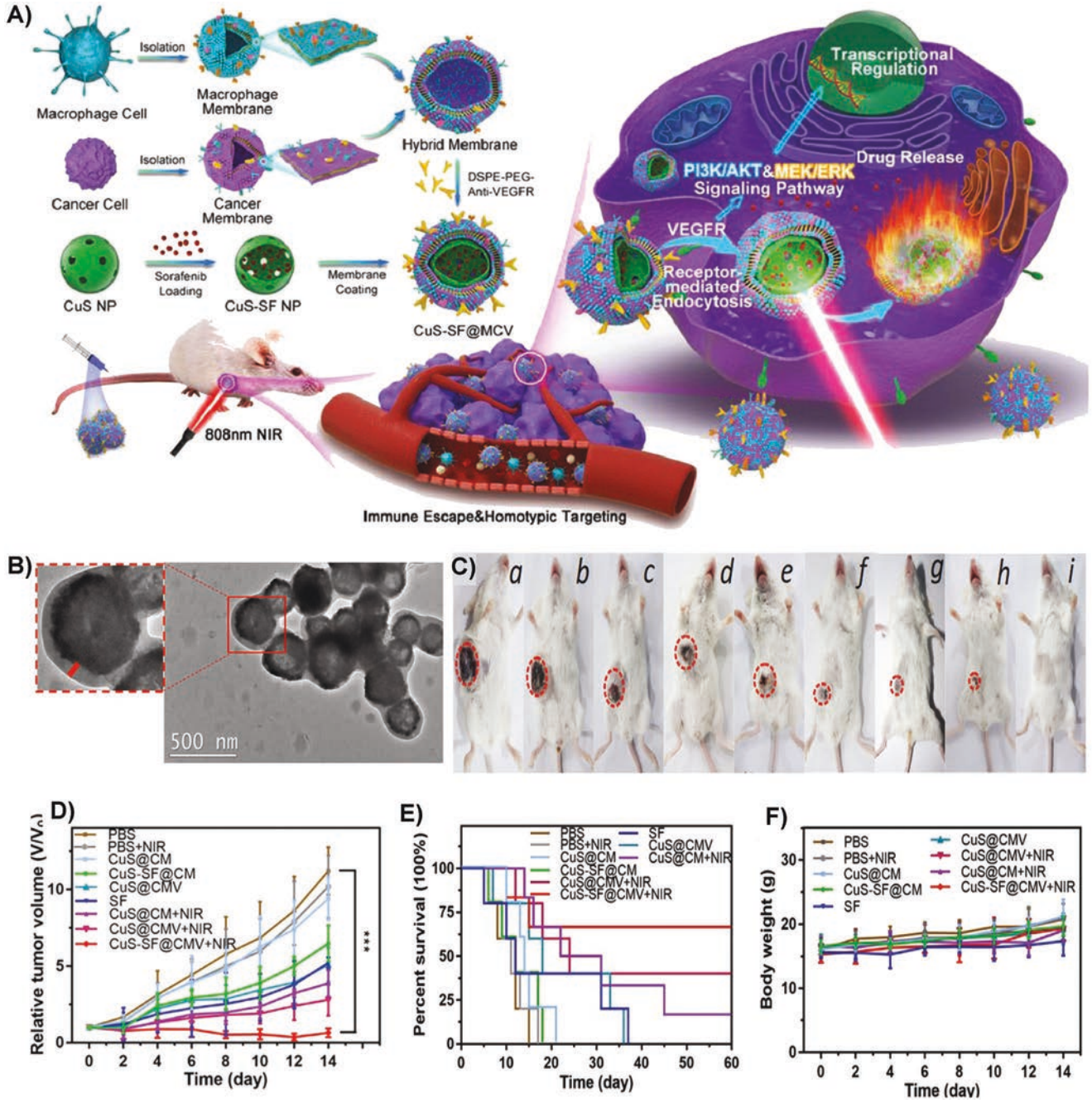

Figure 9. A) Schematic of CuS-SF@MCV preparation for synergistic chemotherapy-PTT in a mouse model of hepatocellular carcinoma. B) TEM images of CuS-SF@MCV showing the core–shell nanostructure. C) Comparative images of tumor size in tumor-bearing mice after different treatments, with CuS-SF@MCV plus NIR being represented in section i (tumors are delimitated by red circles). D) Growth curves of tumor volume and E) survival rate variation (%) in tumor-bearing mice receiving different treatments. F) Body weight changes for each group after intravenous injection in tumor-bearing mice. Reproduced with permission.[129] Copyright 2020, Elsevier. Abbreviations: CuS NP, cooper sulfide nanoparticle; CuS-SF NP, sorafenib-loaded CuS nanoparticle; CuS-SF@MCV, hybrid membrane-coated CuS-SF nanoparticle; NIR, near-infrared; PTT, photothermal therapy; TEM, transmission electron microscopy; VEGFR, vascular endothelial growth factor receptor.

crossing the cell membrane coating and enhance NP internalization by tumor cells. The nanosystem was produced by sequentially wrapping folic acid-conjugated mesoporous silica nanorods coloaded with doxorubicin, L-menthol, and indocyanine green (a photosensitiser and photothermal agent), with a cationic polymer (methoxy poly(ethylene glycol)–poly(β-amino ester) (MPEG-PAE)) and a macrophage-derived membrane.[137]

In a similar approach, a group of researchers harnessed the proton sponge effect of the cationic polymer MPEG-PAE

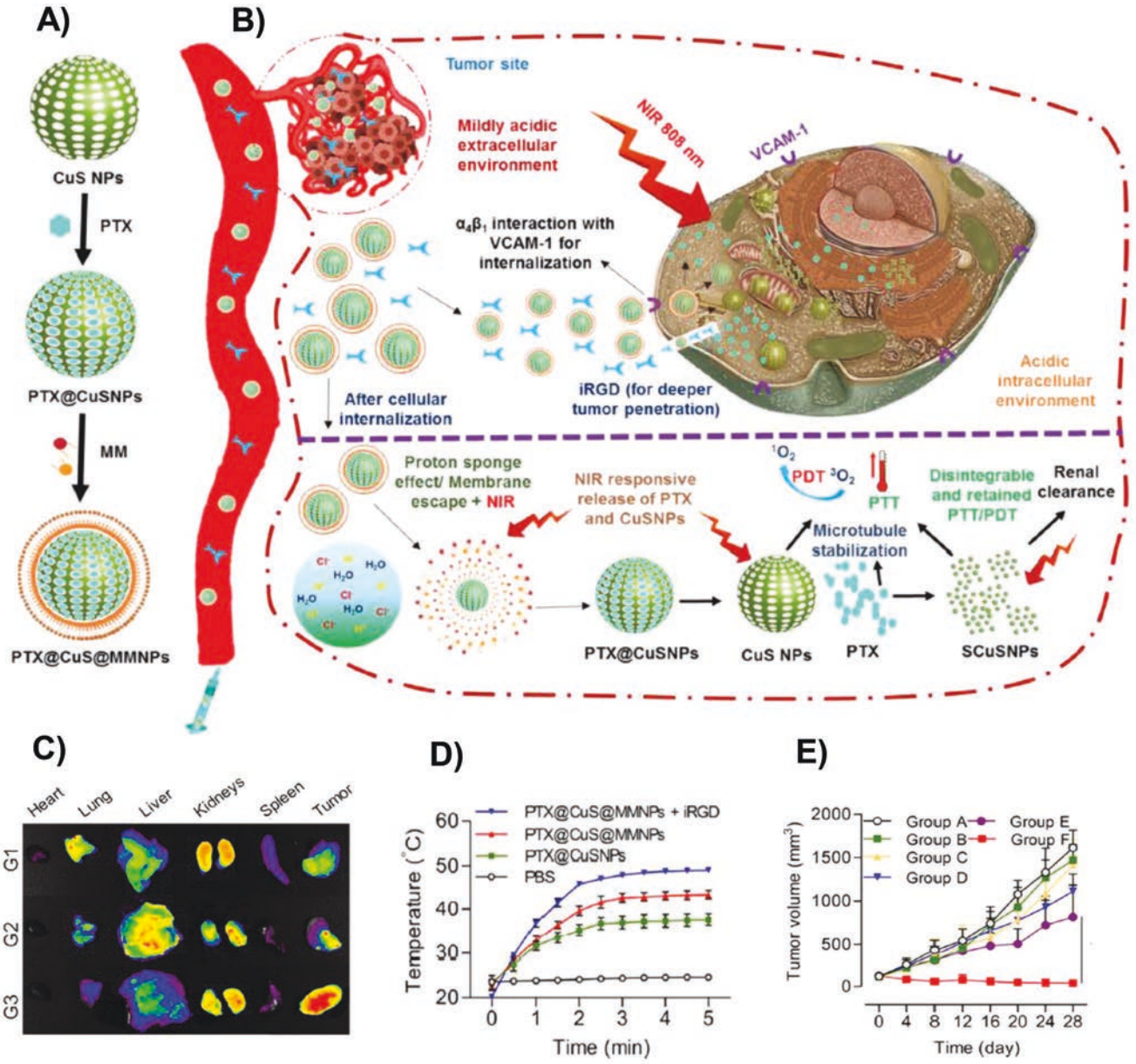

Figure 10. A) Schematic of PTX@CuS@MMNP preparation for synergistic chemotherapy, PDT and PTT against 4T1 tumors. B) PTX@CuS@MMNP tumor penetration and antitumor effects after systemic coadministration of the tumor-targeting peptide iRGD. C) Ex vivo images showing the superior tumor accumulation of PTX@CuS@MMNP plus iRGD (group G3) 24 h after administration. D) Comparative analysis of thermal elevation after different treatments. E) Growth curves of tumor volume of 4T1 tumor-bearing mice after different treatments (Group F represents PTX@CuS@MMNP plus iRGD plus NIR irradiation). Reproduced with permission.[136] Copyright 2020, American Chemical Society. Abbreviations: CuS NP, cooper sulfide nanoparticle; MM, macrophage membrane; NIR, near-infrared; PDT, photodynamic therapy; PTT, photothermal therapy; PTX, paclitaxel; PTX@CuS; PTX-loaded CuS NP; PTX@CuS@MMNP, macrophage membrane-coated PTX@CuS.

to absorb H^+ under acidic conditions of the tumor microenvironment. Such a procedure induced disruption of the cell membrane and allowing uptake of the NPs by tumor cells after specific binding to folic acid receptors that are overexpressed by tumor cells.[110] Upon irradiation by near-infrared light, the indocyanine green produced ROS and generated heat. This, in turn, facilitated the release of doxorubicin, resulting in synergistic antitumor effects that were contributed by chemotherapy, PDT and PTT.[137]

5.1.9. Capture of Circulating Tumor Cells

Macrophages bind to tumor cells within the tumor site and those in circulation via $\alpha 4\beta 1$integrin/VCAM-1 interaction. This enables the tumor cells to survive and spread through the circulation to produce metastases.[15,72] The migration of CTCs through the bloodstream has been recognized as a decisive step in the development of metastasis. Accordingly, detection and enumeration of CTCs in the bloodstream is crucial for cancer

diagnosis, prognosis, and therapy. However, the low occurrence frequency of CTCs in the bloodstream, and the nonspecific binding of white blood cells hamper successful clinical translation of this approach.[138] To address these issues, positively charged magnetic composite nanoparticles were coated with a negatively charged azide-coupled membrane derived from macrophages via electrostatic interaction. An antibody against epithelial cell adhesion molecule (EpCAM), an antigen selectively expressed in adenocarcinoma, was attached to the azide-coupled membrane via click chemistry.[138] The resulting biomimetic immune-magnetosomes were highly effective in recognizing EpCAM-expressing tumor cells because of their surface modification. The MCM coating also helped to reduce nonspecific adsorption and interaction with surrounding white blood cells because of the repulsive effect of white blood cells. The system captured ≈90% of tumor cells dispersed in whole blood in 15 min without any interference from white blood cells.[138]

5.1.10. Cancer Theranostics

Recent developments in the field of cell-mimicking nanotechnology have led to a proliferation of studies exploring MCM-coated NPs as versatile and promising biomimetic nanocarriers for theranostic applications, combining diagnostic imaging and therapeutic activity into a single nanosystem.[139] Recently, theranostic nanoplatforms have been studied for simultaneous tumor imaging and therapy, as they have been shown to be able to deliver both therapeutic drug molecules and imaging agents to targeted tumor sites.[140–142]

By drawing on the concept of biomimetic theranostic nanosystems, a multimodal macrophage-like superparticle was synthesized for the targeted codelivery of drugs and imaging agents to suppress lung metastases of breast cancer, while monitoring the therapeutic efficacy and tumor distribution of NPs in vivo.[143] To this end, doxorubicin and fluorescent quaternary quantum dots (QDs) were coloaded into liposomes, and then cloaked with a macrophage-derived membrane. Similar to the aforementioned studies, the MCM coating enabled the NPs to avoid immune system-mediated clearance and actively target lung metastatic lesions by binding to VCAM-1-expressing cancer cells via the macrophage biomarker $\alpha4\beta1$ integrin. In vivo studies showed that the biomimetic platform efficiently targeted doxorubicin and QDs to cancer cells, leading to the suppression of lung metastasis and stronger fluorescence intensity in tumor tissue. This study made several noteworthy contributions to the development of advanced approaches for the management of lung metastasis of breast cancer, by harnessing the tumor-homing abilities of MCMs for precise in vivo cancer imaging and highly effective antimetastatic treatment.[143]

Similarly, a different study described a theranostic nanosystem, called PTX@MPLMC, opening a new avenue in personalized strategies for cancer management, by combining both diagnostic and therapeutic functions.[144] They first designed persistent luminescence NP (PLNP)@metal–organic framework-derived mesoporous carbon nanocomposites (PLMC), in which $Zn_{1.1}Ga_{18}$ $Ge_{0.1}O_4$: Cr^{3+} PLNPs served as the imaging agent due to its exceptional capacity to emit long-term NIR persistence luminescence upon exposure to light-emitting diodes, which could be harnessed for in vivo persistent luminescence imaging. Then, paclitaxel-loaded PLMC cores were cloaked with macrophage-derived membranes to achieve targeted drug delivery, reduced uptake by the mononuclear phagocytic system, and effective chemotherapy with luminescence imaging guidance. In vivo studies showed an enhanced tumor-targeting ability of PTX@MPLMC, increasing both the tumor accumulation and luminescence intensity in the tumor sites. Collectively, these findings suggest that NIR-activated PTX@MPLMC can optimize the delivery of paclitaxel for inhibition of tumor growth and tumor eradication, while enabling the tracking of PTX@MPLMC in vivo by luminescence signals released from the PLMC inner cores.[144]

In the context of cancer theranostics, silver nanoclusters have also attracted much attention due to their intrinsic cytotoxicity and good fluorescent properties. In this regard, Ag nanoclusters were cloaked with a macrophage-derived membrane to design a biomimetic system for theranostics using a mouse model of Dalton Lymphoma Ascites (DLA).[145] When incubated with DLA tumor cells in vitro, the MCM-coated Ag nanoclusters showed superior efficacy in inducing tumor cell death compared to noncoated Ag nanoclusters, even at lower doses, which highlights the potential of this biomimetic nanosystem as an anticancer agent against DLA tumors. In vivo studies revealed that membrane-coated Ag nanoclusters efficiently homed to DLA tumors, enabling the visualization of tumors by fluorescent signals released by Ag nanocluster cores. Together these findings reveal that the anticancer effect and fluorescent imaging property of Ag nanoclusters coupled with the tumor-targeting ability of MCMs hold great promise for targeted theranostics.[145]

5.2. Inflammatory Diseases

5.2.1. Inflammation-Associated Vascular Diseases

Atherosclerosis: Inspired by the dual-targeting ability of macrophages to inflamed vascular lesions, PLGA NPs containing rapamycin, an mTOR pathway antagonist drug, were wrapped with a macrophage-derived membrane to actively target atherosclerotic lesions and suppress the progression of atherosclerosis.[146] The resulting biomimetic PLGA NPs could not only suppress macrophage-mediated phagocytosis in vitro, but also could actively target atherosclerotic plaques in vivo by binding to VCAM-1 and inflammatory cytokines overexpressed on the inflamed endothelium via their surface-expressed $\alpha4\beta1$ integrin and chemokine receptors, respectively, thereby enabling a more efficient and targeted drug delivery to atherosclerotic lesions compared to noncoated NPs, and efficient reduction of atherosclerotic lesions.[146] The overproduction of ROS in the atherosclerotic lesion has also recently been harnessed for improving targeted drug delivery.[48] Having this in mind, atorvastatin-loaded ROS-responsive NPs were camouflaged with a macrophage-derived membrane to endow the biomimetic nanosystem with intrinsic inflammation-targeting ability and ROS-induced drug release ability, thus providing targeted release of atorvastatin in response to locally produced ROS, and enhanced therapeutic efficacy.[48]

Early detection of atherosclerosis lesions and targeted therapy could contribute to improving antiatherosclerotic treatment, but the current diagnostic and therapeutic modalities are still incapable of fulfilling these requirements.[147] To circumvent these limitations, an MNC inner core was camouflaged with a simvastatin-embedded MCM, to which was attached an apolipoprotein A-I mimetic 4F peptide (AP), for early diagnosis of atherosclerosis via MRI, and for combination therapy by simvastatin and AP (**Figure 11**A).[147] In vitro and in vivo studies showed that the biomimetic nanoplatform efficiently targeted early atherosclerotic lesions due to the inflammation-targeting capabilities of MCMs and the specific interaction of AP with foam cells present in the atherosclerotic plaques. Foam cells are macrophages that have internalized low-density lipoproteins (LDL), and have been directly implicated in the formation of atherosclerotic plaques. The synergistic effects of AP and simvastatin enabled a remarkable suppression of atherosclerotic lesions in vivo by inducing LDL efflux from the foam cells and reducing the inflammatory cytokine levels, respectively (Figure 11B–D). Thus, this biomimetic nanoplatform showed the potential for achieving more efficient and targeted atherosclerosis diagnosis and therapy.[147]

Acute Ischemic Stroke: Acute ischemic stroke ranks as one of the leading causes of death worldwide, and is usually triggered by the obstruction of brain blood vessels by a thrombus. After suffering an acute ischemic stroke, saving the damaged neurons within the ischemic brain regions is the primary goal of most interventions.[148] Having this in mind, fingolimod-loaded manganese dioxide (MnO_2) nanospheres were coated with a macrophage-derived membrane to produce a biomimetic nanosystem, called Ma@(MnO_2 + FTY), for targeting ischemic brain lesions and rescuing damaged neurons (**Figure 12**A).[148] On account of its membrane coating, Ma@(MnO_2 + FTY) showed not only good biocompatibility and longer systemic circulation in vivo, but also a superior ability to target ischemic brain lesions, due to the intrinsic ability of macrophages to target inflammatory sites. This resulted in high accumulation in the ischemic penumbra, where it could protect the damaged neurons by quenching the excessive ROS to produce free O_2, and converting the M1 microglia into the anti-inflammatory M2 microglial phenotype (Figure 12B–D).[148] Indeed, the biomimetic Ma@(MnO_2 + FTY) showed combined neuroprotective effects, since the MnO_2 nanospheres could efficiently convert hydrogen peroxide (H_2O_2) into O_2, which helps to reduce ischemia-associated oxidative stress and suppress the production of proinflammatory cytokines, while the loaded fingolimod could induce the repolarization of M1 microglia to the M2 phenotype to convert the proinflammatory microenvironment into an anti-inflammatory one (Figure 12E). In summary, this biomimetic nanosystem proved to be a promising approach for the synergistic treatment of acute ischemic stroke by exerting neuroprotective effects and promoting the survival of damaged neurons in the ischemic brain.[148]

Vascular Intimal Hyperplasia: Vascular intimal hyperplasia is a common pathological reaction seen in various vascular disorders, and is characterized by the abnormal accumulation and proliferation of vascular smooth muscle cells (VSMCs) in the tunica intima of blood vessels in response to vascular injury.[149] In a recent attempt to develop a biomimetic nanosystem for targeted therapy of vascular intimal hyperplasia, an ROS-responsive amphiphilic NP preparation, called PCM, which could efficiently solubilize the drug rapamycin in its hydrophobic core, was coated with a macrophage-derived membrane.[149] Due to its MCM coating, the biomimetic nanosystem exhibited not only immune evasion ability and longer systemic circulation, but also a superior ability to target the injured blood vessels, mostly owing to the intrinsic ability of macrophages to actively target inflammatory vascular lesions via their surface-expressed $\alpha 4\beta 1$ integrin and CCR2, which interact with VCAM-1 and CCL2, respectively. This enabled the targeted and controlled release of rapamycin in response to locally produced ROS in the inflamed vascular lesions. In summary, the biomimetic nanosystem proved to be an efficient approach for the treatment of vascular intimal hyperplasia, as demonstrated by the efficient inhibition of VSMC proliferation both in vivo and in vitro.[149]

5.2.2. Inflammation Associated Neurodegenerative Disorders

Alzheimer's disease is a well-studied neurodegenerative disorder characterized by progressive accumulation of beta-amyloid (Aβ) in the brain, culminating in the loss of neuronal synapses and neuronal cell apoptosis. Mitochondrial dysfunction, resulting from excessive ROS generation, has been suggested to be a key precipitating event in Alzheimer´s disease, by causing the production and aggregation of Aβ, and therapeutic modalities based on the targeted delivery of antioxidants to neuronal mitochondria have attracted some attention.[150,151]

By taking advantage of the innate ability of macrophages to be recruited to neuroinflammation areas associated with brain injury, a macrophage-mimicking nanosystem was recently designed by camouflaging solid lipid NPs containing genistein, an antioxidant, anti-inflammatory, and neuroprotective flavonoid, with a macrophage-derived membrane to allow the genistein to cross the BBB and target the neuronal mitochondria in the brain (**Figure 13**A,B).[150] By coincorporating triphenylphosphine (TPP), a positively charged mitochondrial-targeting ligand, and rabies virus glycoprotein (RVG29), a specific neuronal-targeting and BBB-crossing ligand, onto the surface of MCMs, the biomimetic nanoplatform could more efficiently penetrate the BBB and selectively enter neuronal cells, to target the mitochondria (Figure 13C,D).[150] In vitro and in vivo studies showed enhanced uptake by neuronal cells, good BBB-penetration ability, enhanced ability to target the negatively charged neuronal mitochondria, and efficient mitochondrial ROS reduction due to the antioxidant effect of genistein (Figure 13E,F). Hence, the combination of TPP, RVG29, and MCMs in a single nanoplatform enabled a significant inhibition of Alzheimer's disease progression by a neuronal mitochondrial-targeted strategy to accomplish effective genistein delivery.[150]

5.2.3. Inflammatory Osteolysis

Inflammatory osteolysis is a condition in which the immune system produces proinflammatory cytokines that increase osteoclast activity and reduce osteogenesis, which ultimately

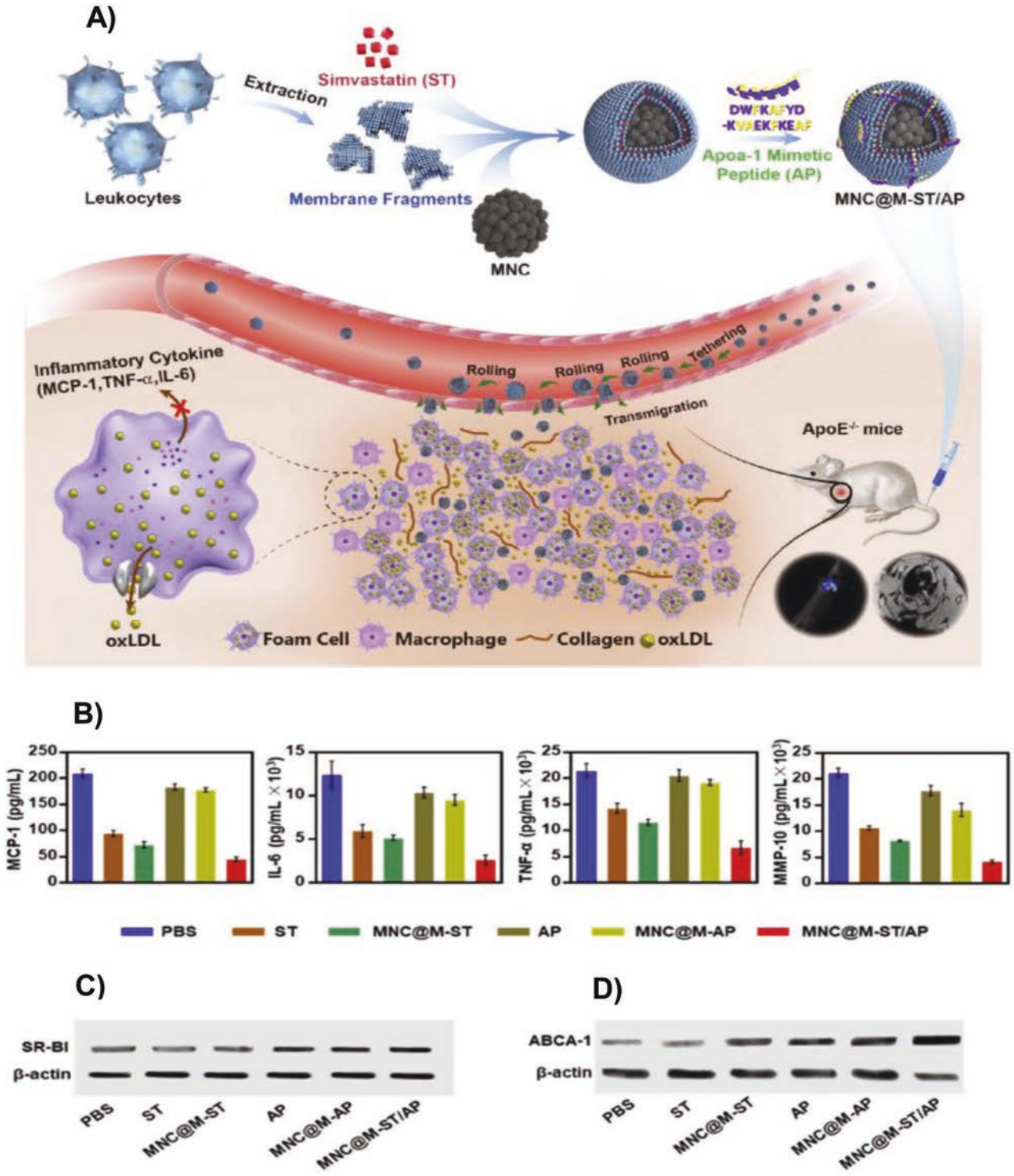

Figure 11. A) Schematic illustration of MNC@M-ST/AP preparation for early detection of atherosclerosis via MRI and targeted therapy by simvastatin and AP. The anti-inflammatory drug simvastatin alleviates inflammation by reducing the levels of inflammatory cytokines, whereas AP induces oxLDL efflux from foam cells via RCT pathways. B) Levels of inflammatory cytokines (MCP-1, IL-6, TNF-α, and MMP-10) in an Apo $E^{-/-}$ mouse model with early atherosclerotic lesions for different treatments. C,D) Levels of the cholesterol-efflux receptors (SR-BI and ABCA-1) in an Apo $E^{-/-}$ mouse model with early atherosclerotic lesions for different treatments. Reproduced with permission.[147] Copyright 2092, Elsevier. Abbreviations: MNC, magnetic nanocluster; MNC@M-AP, AP-attached macrophage membrane-coated MNC; MNC@M-ST, ST-embedded macrophage membrane-coated MNC; MNC@M-ST/AP, ST-embedded AP-attached macrophage membrane-coated MNC; MRI, magnetic resonance imaging; oxLDL, oxidized low-density lipoprotein.

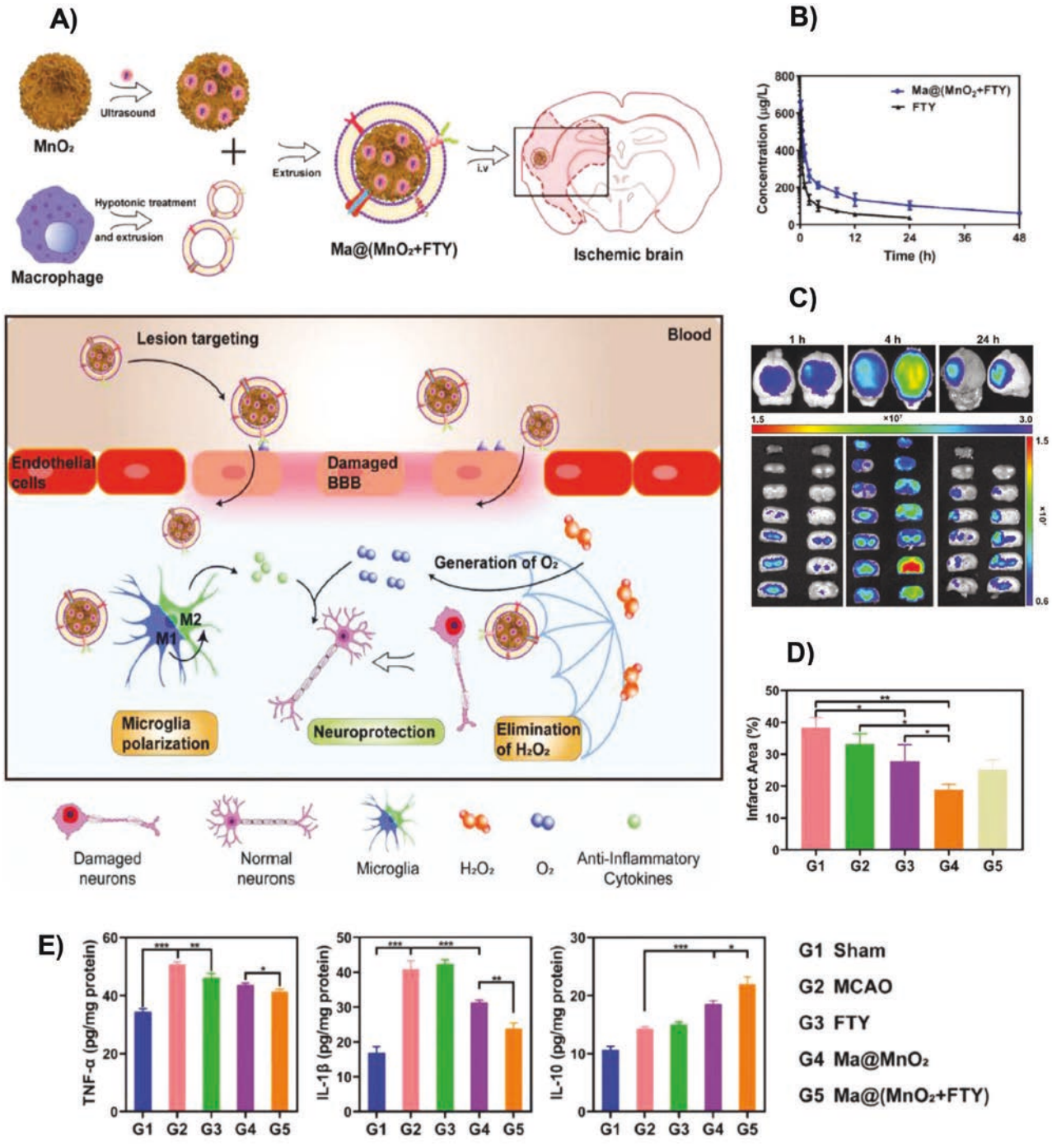

Figure 12. A) Schematic of Ma@(MnO_2 + FTY) preparation by coating FTY-loaded MnO_2 nanospheres with a macrophage membrane to actively target ischemic brain lesions and rescue damaged neurons by converting the local ROS (H_2O_2) in O_2 and suppressing inflammation through inducing the repolarization of M1 microglia in the anti-inflammatory M2 phenotype. B) Concentration of FTY (μg L^{-1}) after intravenous administration of free FTY and Ma@(MnO_2 + FTY). C) Comparative targeting capability to ischemic brain lesions of different treatments at designated times assessed by fluorescence imaging. D) Infarct area (%) measurements assessed by TCC for different treatments, in which Ma@(MnO_2 + FTY) are represented in G4. E) Expression of inflammatory cytokine (TNF-α and IL-1β) and anti-inflammatory cytokine (IL-10) levels for different treatments. Reproduced with permission.[148] Copyright 2021, Wiley-VCH GmbH. Abbreviations: FTY, fingolimod; Ma@(MnO_2 + FTY), macrophage membrane-coated MnO_2 nanosphere containing FTY; Ma@MnO_2, macrophage membrane-coated MnO_2 nanosphere; MnO_2, manganese dioxide.

culminates in bone degradation. Thus, inducing the repolarization of M1 macrophages to the anti-inflammatory M2 phenotype, while at the same time neutralizing proinflammatory cytokines and endotoxins, such as the M1 polarization inducer bacterial lipopolysaccharide, is a promising approach to suppress inflammatory osteolysis.[152]

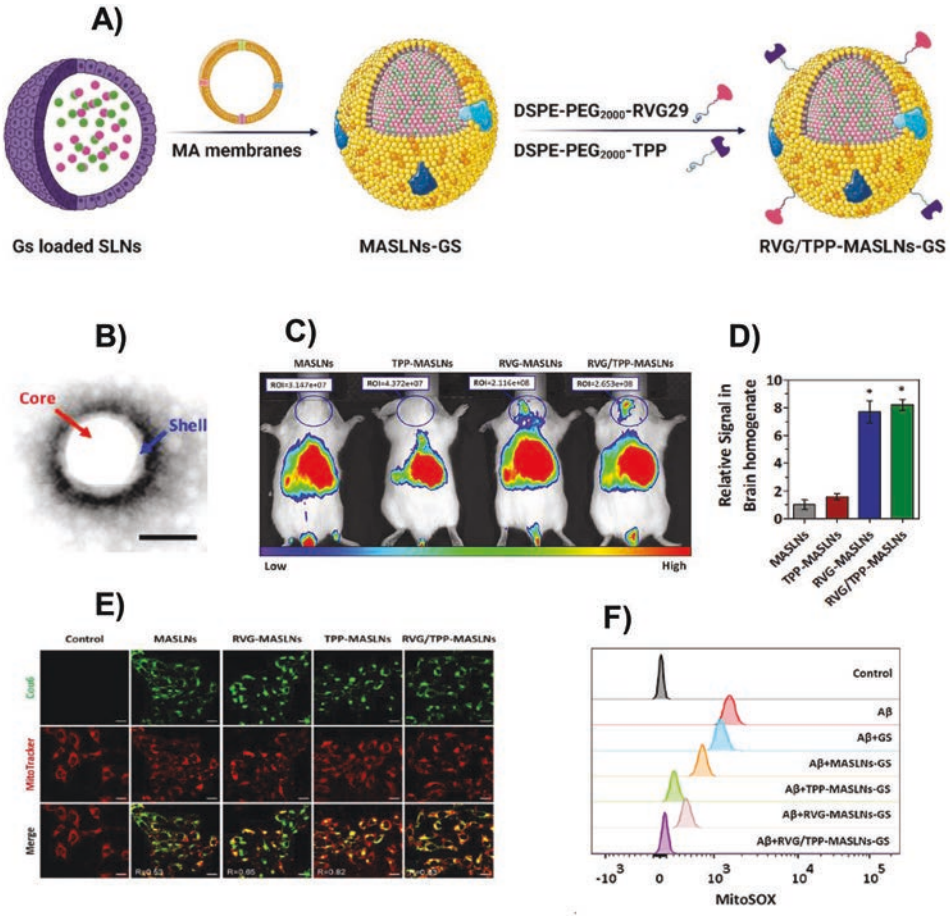

Figure 13. A) Schematic of RVG/TPP-MASLNs-GS preparation to target the neuronal mitochondria for treatment of Alzheimer´s disease. B) TEM images of RVG/TPP-MASLNs-GS showing the core–shell nanostructure. C) In vivo brain distribution of different formulations. D) Relative fluorescence signals of brain obtained for different treatments. E) Evaluation of the mitochondrial-targeting ability of different formulations. The red staining represents the mitochondria and the green staining represents the fluorescent dye (Cou6)-tagged formulations. F) In vitro evaluation of mitochondrial ROS levels for different treatments in Aβ- treated neuronal cells. (B–F) Reproduced with permission.[150] Copyright 2021, KeAi Publishing Communications Ltd. Abbreviations: Aβ, beta-amyloid; GS, genistein; MA, macrophage; MASLNs-GS, macrophage membrane-coated GS-loaded SLNs; ROS, reactive oxygen species; RVG/TPP-MASLNs-GS, macrophage membrane-coated GS-loaded SLNs dual-functionalized with RVG29 and TPP; RVG29, rabies virus glycoprotein; SLN, solid lipid nanoparticle; TEM, transmission electron microscopy; TPP, triphenylphosphine.

In an attempt to develop a biomimetic nanosystem for the treatment of inflammatory osteolysis, recently porous Se@SiO_2 nanospheres were cloaked with a macrophage-derived membrane.[152] Due to the receptors naturally expressed on the MCM surface, including TNF-R, IL6-R, and TLR4, the biomimetic nanosystem, called M-Se@SiO_2, could efficiently bind and neutralize the proinflammatory cytokines TNF-α and IL-6, as well as the bacterial lipopolysaccharide (**Figure 14**A–C). Additionally, the release of selenium (Se) from the M-Se@SiO_2 could induce the polarization of M1 macrophages toward the M2 phenotype, reducing the production of proinflammatory cytokines and increasing the release of anti-inflammatory cytokines, such as IL-10 and IL-4, which in turn can suppress osteolysis and induce osteogenic differentiation (Figure 14D). In vivo studies suggested that M-Se@SiO_2 could effectively inhibit bacterial lipopolysaccharide-induced osteolysis, since the mice treated with bacterial lipopolysaccharide plus M-Se@SiO_2 showed significantly less osteolysis (bone destruction) compared to the other groups, confirming its protective effects against bacterial lipopolysaccharide-induced inflammatory osteolysis (Figure 14E). The biomimetic nanosystem appeared to be an acceptable approach to induce osteogenesis and inhibit inflammatory osteolysis due to its dual functions, including its ability to neutralize endotoxins and proinflammatory cytokines, and also to induce macrophage polarization in the M2 anti-inflammatory phenotype.[152]

5.2.4. Age-Related Macular Degeneration

Age-related macular degeneration (AMD) is an eye disease responsible for irreversible vision loss amongst the older population. The pathological progression of AMD is related to the dysfunction and deterioration of a special monolayer of cells, called the retinal pigmented epithelium (RPE), which causes severe inflammation with the gradual loss of photoreceptors, as well as choroidal neovascularization (CNV) in the advanced stages of the disease, due to the excessive production

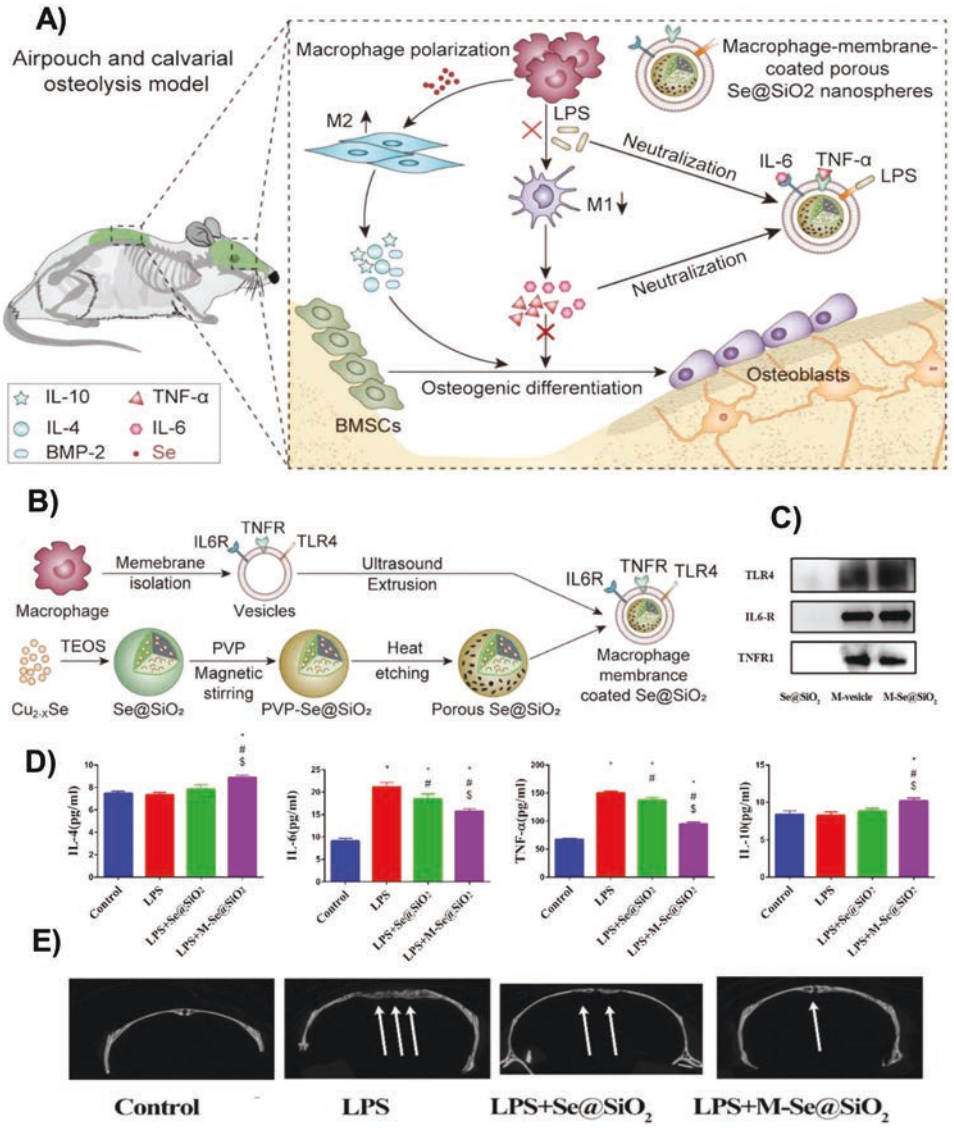

Figure 14. A) Mechanism of inflammatory osteolysis management by M-Se@SiO_2 via inducing the macrophage polarization in the M2 anti-inflammatory phenotype and reducing both the proinflammatory cytokine and LPS levels. B) Schematic illustration of M-Se@SiO_2 preparation. C) Western blotting analysis of TLR4, IL6-R, and TNFR1 in different formulations. D) Levels of proinflammatory cytokines (TNF-α and IL-6) and anti-inflammatory cytokines (IL-10 and IL-4) assessed by ELISA. E) Microcomputed tomography analysis in a mouse model after different treatments (osteolysis is represented by white arrows). Reproduced with permission.[152] Copyright 2021, BioMed Central Ltd. Abbreviations: BMSC, bone mesenchymal stem cell; LPS, bacterial lipopolysaccharide; M-Se@SiO_2, macrophage membrane-coated porous Se@SiO_2 nanospheres; Se, selenium.

of proangiogenic cytokines by the RPE. Over the years, there have been multiple studies indicating the important role of the mTOR signaling pathway in AMD progression, since mTOR activation leads not only to RPE dedifferentiation and loss of photoreceptors, but also increases the production of VEGF, a proangiogenic factor. These findings indicate the potential of mTOR inhibition strategies for suppressing AMD progression by reducing angiogenesis and inflammation, and inducing autophagy.[153]

In an effort to develop new noninvasive and effective therapeutic strategies for AMD, while overcoming the complications associated with the repeated intravitreal injection, a biomimetic nanosystem based on coating PLGA NPs previously loaded with rapamycin with a macrophage-derived membrane nanovesicle preparation was reported (**Figure 15**A).[153] Due its membrane coating, the biomimetic nanosystem showed good ability to evade immune clearance and to cross the blood-retinal barrier after intravenous injection in a mouse model in vivo, resulting in accumulation in the inflamed CNV lesions in the eye, which in turn improved the local concentration of rapamycin for efficient downregulation of the mTOR pathway, thereby inducing suppression of angiogenesis and inflammation, and enhancing autophagy (Figure 15B–D). In summary, this study provided a new promising strategy for AMD treatment based on intravenous administration of biomimetic MCM-coated nanosystems to improve the delivery efficiency of rapamycin toward the inflamed CNV lesions in the eye, by taking advantage of the intrinsic targeting features of macrophages to inflamed tissues, while avoiding the unwanted side effects related to standard intravitreal injection.[153]

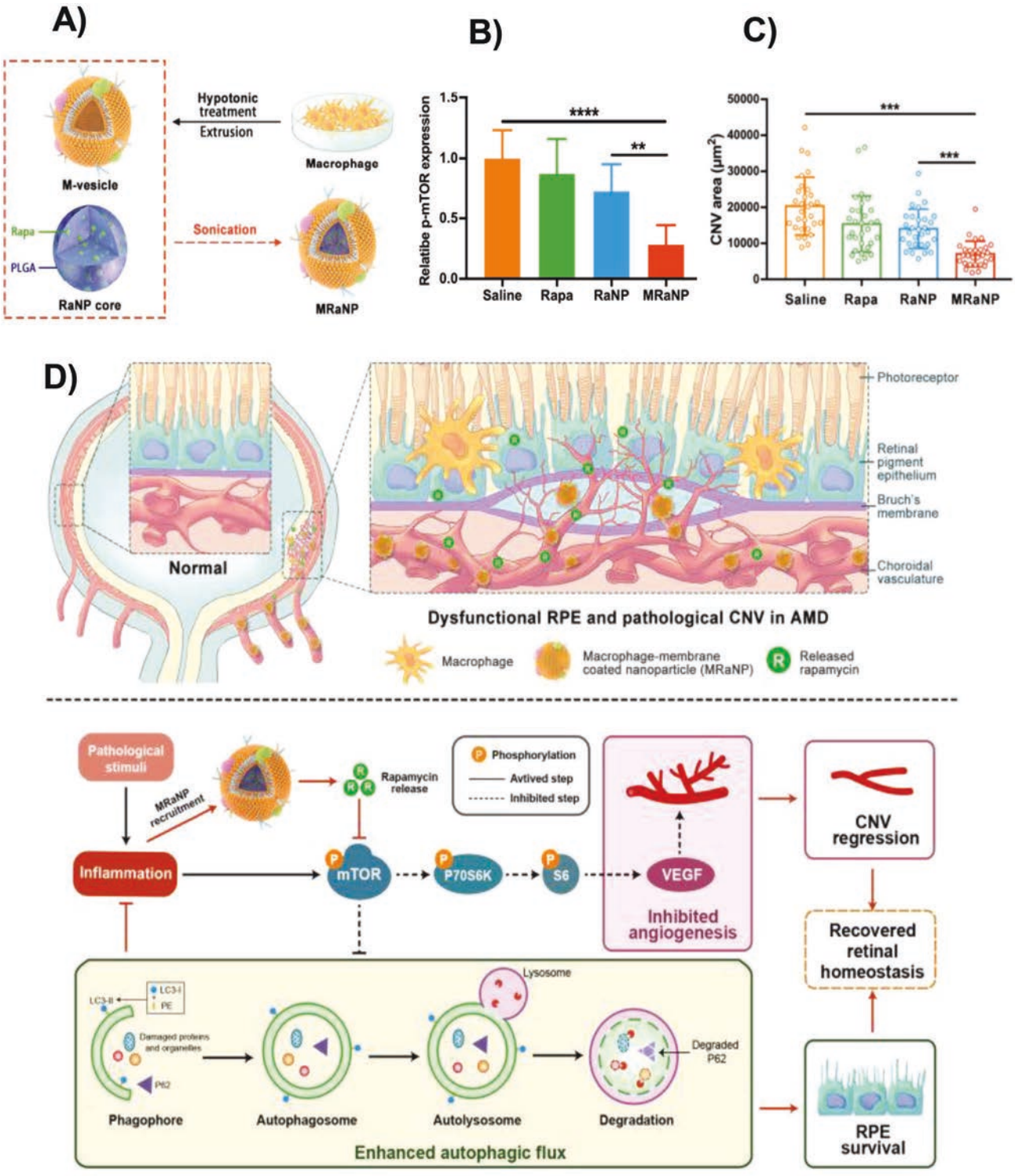

Figure 15. A) Schematic of MRaNP preparation by coating RaNP with a macrophage membrane for targeted delivery of Rapa to inflamed CNV lesions in the eye. B) Comparative analysis of phosphorylated m-TOR (p-mTOR) expression in an LCNV mouse model after different treatments. C) Comparative analysis of CNV lesion area (μm^2) in an LCNV mouse after different treatments. D) Representation of MRaNP targeting the inflamed CNV lesions in the eye after intravenous administration via chemotactic recruitment (up) and mechanism of retinal homeostasis modulation by MRaNP via inhibition of the mTOR signaling pathway (down). Reproduced with permission.[153] Copyright 2021, Elsevier B.V. Abbreviations: AMD, age-related macular degeneration; CNV, choroidal neovascularization; LCNV, laser-induced choroidal neovascularization; MRaNP, macrophage membrane-coated RaNP; PLGA NP, poly(lactic-*co*-glycolic acid) nanoparticle; RaNP, PLGA NP containing Rapa; Rapa, rapamycin; RPE, retinal pigment epithelium.

5.3. Infectious Diseases

5.3.1. Bacterial Infections

Macrophage membrane-coated NPs contain a wide repertoire of pathogen recognition receptors, such as TLR4 and TLR2. These NPs can mimic the bacterial targeting capability of natural macrophage cells.[154] In a novel study, the membranes of macrophages pretreated with *Staphylococcus aureus* were coated onto gold–silver nanocages with good NIR absorption to enhance bacterial targeting and allow antibacterial killing using PTT.[154] It was shown that bacterial pretreatment could significantly increase the amount of bacterial recognition receptors on the membrane surface, thus increasing the ability of the membrane-coated gold–silver nanocages to recognize and bind to bacteria. In vivo studies showed a longer retention time at infected sites in comparison to noncoated counterparts, and efficient hyperthermia-induced bacterial destruction under NIR laser irradiation, thus confirming the antibacterial efficacy of this biomimetic platform against localized bacterial infections.[154]

The absence of effective strategies to manage intracellular bacterial infections, which are more complicated to treat and more severe than extracellular infections, motivated the development of a biomimetic nanosystem by wrapping antimicrobial-agent conjugated NPs (ANPs), composed of triclosan and ciprofloxacin, with macrophage-derived membranes for the treatment of intracellular infections caused by *S. aureus*.[155] The biomimetic NPs were shown to be specifically internalized in vitro by macrophages infected with intracellular *S. aureus*, thus releasing the conjugated antimicrobial agents for efficient intracellular bacterial killing without compromising healthy macrophages, which was attributed to the negative zeta potential and TLR-mediated uptake of the NPs by positively charged *S. aureus*-infected macrophages. Overall, membrane-coated ANPs could provide a superior elimination of intracellular *S. aureus* infection compared to uncoated ANPs or free ciprofloxacin, therefore confirming their ability to mitigate the severity of intracellular bacterial infections.[155]

The increasing worldwide prevalence of multidrug-resistant bacterial infections is rapidly becoming a serious and life-threatening public health problem, due to the slowdown in the development of novel antibiotics and the overuse of existing ones.[156,157] In a similar manner to the aforementioned studies, which highlighted the natural interaction of macrophages with pathogenic agents, another macrophage-mimicking nanoplatform was designed to capture and neutralize bacterial toxins, as well as boost immune response against *Pseudomonas aeruginosa*, a Gram-negative bacterial species responsible for nosocomial pneumonia. They loaded *P. aeruginosa* surface antigens, called PaS-1/-2, into the preassembled MCM-coated PLGA NPs.[156] The experimental studies demonstrated the safety profile of the nanotoxoid formulation, with no obvious signs of toxicity being observed in vivo and in vitro, and its potential for triggering an antibacterial immune response after subcutaneous or intranasal vaccination. In vivo studies showed that this biomimetic vaccine could significantly reduce the bacterial burden in the lungs, thus diminishing the severity of bacterial lung infections and improving the management of bacterial infections.[156]

The concept of using macrophage-mimicking NPs to manage inflammatory disorders has also recently been applied to treat osteomyelitis, a severe bacterial bone infection accompanied by marked inflammation that ultimately results in bone tissue damage.[102] Recently, magnetic composite NPs composed of Fe_3O_4 NPs, titanium dioxide (TiO_2), an antibacterial agent with ultraviolet light-induced ROS generation ability, and calcium phosphate ($Ca_3(PO_4)_2$), which can act as a bone component to promote bone regeneration, were cloaked with macrophage-derived membranes using a state-of-the-art electroporation-based coating technique.[102] In vitro and in vivo studies showed that, by combining the ability of MCMs to neutralize cytokines and bacterial toxins, combined with the ROS-generation capability of TiO_2 and the osteoconductive features of ($Ca_3(PO_4)_2$), the final NPs were able to efficiently alleviate the inflammatory response caused by bacterial infection, stimulate bone tissue formation, and destroy bacteria when irradiated with ultraviolet light, thus achieving anti-inflammatory and antibacterial efficacy against bone infections.[102]

Sepsis is a life-threatening pathological condition associated with an extensive inflammatory reaction in response to bacterial infections.[158] Macrophage-mimicking nanosystems have also been employed for the treatment of sepsis because of their exceptional capacity to absorb and remove bacterial endotoxins and proinflammatory cytokines, due to specific receptors located on the cell membrane.[28,49] Having this in mind, polymeric PLGA NP cores were coated with MCMs to treat sepsis. The biomimetic nanosystem (MΦ-NPs) was able to efficiently neutralize and sequester bacterial lipopolysaccharide and several proinflammatory cytokines in vitro using standardized solutions (**Figure 16**A–D).[49] Western blotting analysis confirmed the presence of CD126, CD120 a/b, and CD119 in the prepared cell membrane-coated NPs, which bind to IL-6, TNF, and IFN-γ (inflammatory cytokines) respectively, as well as the bacterial lipopolysaccharide-binding receptors TLR4 and CD14 (Figure 16E). MΦ-NPs showed superior blood circulation time (which decreased over time) and enhanced uptake by the liver and spleen, which are the main organs of the reticuloendothelial system (Figure 16F,G). In vivo studies showed that MΦ-NPs markedly reduced both proinflammatory cytokine levels and bacterial counts in several organs, and prolonged the survival time of the mice. Overall, these encouraging findings may pave the way for the development of novel strategies for sepsis treatment.[49]

5.3.2. Viral Infections

The ongoing pandemic crisis caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remains a serious global health problem, in which the absence of specific therapeutic regimens greatly contributes to the high mortality and morbidity associated with coronavirus disease 2019 (COVID-19).[26,159] Alveolar macrophages are a crucial primary line of defense against invaders, which express specific membrane receptors involved in cytokine uptake and binding of the spike protein on the coronavirus surface, thus suppressing virus infection by diverting them from target cells and blocking viral cellular entry, while mitigating the excessive inflammatory and immune responses. In a recent effort to develop a

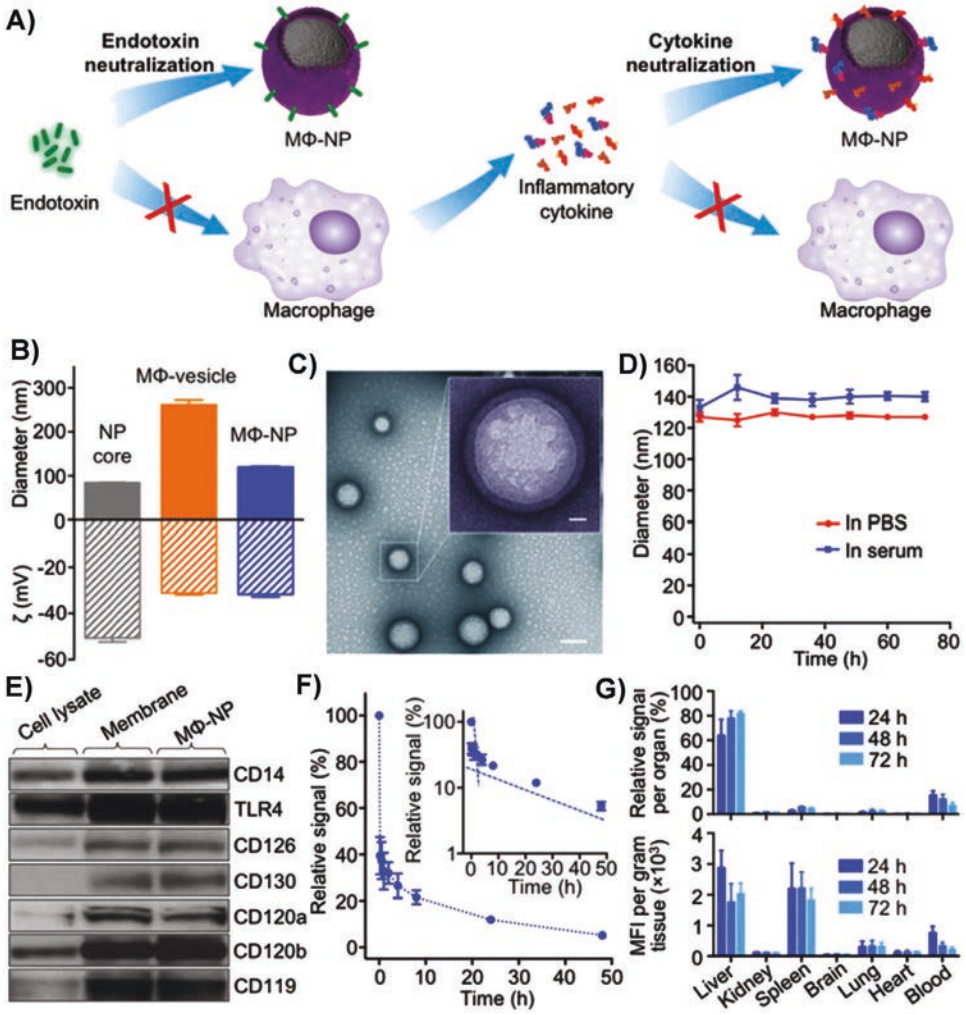

Figure 16. A) Schematic of the mechanism of action of the MΦ-NPs to manage sepsis by neutralizing both endotoxin and proinflammatory cytokines. B) Hydrodynamic size and surface zeta potential measurements assessed by dynamic light scattering before (PLGA NP cores) and after coating polymeric PLGA NP cores with the macrophage membrane (MΦ-NPs). C) TEM images of the MΦ-NPs nanoassembly in which a core–shell nanostructure can be observed. D) Evaluation of MΦ-NPs stability after suspension in 1× PBS or 50% FBS, assessed by monitoring their diameter over 72 h. E) Western blotting analysis of CD126, CD120 a/b, CD119, TLR4, and CD14 in different formulations (macrophage cell lysate, membrane vesicles, and MΦ-NPs). F) Evaluation of the in vivo systemic circulation time of MΦ-NPs after intravenous injection in a mouse model. G) In vivo biodistribution of MΦ-NPs in major organs 24, 48, and 72 h after intravenous injection in a mouse model. Reproduced with permission.[49] Copyright 2017, National Academy of Sciences. Abbreviations: MΦ-NP, polymeric PLGA NP coated with macrophage membrane; PLGA NP, poly(lactic-*co*-glycolic acid) nanoparticle; TEM, transmission electron microscopy.

multifunctional biomimetic nanosystem against COVID-19 infection, polymeric PLGA NP cores containing 2TPE-2NDTA, an efficient photothermal agent, were wrapped with alveolar macrophage-derived membranes (**Figure 17**A).[47] Under NIR irradiation, the biomimetic nanoplatform, called TN@AM NP, was highly efficient in converting NIR light into heat, even at very low concentrations, producing a substantial temperature increase for photothermal viral destruction, due to the high heat sensitivity of SARS-CoV-2. In the experimental studies, the combination therapy with TN@AM NPs and NIR irradiation produced a significant decrease in both proinflammatory cytokine expression and viral count in the lungs, extended the survival time of the infected mice, and reduced lung tissue damage, indicating its potential to be used in clinical practice for the treatment of COVID-19 (Figure 17B–E).[47]

In another recent effort to reduce the excessive inflammatory response associated with coronavirus infection, macrophage-derived membranes were decorated onto PLGA NPs containing lopinavir, an antiviral drug, constructing a nanosystem with both antiviral and anti-inflammatory potential (**Figure 18**A).[160] The biomimetic nanoplatform expressed cytokine binding receptors, including IL-6R and IL-1βR, and could effectively absorb inflammatory cytokines and suppress macrophage and neutrophil activation, thereby alleviating the strong inflammatory response (Figure 18B,C). In addition, the presence of angiotensin-converting enzyme 2 (ACE II) on the MCM enabled the nanosystem to specifically bind to the spike protein on the coronavirus surface, thereby allowing the targeted drug delivery to sites of viral infection. In vivo studies showed superior reduction of proinflammatory cytokine levels and

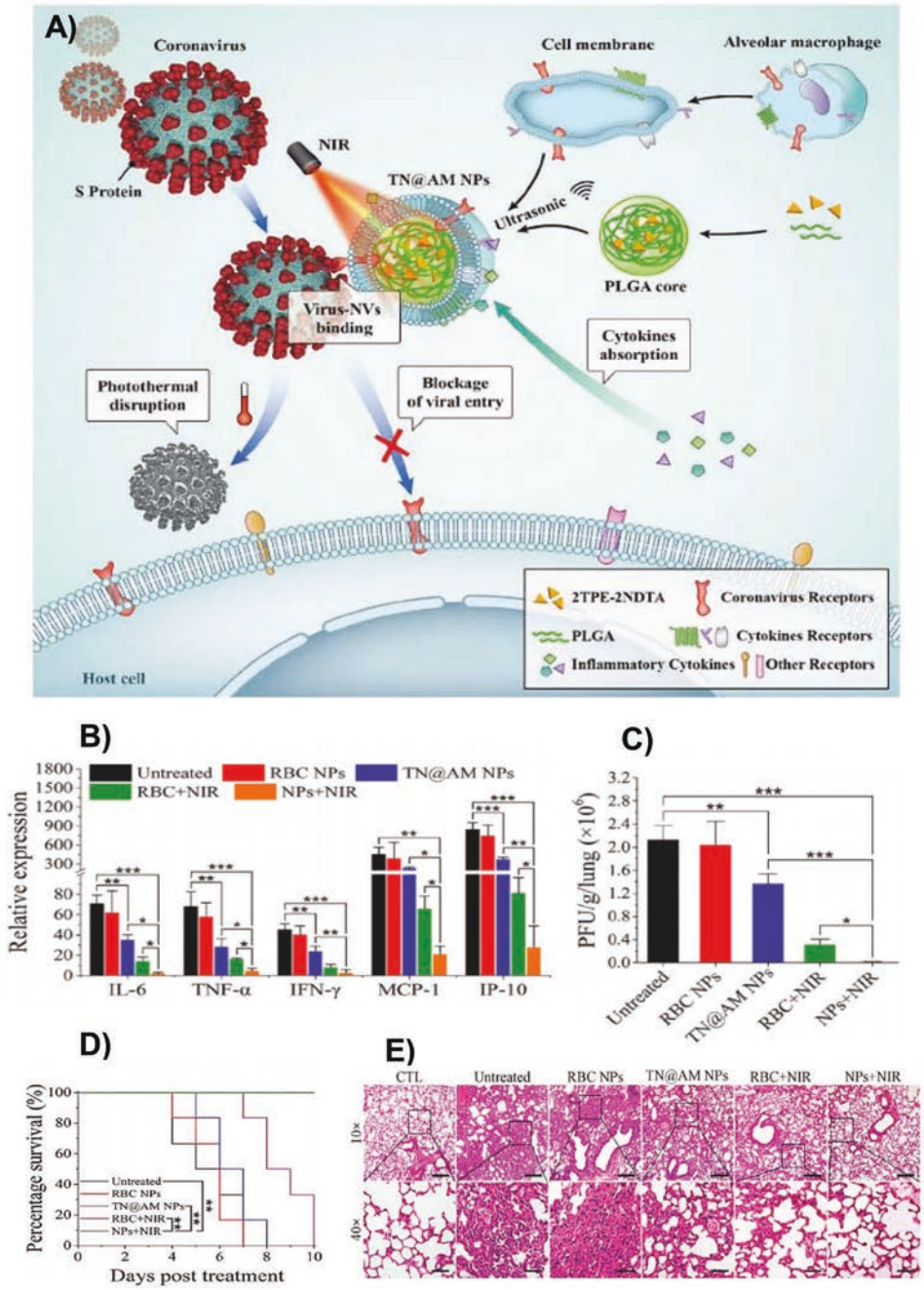

Figure 17. A) Schematic of alveolar macrophage-mimicking PLGA NPs (TN@AM NPs) preparation for targeted PTT against coronavirus infection. B) Relative mRNA expression for IL-6, TNF-α, IFN- γ, MCP-1, and IP-10 (proinflammatory cytokines) in the lungs. C) Representation of lung viral count after 5 days of different treatments. D) Analysis of mice survival (%) after each treatment. E) H&E staining images of pulmonary tissue recorder after 5 days of different treatments. Reproduced with permission.[47] Copyright 2021, Wiley-VCH GmbH. Abbreviations: NIR, near-infrared; PLGA NP, poly(lactic-*co*-glycolic acid) nanoparticle; PTT, photothermal therapy; TN@AM NP, alveolar macrophage membrane-coated PLGA nanoparticle containing 2TPE-2NDTA.

efficient viral destruction, which resulted in prolonged survival time of infected mice and reduced the inflammation-induced lung tissue damage (Figure 18D–F). Hence, this biomimetic platform showed great potential for alleviating strong inflammatory responses for the treatment of COVID-19.[160]

6. Challenges in Clinical Translation and Future Prospectives

Immune cell–mimetic nanoplatforms are promising candidates for targeted drug delivery and immune modulation. Despite the

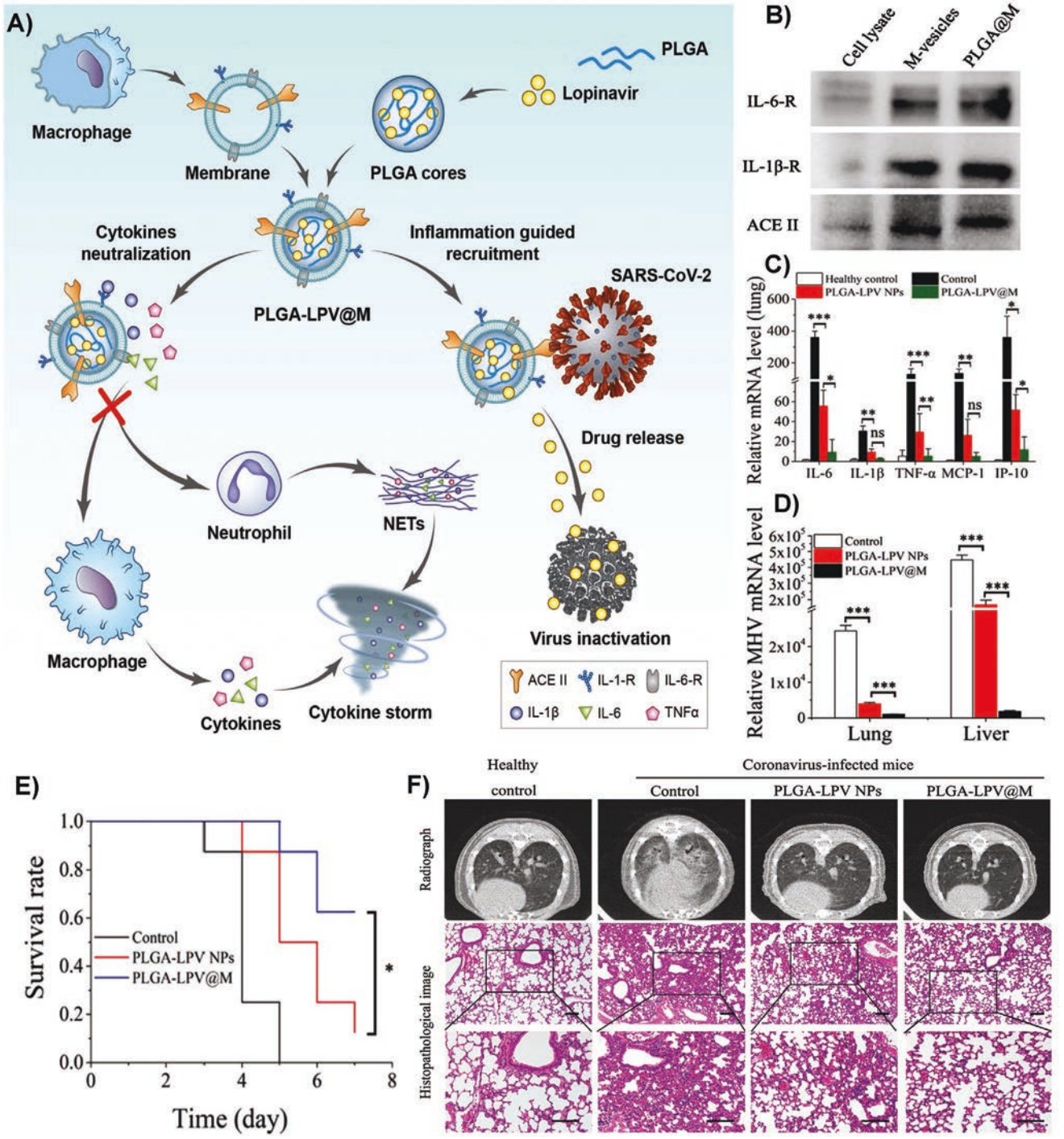

Figure 18. A) Schematic of PLGA-LPV@M preparation for antiviral and anti-inflammatory therapy against coronavirus infection. B) Expression of three surface markers (IL-6R, IL-1β R, and ACE II) assessed by western blotting. C) Relative mRNA expression for IL-6, IL-1β, TNF-α, MCP-1, and IP-10 (proinflammatory cytokines) in the lungs. D) Representation of viral loads (mRNA levels) in lung and liver tissues in coronavirus-infected mice treated with PLGA-LPV NPs and PLGA-LPV@M. E) Survival rate (days) of coronavirus-infected mice treated with PLGA-LPV NPs and PLGA-LPV@M. F) Radiograph (up) and histological (down) analysis of lung tissue in coronavirus-infected mice after different treatments. Reproduced with permission.[160] Copyright 2021, BioMed Central Ltd. Abbreviations: ACE II, angiotensin-converting enzyme 2; PLGA NP, poly(lactic-*co*-glycolic acid) nanoparticle; PLGA-LPV NP, lopinavir-loaded PLGA nanoparticle; PLGA-LPV@M, macrophage membrane-coated PLGA-LPV nanoparticle.

bright future for immune cell–mimetic nanosystems and the tremendous progress made in recent years by camouflaging NPs with macrophage-derived membranes, there are salient issues that need to be overcome before these systems can be approved as standard approaches in clinical practice.[18,42] The main challenges to the successful clinical translation of

MCM-coated NPs mainly derive from the novelty and infancy of this biomimetic technology, including 1) the high complexity of the preparation methods, 2) the heterogeneity of white blood cell functions depending on the cell source (e.g., gender, age, and health conditions), 3) possible epigenetic modification of white blood cells during isolation and purification procedures, 4) immunogenicity, 5) poor reproducibility, 6) issues related to large-scale production, and 7) safety concerns regarding the possibility of coating techniques damaging the integrity and structure of membrane proteins and compromise the biofunctionality of cell membranes.[18,21,39,42,66,101] Furthermore, the lack of understanding of the triggering mechanisms of macrophage migration and polarization, and the high complexity of the immune response within the tumor microenvironment, are concerns that need to be addressed, likely through the use of imaging techniques that can monitor the distribution and accumulation of macrophages in pathological tissues.[41,42]

Using immune cell membranes to functionalize the NPs via top-down approaches has emerged as a versatile and very promising strategy to prolong the blood circulation time in vivo, and achieve a more precise and efficient accumulation of these nanosystems in inflamed, infectious, and neoplastic tissues.[23,28] However, despite these advantages, producing macrophages on a clinical scale suitable for universal use is a demanding task, due to immunological and safety concerns arising from the presence of proteins involved in triggering immune responses on the cell membranes (e.g., MHC molecules). Therefore, due to the high risk of immune rejection when using allogeneic cells, human macrophages should be genetically modified after being extracted to reduce undesirable side effects.[21,41] Another option consists of using cells derived from each individual patient, termed autologous cells, as membrane sources for NPs coating, which could significantly improve the safety profile of these bioinspired nanosystems. Since the immune system recognizes these modified autologous cells as "self," developing personalized therapies based on autologous cell membranes hold great future promise for drug delivery purposes.[18,23]

Another challenge concerns the absence of standardized protocols for macrophage extraction and purification, which may be responsible for poor batch-to-batch consistency. Ensuring reproducibility between batches is very challenging, not only because white blood cells may undergo changes in gene expression during in vitro manipulation, but also their functions may fluctuate according to the source.[18,39] Therefore, there is an emerging need to develop novel large-scale production techniques that can reduce batch-to-batch variability and ensure the properties and quality of the macrophages.[41,42]

To date, several fusion techniques have been proposed for coating immune cell membranes onto NPs, however the different efficiency of these methods and the current lack of standardized protocols may prevent the wider use of these approaches.[23] Moreover, another issue related to the coating method, is the possible disruption of cell membranes and loss of biofunctionality of the membrane proteins, which may compromise their natural function and trigger a strong immune response against the damaged protein markers, thus raising both efficacy and safety issues. Hence, the development of optimized cell membrane coating protocols is a key step toward the clinical translation of these biomimetic nanosystems.[23,66] In addition, the development of standardized criteria and quality control parameters for cell membrane-coated NPs, is also urgently needed to avoid the presence of microorganisms, toxins, or other contaminants.[21,23,41,101]

In addition to directly transporting therapeutic drugs to target sites, cell-based delivery platforms can also be designed to deliver genes that encode therapeutic proteins after exposure to a particular trigger. In this approach, the gene is selected according to the disease mechanism, and then inserted downstream to a promotor, triggering gene expression and systemic release of the protein of interest.[18,23,30] These "cell-factories" are a very promising strategy for achieving a more effective therapeutic dose, better therapeutic results, and improved patient acceptance and satisfaction, by reducing the number of parenteral injections required.[18,23]

Due to the remarkable features of immune cell membrane-coated NPs, more and more research in the future will focus on optimizing and improving their targeting capability, and therefore further advances in cancer-targeted therapy can be expected owing to the development of biomimetic systems with even greater efficiency and tumor specificity.[23] However, despite emerging evidence of better therapeutic efficacy and reduced toxicity in vivo, these macrophage-mimicking nanosystems still face some critical problems that may compromise their clinical translation. Thus, given the enormous potential of these nanosystems to revolutionize therapy and diagnosis of various diseases in the future, these limitations must be urgently addressed before these strategies can be successfully implemented in clinical practice.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

biointerfaces, biomimetic nanoparticles, cancer, cell membrane-coated nanosystems, inflammation, macrophages

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